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The role of progesterone signaling in the pathogenesis of uterine leiomyoma

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Abstract

Uterine leiomyomas are benign tumors that originate from the myometrium. Evidence points to ovarian steroid hormones, in particular, progesterone as major promoters of leiomyoma development and growth. While progesterone action in leiomyomas involves the classical nuclear receptor effects on gene regulation, there is growing evidence that signaling pathways are directly activated by the progesterone receptor (PR) and that PR can interact with growth factor signaling systems to promote proliferation and survival of leiomyomas. Studies investigating the genomic and non-genomic actions of PR and its role in leiomyoma growth are summarized here. Studies testing various selective progesterone receptor modulators for the treatment of leiomyomas are also highlighted. An increased understanding of the mechanisms associated with progesterone-driven growth of leiomyomas is critical in order to develop more efficient and targeted therapies for this prevalent disease.

Keywords

Progesterone receptor; uterine leiomyoma; leiomyoma; progesterone

Uterine Leiomyomas

Uterine leiomyoma, also frequently referred to as uterine fibroids, are smooth muscle cell tumors originating from the myometrium. Leiomyomas can occur in 70–80% of women and 30% of women with leiomyoma will seek treatment due to morbidities such as abdominal pain and heavy uterine bleeding[1]. Due to health disturbances, leiomyoma are the number one gynecological reason that women are admitted to the hospital to undergo hysterectomy. Approximately 600,000 hysterectomies are performed each year in the United States of which 200,000 are due to leiomyoma [2]. Direct healthcare costs for the management of leiomyoma are estimated to be over \$2 billion annually [2].

The natural history of uterine leiomyoma is complex. Leiomyoma generally become symptomatic in woman during her 30's and will usually regress after menopause. Uterine leiomyoma can cause heavy menstrual bleeding leading to anemia, abdominal pain and

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pressure, urinary frequency, painful intercourse, and fertility complications. Multiple leiomyoma can occur and at times can become extremely large. Tumors show a large degree of heterogeneity even those within the same patient which is evident in growth rates and protein expression. Moreover, expression of proteins can vary within a single tumor depending on the area. The heterogeneity of tumors was demonstrated in The Fibroid Growth Study which followed the size of 262 leiomyoma from 72 women for up to 12 months using MRI [3]. Researchers found that leiomyoma from the same woman can grow at different rates with some even regressing spontaneously. The average growth rate was 9% over 6 months, and growth was not dependent upon location or tumor size. The number of tumors sharing a uterus did affect leiomyoma growth rate, although single leiomyoma grew faster than leiomyomas sharing a uterus. Interestingly, growth rates of leiomyoma differed between races when age was taken into account. White women older than 45 had slower growing tumors compared to women less than 35, while tumors from black women did not show a decrease in growth rate with age.

Etiology and Treatment

Despite the prevalence of leiomyoma, the causes remain unknown. Race is a risk factor for leiomyoma development. African American women have a greater chance of being affected by leiomyoma, which can occur at an earlier age [4]. Symptoms are more severe and surgeries are performed at an earlier age compared to Caucasian women. Women of all races have a greater chance of having leiomyoma as they age. Oral contraceptive (OCP) use protects against symptomatic tumors [5]. Women who have had children are at less risk for leiomyoma than nulliparous women, while early age at first menstrual cycle increases leiomyoma risk. General measures of health may also be predictive of leiomyoma affliction. Obesity, high blood pressure, a diet high in red meat, and alcohol consumption are associated with leiomyomas, while smoking decreases risk for unknown reasons [5–7].

Treatments for leiomyoma are limited. Heavy bleeding can be controlled with OCPs and endometrial ablation. However women with heavy bleeding and pain undergo surgical options to treat their leiomyoma due to failure of previous treatments. Gonadotropin releasing hormone agonists (GnRHa) can shrink tumor volume and improve other symptoms, but are only approved for 3–6 months prior to surgery due to adverse side effects, such as decreased bone mineral density. Levonorgestrel intrauterine systems (LNG-IUS) can also reduce bleeding association with leiomyomas [8–11]. Surgical options include myomectomy (removing the tumors) and hysterectomy. Less invasive procedures include uterine artery embolization, which blocks blood flow to the tumor by embolus [12][13] and magnetic resonance guided focused ultrasound surgery (MRgFUS) which uses focused ultrasound energy to thermally destruct leiomyoma tissue [14]. Non-hysterectomy procedures are associated with a high rate of symptom recurrence from growth of pre-existing or new tumors. For example, up to 59% of women require a second surgery after myomectomy [15]. Despite the risk of recurrence, women choose alternatives to hysterectomy in order to preserve fertility and/or to retain the uterus for personal reasons. Given the heterogeneity of leiomyomas and lack of effective therapies, identifying additional pathways that are involved in tumor growth are attractive for therapy development. In the future, classes of leiomyomas may be differentiated by molecular pathways for the best treatment available.

Clinical evidence for the role of progesterone in leiomyoma

While it is thought that the initial events that trigger leiomyoma tumorigenesis involves somatic mutations, it is evident that the development and growth of leiomyoma are highly dependent on ovarian steroid hormones. The incidence of leiomyoma in women during their

reproductive years and its regression after menopause, strongly supports leiomyoma dependence on ovarian steroids. When women are given GnRH agonists, a reduction in leiomyoma size occurs, implicating ovarian hormones in leiomyoma growth. While estrogen has been considered the major mitogenic factor in the uterus, there is growing evidence from clinical, biochemical, histological, and pharmacological studies that progesterone and its receptor, PR, play a key role in uterine leiomyoma growth and development [16]. For instance, higher mitotic activity in leiomyomas during the secretory phase compared to the proliferative phase of the menstrual cycle has been reported [17,18]. During pregnancy, it has been shown that the size of the leiomyomas increase in volume during the first 10th week of gestation [19–22]. Those investigators that followed the leiomyoma size longitudinally after the first trimester, however, did not observe a further difference between the second and third trimester [20–22]. Treatment of women with progesterone resulted in increased cellularity and mitotic activity in the leiomyomas [23]. Treatment of postmenopausal women with estrogen and progestins resulted in proliferative activity in the leiomyomas that was equal to that observed in premenopausal women whereas estrogen only treatment caused a very low proliferative activity [24]. Several studies have shown that while GnRH agonists can reduce leiomyoma size, progestin add-back therapy prevents this reduction [25–27] strongly implicating progesterone to be pro-mitotic for leiomyomas. Clinical trials have shown mixed results concerning reduction in uterus size with the progestin releasing intrauterine device, the levonorgestrel intrauterine system (LNG IUS). Several studies have found that uterine volume can be reduced in as short as 3 months with a reduction in leiomyoma volume reduced from 6–12 months of LNG IUS use [28–30]. However, other studies find a reduction in menstrual blood loss, but not leiomyoma volume [8–11]. Larger trials should be conducted to determine if LNG IUS improves blood loss and leiomyoma burden. The mechanism of leiomyoma size reduction after LNG IUS insertion is unknown. Increased local rather than systemic progestin may have unexpected results on leiomyoma biology. For instance, women with LNG IUS compared to women taking oral contraceptives have lower endometrial PR and ER expression [31]. In addition, LNG reduced serum progesterone levels and may reduce uterine artery blood flow which could explain changes in leiomyoma tumor size [32]. In support of these observations, Xu et al [33] demonstrated in vitro that LNG treatment reduced leiomyoma cell viability and increased apoptosis. Additional studies are needed to assess the cellular effects of LNG on leiomyoma and myometrial cells.

The use of antiprogestins or selective progesterone receptor modulators provides yet the strongest evidence for the in vivo mitogenic effect of progesterone on leiomyoma growth. Mifepristone, otherwise known as RU486, can effectively reduce uterine volume, bleeding, and abdominal discomfort associated with uterine leiomyoma [34–41]. Currently, RU486 is only approved for medical abortion in the United States. Therefore, some stigma revolves around the use of RU486 as a long term medical treatment. However, doses of RU486 used for medical abortion are much higher than that used in leiomyoma trials. For instance, 5mg RU486 daily can reduce leiomyoma size and symptoms in 6 months while medical abortion uses up to 600mg of RU486 as a single dose. One study followed women an average of 6 months after treatment stopped. Authors noted that regrowth of tumors occurred but more slowly than with GnRHa therapy [42]. Long term studies with follow up are required to evaluate safety of continuous or intermittent long term, low dose RU486.

Selective progesterone receptor modulators (SPRM) have been and continue to be tested for its effects on leiomyomas. SPRMs can function as agonists or antagonists to PR depending upon the cell type and molecular environment. SPRM are selective because only certain progesterone ligand responses will occur. Clinically, the SPRMs are attractive because of reduced side effects on non- target tissues, such as the breast and brain. Asoprisnil (J687) is a SPRM that was tested in 129 women with leiomyoma [43]. J687 reduced bleeding and

decreased leiomyoma tumor volume [43,44]. *In vitro* effects of Asoprisnil are reduced proliferation, induction of apoptosis, reduction of extra-cellular matrix (ECM) deposition, and reduction of growth factor expression [45–50]. Phase III clinical trials testing the safety of Asoprisnil have been completed. Ulipristal, CDB-2914, is another SPRM that can reduce leiomyoma symptoms. Preclinical testing in primary cells suggests that CDB-2914 inhibits proliferation, induces apoptosis, alters ECM regulation only in leiomyoma cells, and may reduce angiogenesis [51–54]. A small trial revealed that CDB-2914 reduced uterine volume and bleeding [55]. Larger trials for CDB-2914 are underway to treat uterine leiomyoma. Proellex, CDB-4124 has been tested in two clinical trials for symptomatic leiomyoma. A reduction in blood loss and leiomyoma-related symptoms was observed and leiomyoma volume was reduced (reviewed [56]). The effects of the SPRMs on the endometrium are usually examined given the tissue's responsiveness to progesterone. Endometrial changes that do occur with SPRM described a new type of endometrial pathology now referred to as “non-physiologic effect” [57] [49,58,59]. Studies have determined that SPRM-mediated changes of the endometrium are non-proliferative and not a cancer precursor [60–63]. Thus, the use of SPRMs as a form of treatment for leiomyoma hold promise.

Progesterone receptor regulation in leiomyoma

The physiologic actions of progesterone are mediated by interaction with the progesterone receptor (PR), a member of the nuclear hormone superfamily of ligand-activated transcription factors [64,65]. There are two predominant PR isoforms, designated PR-A and PR-B. Although data are conflicting, several investigators have shown that levels of PR in leiomyomas do not change during the menstrual cycle and that increased concentration of both PR-A and PR-B occur in leiomyoma tissue compared to myometrial tissue [17,66,67]. As in other cell types, leiomyoma cells respond to estradiol and expression of both PR isoforms increase [68]. Accordingly, overexpression of dominant-negative ER decreased PR expression in human leiomyoma cells [69]. Recent work by Ishikawa et al. [70], suggested that estradiol maintains PR levels and that it is progesterone through its receptor that promotes leiomyoma growth. Regulation of PR expression in leiomyomas has not been studied in any great detail. Several groups have looked at the role of estrogen in upregulating PR in leiomyomas. Aromatase is an enzyme that converts testosterone to estradiol. Aromatase is expressed more highly in leiomyomas and leiomyomas are capable of making their own estradiol [71–73]. Aromatase expression of leiomyomas also varies with ethnicity. For instance, African American women showed significantly higher aromatase mRNA than Caucasian and Japanese women [72]. Contrary to the assumption that aromatase activity, leading to increased estradiol, should increase PR levels, this correlation was not what was observed in this study. In fact, PR mRNA levels in leiomyomas were significantly higher in Japanese women compared with African-American or Caucasian-American women. Further studies are necessary to correlate aromatase activity and PR function. Importantly aromatase inhibitors can decrease leiomyoma size and symptoms and are currently being tested as leiomyoma therapy. Aromatase inhibitors are attractive potential therapies because they do not decrease serum estradiol levels or cause menopausal-type side effects like GnRH α [74,75]. Catechol-*O*-methyltransferase (COMT) metabolizes estrogen into an inactive form. COMT alleles have been associated with different levels of serum estrogen in women [76] leading researchers to question a possible role for COMT in leiomyoma. 2-methoxyestradiol (2ME) is a product of COMT activity and can inhibit leiomyoma cell proliferation [77]. COMT overexpression and high 2ME levels disrupt microtubule dynamics which can affect steroid receptor cellular distribution and transcriptional potential. Total PR levels dropped with high COMT expression suggesting that disruption of PR levels via estrogen metabolism may inhibit leiomyoma cell proliferation.

Activation of signaling pathways in leiomyoma by estrogen and progesterone

It has been well documented in various cell types that both estradiol and progesterone can act through its classical receptors to rapidly activate signaling pathways in a non-genomic manner. Given the important role estradiol (E2) and progesterone play in leiomyoma growth, the ability of hormones to rapidly activate signaling pathways as potential mechanisms of action has been explored, although data are limiting. Barbarisi et al [78] demonstrated a rapid activation of the MAPK pathway by estradiol in primary leiomyoma cells. In addition, rapid protein tyrosine phosphorylation of a subset of intracellular proteins, such as GAP, PI3K, and PLCgamma also occurred. Interestingly, activation of this pathway was related to E2-induced PDGF secretion and it was proposed that PDGF, alone or in association with other growth factors, was the main growth factor involved in the proliferation response of leiomyoma cells to E2 stimulation. Another study demonstrated rapid increase of phosphorylated protein kinase C alpha (PKC alpha) and ERK1/2 by E2 in immortalized uterine smooth muscle which corresponded to increased proliferation [79]. The interaction between ERalpha and signaling pathways was suggested in a study that showed higher ER-alpha phosphorylation in leiomyoma tissues derived from patients in the proliferative phase of the menstrual cycle which correlated with an increased phosphorylation of p44/p42 MAPK proteins in leiomyoma [80]. Phosphorylated p44/42 colocalized with ER-alpha phosphorylated on serine 118 suggesting that MAPK may phosphorylate ER-alpha in leiomyoma.

Studies have demonstrated that progesterone can have rapid, membrane initiated effects independent of gene transcription to alter production of second messenger and cell signal transduction pathway. In breast cancer cells, progesterone triggers rapid non-genomic effects of progesterone through nuclear PR by directly binding to the SH3 domain of Src kinase to activate its kinase activity [81]. Similarly, progesterone-mediated regulation of the PI3K/AKT pathway has been demonstrated in breast cancer cells and in rat endometrial stromal cells [81–85]. In leiomyoma cells, progesterone, through its receptor, can rapidly activate the PI3K/AKT pathway [86]. Specifically, levels of phosphorylated-AKT and its downstream effectors, p-GSK3b, and p-FOXO1 rapidly increased with progestins in leiomyoma cells. AKT phosphorylation was abrogated by progesterone receptor antagonist RU 486 and the PI3K inhibitor LY290004 in primary leiomyoma cells demonstrating dependence on PR and PI3K. Furthermore, an AKT inhibitor decreased leiomyoma cell viability and promoted apoptosis despite the presence of progestins. The PI3K/AKT pathway has been highlighted as a potential promoter of leiomyoma growth in recent years. Phosphorylated AKT (p-AKT) levels are higher in leiomyoma tumors than matched myometrium [87]. Tumors from menopausal women also showed reduced p-AKT levels compared to pre-menopausally derived tumors. AKT effectors such as, GSK3b and FOXO1 have also been shown to be more highly phosphorylated in leiomyoma tumors versus myometrium [88,89]. Specifically, levels of pSer256-FOXO1 were higher in leiomyoma than in matched myometrium and interestingly, the pSer256-FOXO1 was localized mostly in the nuclear fraction presumably due to inadequate shuttling of FOXO1 by 14-3-3 [88]. Levels of pGSK3alpha and cyclin D2 proteins were elevated significantly in the leiomyoma compared with the normal myometrium [89]. A negative regulator of AKT is PTEN and the less active phospho-PTEN was found to be more prevalent in leiomyomas compared to matched myometrium during the menstrual cycle [90]. Levels of p-PTEN did not differ in leiomyoma from myometrium in tissues from postmenopausal women, strongly implicating the role of steroid hormones for increased p-PTEN and pAKT [90]. Importantly, inhibition of PI3K, an upstream positive regulator of AKT, reduces leiomyoma and myometrial cell

line proliferation and cell cycle progression [91]. GnRHa therapy decreased PI3K activity and AKT phosphorylation supporting that AKT activation is hormone-dependent [92].

Regulation of Growth Factors and their Receptors by progesterone

As demonstrated in many cell systems, hormone and growth factor signaling pathways are interconnected, working together towards the regulation of physiological processes including proliferation, apoptosis, and differentiation [93–95]. This has also been the focus of research in leiomyoma cells, which has been recently reviewed [96–98]. Growth factor receptors, also known as receptor tyrosine kinases (RTK), are transmembrane receptors that are activated by an extracellular ligand or growth factor. Once a growth factor has bound to the receptor the receptor dimerizes or undergoes a conformation change inducing autophosphorylation and activation of kinase activity, including PI3K, Ras-MAPK, and JAK-STAT [99].

Much research has been done characterizing the differential expression of various growth factors and their receptors in leiomyoma and myometrium. To briefly highlight some of those findings, leiomyoma expressed higher insulin receptor (IR), insulin-like growth factor receptor-I (IGFRI), IGF-II, epidermal growth factor (EGF), platelet derived growth factor (PDGF) and its receptors, and transforming growth factor B ligands (TGFB) and its receptors [100–105] than myometrium. One study performed a phospho-RTK array on leiomyoma and myometrial tissues from 10 patients and found higher expression of phospho-RTKs belonging to the EGF, FGF, IGF-I, HGF, and PDGF growth factor receptor gene families in leiomyomas. Studies have shown that growth factors can induce proliferation of leiomyoma cells. TGFB1 and PDGF increased leiomyoma cell proliferation in culture [106,107]. Leiomyoma cells grown subcutaneously and treated with PDGF grew larger tumors than untreated mice [107]. Furthermore, inhibiting PDGFR with lentiviral RNA reduced tumor size of subcutaneous leiomyoma lesions. Lee and Nowak [108] found that myometrial cells treated with high concentrations of TGFB ligands resulted in a reduction of proliferation, which was expected at the high concentrations. Leiomyoma cells, on the other hand, did not respond to the same treatments indicating a dysregulation of TGFB signaling in leiomyoma cells. The EGFR inhibitor AG1478 reduced leiomyoma and myometrial cell proliferation by possibly arresting cells in G1 [109]. AG1478 effects were not blunted by the presence of progesterone or estradiol indicating that AG1478 would still be effective in cycling women.

Progesterone can regulate expression of growth factor signaling proteins. During the secretory phase of the menstrual cycle EGF mRNA was higher in leiomyoma than myometrium [110]. Progesterone, but not estradiol treatment stimulated EGF protein expression [111]. In addition, the SPRM, asoprisnil decreased EGF mRNA expression [47,112]. Conversely, progesterone reduces the expression of IGF-I mRNA, while IGFRI levels remained constant [113]. Similarly, women in the proliferative phase, had the highest amounts of IGF-I in their leiomyoma tissues [114]. IGF-I mRNA levels have been shown to negatively correlate with PRB levels in leiomyoma [115]. Since it has been shown that IGF-I treatment can increase leiomyoma proliferation [103,116,117], it remains to be clarified how progesterone regulation of IGFs contributes to leiomyoma growth. The differential expression of PDGFs in leiomyoma and myometrial cells is conflicting depending on the study [105,118–122]. It has been shown that increased PDGF-BB expression occurs during the secretory phase compared to the proliferative phase in leiomyomas [118]. TGF beta receptors type I–II and TGF beta 1, 2, 3 have been found in myometrium [123,124] but expression in leiomyoma remains discrepant^{118; 119}. Both increased and decreased expression of TGF beta 1 mRNA in leiomyoma compared to myometrium, have been demonstrated [106,123,125]. The expression of TGF beta 3 has been shown to be

consistently higher in leiomyoma compared to myometrium [126] and peak levels of were found during the secretory phase of the menstrual cycle suggesting progesterone involvement [106,108]. In support of this, the SPRM, asoprisnil, decreased TGF beta 3 mRNA in leiomyoma cells [47,112]. It is evident that the crosstalk between the progesterone and growth factor signaling pathways has been understudied in leiomyomas. Given the important role progesterone plays in leiomyoma development and growth, and the common dysregulation of growth factor signaling that occurs in this disease, it will be critical to study the mechanisms associated with the interconnection between progesterone and growth factors.

Regulation of genes associated with proliferation and apoptosis by progesterone

Leiomyomas show higher levels of the proliferating cell nuclear antigen (PCNA), which is associated with cell proliferation, than myometrium throughout the menstrual cycle [111]. Treatment of leiomyoma cells with estradiol or progesterone increased PCNA expression compared to untreated cells [127]. Asoprisnil and CDB-4124 decreased PCNA in cultured leiomyoma cells with no effect on myometrial cells [47]; [128]. Studies have shown that progesterone can increase expression of the anti-apoptotic BCL-2 gene [129,130]. Direct binding of liganded PR to the BCL-2 promoter enhances its transcription in primary leiomyoma cells [130]. Accordingly, asoprisnil decreased BCL-2 expression with a corresponding increase in TUNEL staining, cleaved caspase 3, and cleaved PARP supporting a role for progesterone receptor in preventing apoptosis in these cells [45,47]. While it is evident that progesterone plays a key role in proliferation and apoptosis in leiomyomas, identification of the genes associated with these physiological processes have not been studied in great detail. Seminal work by Yin et al [131] used the chromatin immunoprecipitation (ChIP)-cloning approach to identify PR target genes in primary uterine leiomyoma cells. Eighteen novel PR-binding sites were identified, one of which was located 20.5 kb upstream of the transcriptional start site of the Krüppel-like transcription factor 11 (KLF11) gene. Upon confirmation analysis, KLF11 mRNA levels were minimally downregulated by progesterone but robustly upregulated by the progesterone antagonist RU486. Promoter activity of both the basal and distal PR binding regions of KLF-11 was upregulated by RU486. These regions also contained multiple Sp1-binding sequences and lacked classic progesterone response elements. RU486 stimulated recruitment of Sp1, RNA polymerase II, PR, and the coactivators SRC-1 and SRC-2 to the distal region and basal promoter. Gene knockdown studies revealed that KLF-11 inhibits proliferation of leiomyoma cells and that its expression is significantly lower in leiomyoma tissues compared with adjacent myometrial tissues. Another novel progesterone receptor target gene identified by ChIP-cloning was the L-type amino acid transporter 2 (LAT2) [132]. Progesterone significantly induced LAT2 mRNA levels, which was blocked by cotreatment with RU486. LAT2 forms heterodimeric complexes with 4F2 heavy chain (4F2hc) and it was observed in this study that while progesterone did not alter 4F2hc mRNA levels, RU486 significantly induced 4F2hc mRNA expression. Small interfering RNA knockdown of LAT2 or 4F2hc markedly increased leiomyoma cell proliferation. Levels of LAT2, but not 4F2hc were higher in leiomyoma tissues compared with matched myometrial tissues. Much more work needs to be done to determine which genes are regulated by progesterone to promote leiomyoma growth.

Recently investigators have uncovered that microRNAs (miRNAs) may play a role in leiomyoma pathogenesis [133–139]. MiRNAs are small non-coding RNA's that inhibit translation mostly through binding to target mRNA 3' UTR. Regulation of miRNAs by hormones has been investigated. With the use of microarrays, Pan et al [138] discovered miRNAs that were differentially expressed in myometrial and leiomyoma cells. Among

those identified, the regulation of miR-20a, miR-21 and miR-26a in response to various hormonal and antagonist treatments were analyzed. Specifically, medroxyprogesterone acetate (MPA) treatment increased the expression of miR-21 and inhibited miR-26a in leiomyoma cells whereas miR-20a expression was not significantly affected as compared to untreated controls. RU-486 significantly increased miR-21 and inhibited miR-26a expression in leiomyoma cells. Additional studies with miR-21 revealed that its expression was elevated in leiomyomas during the secretory phase of the menstrual cycle [134]. In addition, miR-21 was found in women who received Depo-Provera and oral contraceptives, but reduced with GnRHa therapy. This field of research is still in its infancy in leiomyomas, and given that hormonal regulation of miRNAs is an emerging field that is being studied in other hormone-dependent diseases, it is appropriate to include leiomyomas in these investigations.

Animal model insights to elucidate the role of progesterone in leiomyoma

Animal models have been used to study leiomyoma growth and behavior in an in vivo setting. The Eker rat has been used to study leiomyomas due to the spontaneous development of smooth muscle tumors of the uterus [140–142]. These rats are heterozygous for tuberin (TSC2), a tumor suppressor gene, where its gene product is regulated by various signaling molecules, such as AKT and it also directly affects the mTOR complex 1. Smooth muscle tumors in the Eker rat are phenotypically and biochemically similar to the human disease, they are benign, and they are responsiveness to hormones, expressing both ER and PR. Cells from these tumors proliferated more quickly and underwent apoptosis more slowly than myometrial cells during the estrus cycle [143]. The authors also documented that unlike myometrial cells, leiomyoma cells did not respond properly to hormonal signals. Interestingly, Cook et al [144] demonstrated that exposures of the Eker rat to xenoestrogen during the development of the myometrium resulted in reprogramming the response of this tissue to estrogen to promote leiomyoma development. The dependence of the leiomyoma tumors in the Eker rat to hormones was further demonstrated using the ELT3 cells, which were derived from the rat tumors. Expression of a dominant negative-ER (DN-ER) altered expression of hormonally responsive genes and proteins by inhibiting ligand-dependent wild type ER transcriptional activity. Genes and proteins associated with proliferation, cyclin D1, Cox2, PCNA, VEGF, and EGF decreased, as well as the anti-apoptotic protein, Bcl2. PRA and PRB expression were significantly reduced with DN-ER with PRA levels being more affected than PRB. Subcutaneously injected DN-ER expressing ELT3 cells into nude mice [145] resulted in tumors significantly smaller than empty vector expressing tumors. Tumors injected with DN-ER showed decreased proliferation and apoptosis by two weeks. These data demonstrate that estrogen and progesterone responsive genes can be manipulated by altering ER transcription and that targeting ER could be developed further for leiomyoma therapy.

In recent years, mouse models to study human leiomyomas have been established by three groups. First, Suo et al [107] grew human leiomyoma xenografts, subcutaneously in Rag2^{-/-}γc^{-/-} double knock out female mice. Before grafting, a green fluorescence protein (GFP)-luciferase (LUC) fusion gene was introduced into leiomyoma cells using lentiviral-mediated gene transfer to allow for the monitoring of transplanted leiomyoma cells in mice using bioluminescence-based whole animal imaging. The authors found that in the presence of estradiol, freshly dissociated leiomyoma cells, but not the leiomyoma-derived smooth muscle cells grown in ex vivo cultures, can generate stable xenografts in subcutaneous Matrigel implants. In contrast, myometrial cells did not form xenografts under the same experimental conditions. Second, human leiomyoma tissues have been transplanted in the NOD/SCID/gc-null mice subcutaneously with estrogen supplementation [146]. After 4 and 8 weeks, the histological architecture as well as markers of uterine leiomyoma, including ER,

PR, and α -smooth muscle actin were maintained. Finally, the role of progesterone in leiomyoma growth was shown using yet another mouse model, where human leiomyoma cells were grafted under the renal capsule of nonobese diabetic-scid IL2R^{gnull} female mice [70]. Leiomyoma tissue pieces or leiomyoma cells embedded in collagen were grafted under the kidney capsule in immunodeficient mice which were ovariectomized and supplemented with estrogen only, progesterone only, or estrogen with progesterone in the form of hormone pellets inserted under the skin. Myometrial tissues were also examined but failed to grow under any conditions supporting that the myometrium is a non-proliferative tissue in the absence of pregnancy. Interestingly, leiomyoma grafts grew only in the presence of both estrogen and progesterone (E+P). In addition, even though tumor volume and proliferation were higher in the presence of E+P, cell density was lower indicating an increased deposition of ECM. Importantly, tumor volume, proliferation, and ECM were dependent upon the progesterone receptor as determined by RU486, treatment. This significant finding demonstrated that estrogen and its receptor serve to maintain PR levels, but leiomyoma growth is dependent upon progesterone actions in human tissues and cells. The authors also determined that hormones were required for leiomyoma tumor maintenance, in that upon removal of the hormone pellets, tumor volume, proliferation, and ECM deposition were blunted. Additionally, the highest proliferation index was during the short period with hormones indicating that proliferation is an early event in leiomyoma growth. With the various mouse models described, further testing of hormones and other pharmacological compounds on human leiomyoma tissues can be done in an in vivo system.

Conclusions

Despite the prevalence of uterine leiomyomas in women and the degree to which the leiomyoma cause morbidity, relatively little is known about this disease. As described, there is abundant preclinical and clinical evidence to implicate progesterone in promoting growth of leiomyomas. Much of the investigation, thus far, has focused on differential expression of proteins and genes during the menstrual cycle and in response to exogenous progesterone or progestins, as well as the effects of hormones and growth factors on physiological processes associated with leiomyoma pathology, such as proliferation and apoptosis. There is much more to be learned in terms of how progesterone promotes proliferation, the repertoire of genes involved, and how progesterone and growth factor signaling pathways crosstalk in leiomyomas. These mechanisms need to be investigated in detail. The use of SPRMs for the treatment of leiomyoma holds promise and underscores the crucial role progesterone plays in leiomyomas. Depending on the outcome of the clinical trials, SPRMs could become an alternative option to surgery for women with leiomyoma. Given the high incidence of leiomyoma in women, the morbidity that is associated with this disease and the financial burden of over 200,000 hysterectomies performed for leiomyomas each year in the US alone, it is imperative that alternate therapies are developed. This can only be done with a better understanding of the molecular mechanisms associated with key players, such as progesterone, in this disease.

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