REVIEW ARTICLE

MECHANISMS OF DISEASE

Dan L. Longo, M.D., *Editor*

Uterine Fibroids

Serdar E. Bulun, M.D.

From the Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago. Address reprint requests to Dr. Bulun at Prentice Women's Hospital, 250 E. Superior St., Ste. 03-2306, Chicago, IL 60611, or at s-bulun@northwestern.edu.

N Engl J Med 2013;369:1344-55.
DOI: 10.1056/NEJMra1209993

Uterine fibroids (leiomyomas) represent the most common tumor in women. These lesions disrupt the functions of the uterus and cause excessive uterine bleeding, anemia, defective implantation of an embryo, recurrent pregnancy loss, preterm labor, obstruction of labor, pelvic discomfort, and urinary incontinence and may mimic or mask malignant tumors. By the time they reach 50 years of age, nearly 70% of white women and more than 80% of black women will have had at least one fibroid; severe symptoms develop in 15 to 30% of these women.[1,2] Uterine fibroids in black women are significantly larger at diagnosis than those in white women, are diagnosed at an earlier age, and are characterized by more severe symptoms and a longer period of sustained growth.[3-5] Approximately 200,000 hysterectomies, 30,000 myomectomies, and thousands of selective uterine-artery embolizations and high-intensity focused ultrasound procedures are performed annually in the United States to remove or destroy uterine fibroids. The annual economic burden of these tumors is estimated to be between $5.9 billion and $34.4 billion.[6]

There may be one predominant uterine fibroid or a cluster of many fibroids (Fig. 1). Very large fibroids can cause the uterus to expand to the size reached at 6 or 7 months of pregnancy. The location and size of the fibroid in the uterus are critical determinants of its clinical manifestations. As compared with other fibroids, submucous fibroids that extend into the uterine cavity are the most disruptive to endometrial integrity, implantation, and the capacity of the myometrium to contract and stop menstrual bleeding from the endometrial blood vessels; thus, even small submucous fibroids are associated with excessive or irregular bleeding, infertility, and recurrent pregnancy loss. In contrast, subserous fibroids that grow out into the peritoneal cavity can exert pressure that is sensed by the patient as pelvic discomfort only if they reach a certain size. Intramural fibroids that reside in the myometrial wall represent an intermediary group. Regardless of their size or location, fibroids may have paracrine molecular effects on the adjacent endometrium that are extensive enough to cause excessive uterine bleeding or defective implantation.[7]

Uterine fibroids are monoclonal tumors that arise from the uterine smooth-muscle tissue (i.e., the myometrium).[8] Histologically, fibroids are benign neoplasms composed of disordered smooth-muscle cells buried in abundant quantities of extracellular matrix (Fig. 1). The cells proliferate in vivo at a modest rate. Formation of the extracellular matrix also accounts for a substantial portion of tumor expansion. Uterine fibroids are almost always benign.[9]

A striking feature of uterine fibroids is their dependency on the ovarian steroids estrogen and progesterone.[10] Ovarian activity is essential for fibroid growth, and most fibroids shrink after menopause. The sharp elevations and declines in the production of estrogen and progesterone that are associated with very early pregnancy and the postpartum period have a dramatic effect on fibroid growth.[11-13] Gonadotropin-releasing-hormone (GnRH) analogues, which suppress ovarian activity and reduce circulating levels of estrogen and progesterone, shrink fibroids and reduce associated uterine bleeding.[14]

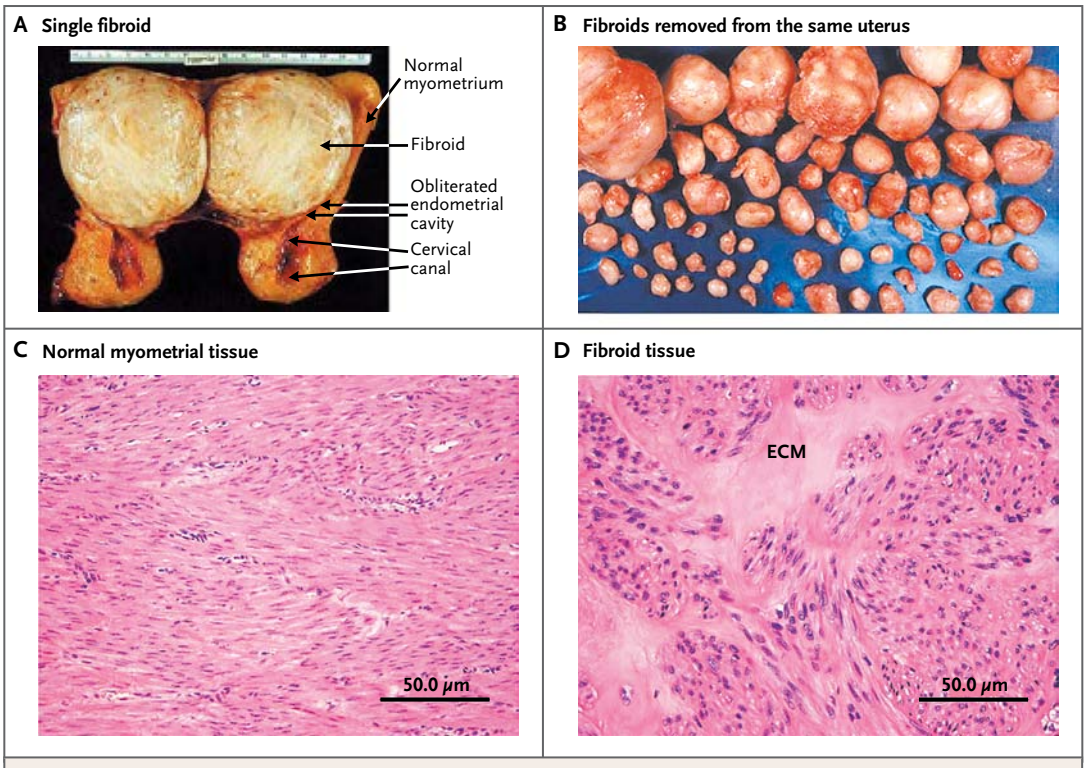


Figure 1. Gross Anatomical and Histologic Features of Fibroids.

A single large fibroid may occupy the entire uterine fundus (Panel A, bivalved) and obliterate the endometrial cavity, but many fibroids of varying size can also grow in a single uterus (Panel B). Normal myometrium contains well-organized bundles of smooth-muscle cells with relatively small nuclei and abundant cytoplasm (Panel C, hematoxylin and eosin). With the growth of fibroid tissues, islands of disordered smooth-muscle cells separated by abundant extracellular matrix (ECM) (Panel D; hematoxylin and eosin) are prominent in fibroid tissue. Fibroid smooth-muscle cells contain fairly large and conspicuous nuclei. Photographs in Panels B, C, and D are courtesy of Dr. Jian-Jun Wei, Northwestern University, Chicago.

A limited number of genetic defects transmitted by germ cells have been associated with familial uterine fibroid syndromes.[15] Most notable are germline mutations causing fumarate hydratase deficiency, which predisposes women to the development of multiple uterine fibroids.[16] In addition, a variety of somatic chromosomal rearrangements have been described in up to 40% of uterine fibroids.[17] Recently, whole-genome sequencing showed that chromosomal rearrangements are often complex, best described as single events consisting of multiple chromosomal breaks and random reassembly.[18] In an earlier study, a somatic single-gene defect was found in a majority of uterine fibroid tumors; this group of mutations affects the gene encoding mediator complex subunit 12 (*MED12*).[19]

There are also genomewide differences in DNA methylation between fibroid tissue and the adjacent normal myometrium.[20] A large number of other molecular defects involving transcriptional and posttranscriptional events, microRNAs (miRNAs), and signaling pathways have also been described.[21-28] Although some of the effects of uterine fibroids on cell proliferation, apoptosis, and extracellular matrix formation have been identified, little is known about their effects on other cellular processes in fibroid growth, such as autophagy and senescence. This review focuses on some recent developments in fibroid research, including the role of stem cells, somatic genetic and epigenetic defects, and the action of estrogen and progesterone and their cross-talk with various signaling pathways.

CELLULAR ORIGINS

The cellular origin of uterine fibroids remains unknown. Several observations support the notion that each fibroid originates from the trans-

formation of a single somatic stem cell of the myometrium under the influence of ovarian hormones. Early genetic studies suggest that fibroids are monoclonal tumors.[8] Human and mouse myometrial tissues contain multipotent somatic stem cells. By means of asymmetric division, this subset of tissue cells undergoes self-renewal and produces daughter cells under the influence of reproductive hormones (possibly ovarian hormones); this process is responsible for regeneration.[29-31] Human uterine fibroid tissue contains fewer stem cells than normal myometrium.[32,33] However, stem cells derived from fibroid tissue — not the myometrium — carry *MED12* mutations, which suggests that at least one genetic hit initially transforms a myometrial stem cell, which subsequently interacts with the surrounding myometrial tissue to give rise to a fibroid tumor (Fig. 2).[33]

In vivo experimental models reveal that the growth of human fibroid tumors that are dependent on estrogen and progesterone requires the presence of multipotent somatic stem cells.[33,34] As compared with the main fibroid-cell population or with normal myometrial cells, fibroid stem cells express remarkably low levels of receptors for estrogen and progesterone. The growth of fibroid stem cells requires the presence of myometrial cells with higher levels of the estrogen and progesterone receptors and their ligands, suggesting that the action of steroid hormones on fibroid stem cells is mediated by myometrial cells in a paracrine fashion.[33,34] It is likely that this paracrine interaction with the surrounding cells supports the self-renewal of fibroid stem cells (Fig. 2). Both myometrial and fibroid multipotent somatic stem cells lack markers for smooth-muscle cells, and in addition to their differentiation into smooth-muscle cells in vivo, they can be induced to differentiate into cells with adipogenic and osteogenic lineages.[31,34]

Signaling by the wingless-type MMTV integration site family (WNT)–β-catenin pathway seems to play a role in somatic stem-cell function in the myometrium and in uterine fibroid tissue. Overall, total β-catenin levels in the myometrium and fibroid tissue are similar.[35] But because the key effects of β-catenin are probably manifested at the level of stem cells, which make up a very small fraction of fibroid or myometrial tissue, differences in β-catenin levels would not be detected when whole fibroid and myometrial tissues are compared. In mice, selective deletion of β-catenin in uterine mesenchyme during embryonic development substantially reduces uterine size and replaces the uterus with adipocytes, disrupting entirely the normal myometrial differentiation or regeneration of smooth muscle. This observation suggests that β-catenin plays a key role in stem-cell renewal and in the differentiation of stem cells into the smooth-muscle phenotype observed in myometrial and fibroid tissues.[29] Conversely, selective overexpression of constitutively activated β-catenin in uterine mesenchyme during embryonic development and in adult mice gives rise to fibroidlike tumors in the uterus.[36]

Complex mechanisms regulate the biologic functions of β-catenin. Secreted WNT proteins bind to cell-surface receptors of the Frizzled family, causing the activation of a cascade of proteins that leads to decreased β-catenin degradation in the cytosol and ultimately changes the amount of β-catenin that reaches the nucleus.[37] Having escaped degradation, cytoplasmic β-catenin is able to enter the nucleus and interact with chromatin and the family of T-cell transcription factor (TCF) proteins to regulate the expression of a large number of genes and alter key cellular functions, such as cell fate, tumorigenesis, and differentiation.[37] The size and number of fibroidlike tumors driven by β-catenin increase with parity in mice, suggesting that ovarian hormones may interact with the WNT–β-catenin pathway to accelerate tumorigenesis.[36] The activated WNT–β-catenin pathway has also been shown to stimulate the expression of transforming growth factor β3 (TGF-β3), which induces cell proliferation and the formation of extracellular matrix in human fibroid tissue.[36,38] Fibroid-tissue–derived TGF-β3 may also suppress the expression of local anticoagulant factors in adjacent endometrial cells, which results in the prolonged menstrual bleeding associated with fibroids.[7] These observations indicate that there are critical interactions among activated WNT–β-catenin and TGF-β pathways, estrogen and progesterone, and stem-cell renewal and that these interactions ultimately give rise to the clonal formation of uterine fibroid tumors (Fig. 3).

GENETIC FEATURES

Hereditary syndromes and somatic chromosomal aberrations associated with uterine fibroids

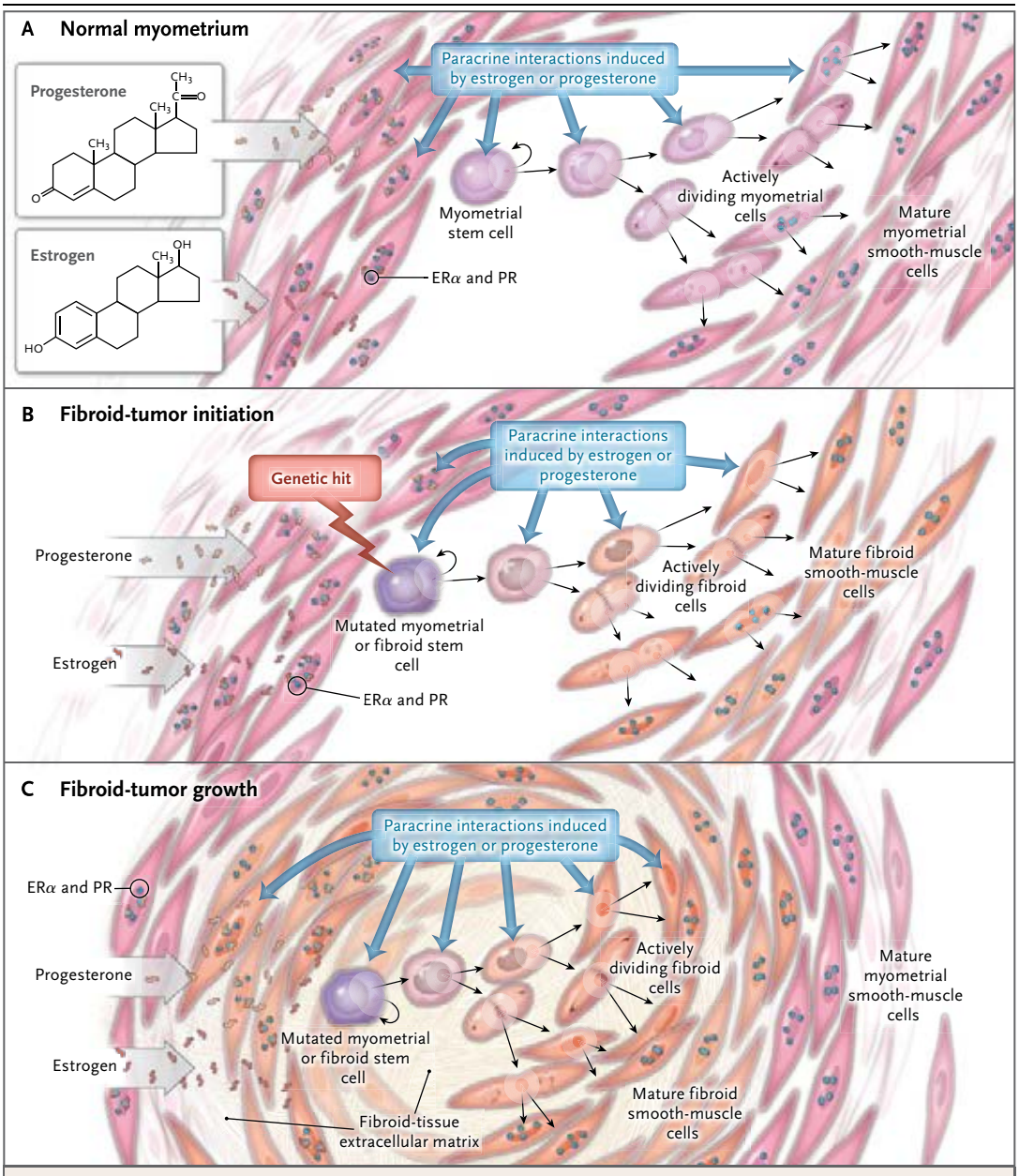


Figure 2. Tumorigenesis of Fibroids.

Both normal myometrial tissue and fibroid tissue contain pools of cells with the capacity for self-renewal; these populations are referred to as stem cells. A stem-cell population is responsible for the proliferation of normal myometrial smooth-muscle cells (Panel A). This process accounts in part for the physiologic enlargement of the uterus during pregnancy. Mature myometrial cells express much higher levels of estrogen receptor α (ERα) and progesterone receptor (PR) than do stem cells. Thus, it is likely that estrogen- and progesterone-dependent cell proliferation is primarily mediated by the ERα and PR that reside in mature cells. Paracrine factors, such as WNT ligands, that are released by mature cells may act on stem cells to induce their self-renewal and proliferation. A genetic hit, such as a MED12 mutation or a chromosomal rearrangement affecting HMGA2, may transform a myometrial stem cell into a fibroid stem cell (Panel B). This fibroid cell may self-renew and start dividing in an uncontrolled fashion until it differentiates into a mature fibroid smooth-muscle cell. During this process, fibroid smooth-muscle cells acquire many epigenetic and phenotypic abnormalities. ERαs and PRs are concentrated primarily in mature fibroid cells and pass on estrogenic or progestogenic signals to stem cells through paracrine mechanisms. The single, transformed fibroid stem cell eventually gives rise to a benign fibroid tumor with well-demarcated margins, which expands within the myometrial tissue (Panel C). Extracellular-matrix formation contributes substantially to tumor expansion.

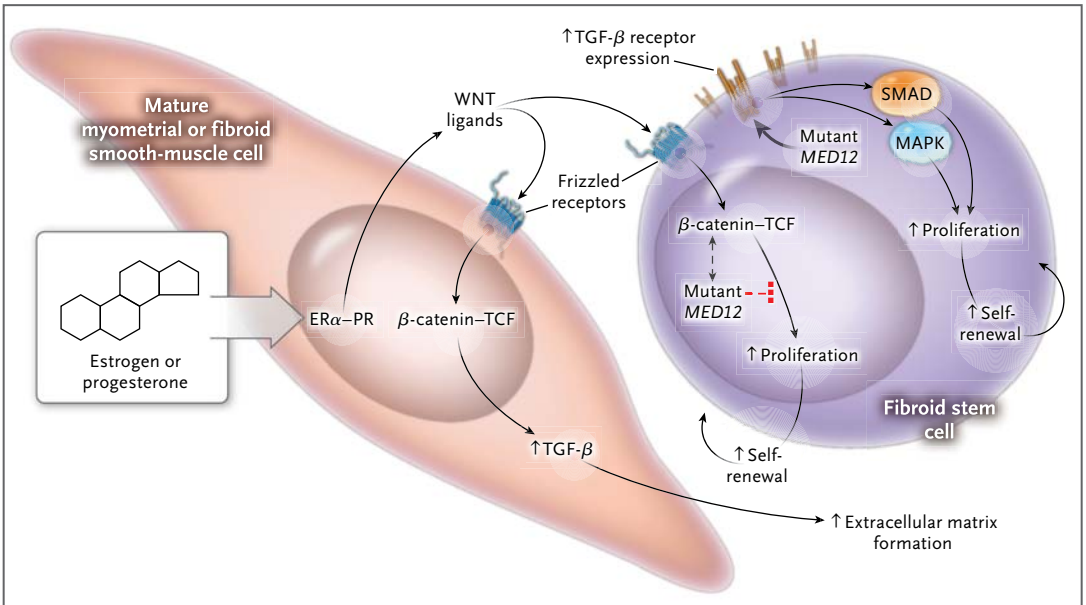


Figure 3. Interactions among Ovarian Hormones, the β-Catenin and TGF-β Pathways, and *MED12* in Fibroid Cells.
Since ERα and PR levels are remarkably high in mature myometrial cells and fibroid cells as compared with stem cells, estrogen and progesterone probably send signals to fibroid stem cells through hormone receptors in mature cells in a paracrine fashion. Estrogen and progesterone may increase secretion of WNT ligands from mature smooth-muscle cells surrounding the stem cells. In both cell types, WNT, acting through the Frizzled family of receptors, activates the β-catenin–T-cell transcription factor (TCF) pathway, which induces the production of transforming growth factor β (TGF-β) in mature cells and leads to excessive formation of extracellular matrix. In stem cells, nonmutant *MED12* may act as a physiologic modifier of β-catenin action, whereas mutant *MED12* (or the absence of *MED12*) may lead to the failure to accomplish this function. The absence of *MED12* or the presence of the mutant form in stem cells has also been linked to increased expression of the TGF-β receptor, which leads to the activation of its downstream signaling. This in turn activates the mothers against decapentaplegic homologue (SMAD) and mitogen-activated protein kinase (MAPK) family proteins, mediating stem-cell self-renewal and proliferation.

have been reviewed previously.[15,39] Analysis of single-nucleotide polymorphisms in peripheral-blood DNA has revealed three chromosomal loci — 10q24.33, 22q13.1, and 11p15.5 — associated with uterine fibroids.[40] Somatic mutations involving high-mobility group AT-hook 2 (*HMGA2*) and *MED12* are discussed here. Rearrangements involving chromosome 12q14-15 are observed in 7.5% of fibroids. Most of the 12q15 breakpoints are located upstream of the *HMGA2* gene promoter, giving rise to full-length HMGA2 overexpression, and are strongly associated with large fibroids.[17] Hmga2 expression in murine neural stem cells suppresses cyclin-dependent kinase inhibitor 2a (Cdkn2a), which encodes the proteins p16Ink4a and p14Arf, negative regulators of their self-renewal.[41] In fibroid cells, HMGA2 appears to inhibit senescence by down-regulating p14ARF.[42] Intriguingly, uterine fibroids are deficient in the Let-7 miRNA that targets and suppresses HMGA2.[43] Thus, alterations in the Let7–HMGA2–p14ARF pathway in fibroid stem cells may favor self-renewal and offset senescence.

In their study of 225 fibroid tumors from 80 patients, Mäkinen et al. found that approximately 70% contained heterozygous somatic mutations that affect *MED12* on the X chromosome.[19] The mutated allele was either predominantly or exclusively expressed in affected tumors.[44] Other studies confirmed these findings and established that mutations in *MED12* are also present in small subsets of other mesenchymal tumors of the uterus or in other tissues, although the uterine fibroid remains the most frequently affected tumor.[44-47]

MED12 encodes a subunit of the mediator complex, which consists of at least 26 subunits and regulates transcription initiation and elongation by bridging regulatory elements in gene promoters to the RNA polymerase II initiation

complex.[19] The mediator complex is highly conserved in all eukaryotes and is required for the transcription of almost all genes in yeast.[48] MED12, together with MED13, cyclin-dependent kinase 8 (CDK8), and cyclin C, also forms a mediator subcomplex (the CDK8 module) that regulates transcription.[48] MED12 binds directly to β-catenin and regulates canonical WNT signaling.[49] Because MED12 limits β-catenin–dependent tissue growth during embryonic development, a critical question is whether the absence of MED12 or the presence of a defective version in uterine fibroid stem cells or the main fibroid-cell population causes β-catenin pathway–dependent tumor growth.[50,51] The expression of WNT4, an activator of β-catenin, is markedly elevated in fibroids with *MED12* mutations as compared with those without these mutations (Fig. 3).[47]

In a further twist, MED12 deficiency activates the TGF-β pathway, leading to drug resistance and fibroid-cell proliferation mediated by members of two signaling protein families in cancer cells: the mothers against decapentaplegic homologue (SMAD) and mitogen-activated protein kinase (MAPK) (Fig. 3).[52] It is postulated that MED12 deficiency in somatic stem cells may trigger these events.[48] These observations point to a mechanism involving *MED12* mutations, WNT–β-catenin activation, and hyperactive TGF-β signaling that supports stem-cell renewal, cell proliferation, and fibrosis in uterine fibroid tissue (Fig. 3).[48,53,54]

EPIGENETIC FEATURES

Epigenetic mechanisms such as DNA methylation and histone modification may be inherited and may regulate gene expression independently of the primary DNA sequence. DNA methyltransferases catalyze the covalent addition of a methyl group to a cytosine in a cytosine–guanine sequence. As the degree of methylation of cytosine–guanine sequences in a gene promoter increases, its expression decreases. This mechanism is particularly important for differential gene expression in stem cells.[55-57]

The aberrant expression of specific DNA methyltransferases in uterine fibroid tissue as compared with normal myometrial tissue prompted further research into DNA methylation in these tumors.[58] Genomewide profiling of DNA methylation and messenger RNA (mRNA) expression in uterine fibroid tissue and matched normal myometrial tissue from 18 black women revealed 55 genes in the two tissue types in which there were differences affecting promoter methylation and mRNA transcription.[20] The majority of these genes (62%) displayed hypermethylation at promoter sites that were associated with their silencing in the fibroid tissues.[20] A large number of tumor suppressors, including the gene encoding the transcription factor Krüppel-like factor 11 (KLF11), were among these hypermethylated and repressed genes.[20] *KLF11,* also a target of progesterone or antiprogestins in uterine fibroid tissue, probably plays a distinct role in the fibroid development.[20,59] These observations point to the important contribution of promoter methylation-mediated gene silencing in the pathogenesis of uterine fibroids.

ESTROGEN

A large body of experimental data and circumstantial evidence suggests that estrogen stimulates the growth of uterine fibroids through estrogen receptor α.[60] The primary roles of estrogen and estrogen receptor α in fibroid growth are permissive in that they enable tissue to respond to progesterone by inducing the expression of progesterone receptor (Fig. 4).[10] Fibroid tissue is exposed to ovarian estrogen and to estrogen produced locally through the aromatase activity in fibroid cells.[61]

In fibroid tissue, multiple promoters controlled by a diverse set of transcription factors contribute to the expression of a single aromatase protein that converts circulating precursors into estrogens.[62] The mechanism underlying gonadotropin-independent expression of aromatase in fibroid tissue is not completely understood.[63] It is likely that local aromatase activity in fibroids is clinically relevant because fibroid tissue from black women — who have an increased prevalence of uterine fibroids and an earlier age at diagnosis, as compared with white women — contain high levels of aromatase, which result in elevated levels of estrogen in tissue.[64,65] Most important, aromatase inhibitors are as effective as GnRH analogues in shrinking fibroid volume, despite stable levels of circulating estrogen. These observations suggest that the inhibition of aromatase in fibroid tissue is a key mechanism in hormone-dependent fibroid growth (Fig. 4).[66]

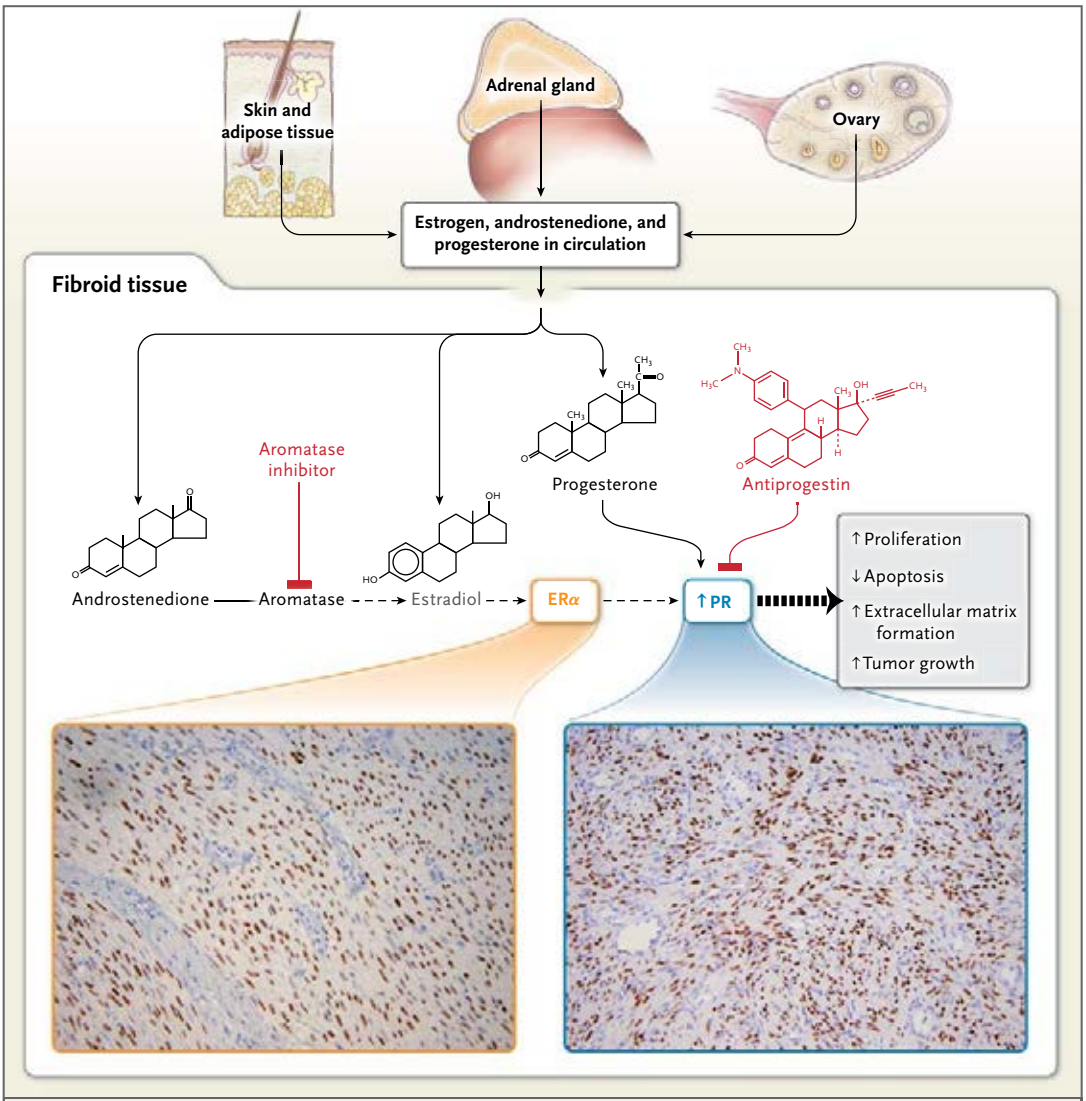


Figure 4. Biologic Effects of Estrogen and Progesterone on Fibroid Tissue.

In peripheral tissues (skin and adipose tissue) and the ovaries, aromatase catalyzes the formation of estrogen, which reaches uterine fibroid tissue through the circulation. In addition, aromatase in fibroid tissue converts androstenedione of adrenal or ovarian origin to estrogen locally. The biologically potent estrogen, estradiol, induces the production of PR by means of ERα. PR is essential for the response of fibroid tissue to progesterone secreted by the ovaries. Progesterone and PR are indispensible to tumor growth, increasing cell proliferation and survival and enhancing extracellular-matrix formation. In the absence of progesterone and PR, estrogen and ERα are not sufficient for fibroid growth. Immunohistochemical staining in fibroid tissue (insets, brown) indicates nuclear localization of ERα or PR in smooth-muscle cells. The fact that an aromatase inhibitor or antiprogestin shrinks fibroid tumors provides support for this mechanism of fibroid growth.

PROGESTERONE

An in vivo model in which human fibroid tissue was grafted under the kidney capsule in mice revealed that progesterone and its receptor were essential and sufficient for tumor growth, as indicated by the stimulation of cell proliferation, the accumulation of extracellular matrix, and cellular hypertrophy.[10] A number of clinical observations also support these findings. The use of progestins in hormone-replacement regimens stimulates the growth of fibroids in postmeno-

pausal women in a dose-dependent manner, and the addition of progestins to GnRH agonists diminishes the inhibitory effects of these agonists on leiomyoma size.[67,68] The strongest evidence supporting the in vivo growth-stimulating effects of progesterone on fibroids comes from clinical trials of three different antiprogestins, each of which showed that treatment consistently reduced tumor size (Fig. 4).[69-72]

Progesterone receptor, a ligand-activated transcription factor, mediates the actions of progesterone and antiprogestins and exerts broad biologic effects as a master regulator of hundreds of genes at any given time (Fig. 5).[73] Across the genome of fibroid smooth-muscle cells, the antiprogestin RU486–bound progesterone receptor interacts with more than 7000 DNA sites, most of which lie very far from transcription start sites.[74] More than 75% of RU486-regulated genes contain a progesterone-receptor–binding site that is more than 50,000 bp from their transcription start sites; these genes control cell growth, focal adhesion, and the functioning of the extracellular matrix.[74] This mechanism, in which genes are regulated by the progesterone receptor, contrasts with that seen in breast-cancer cells, in which the majority of genomic targets of the RU486-bound progesterone receptor reside within 5000 bp of a regulated gene.[74] These observations underscore the complexity of progesterone and antiprogestin action and account for the difficulties in identifying a single progesterone-receptor target gene for use as an effective therapeutic strategy.

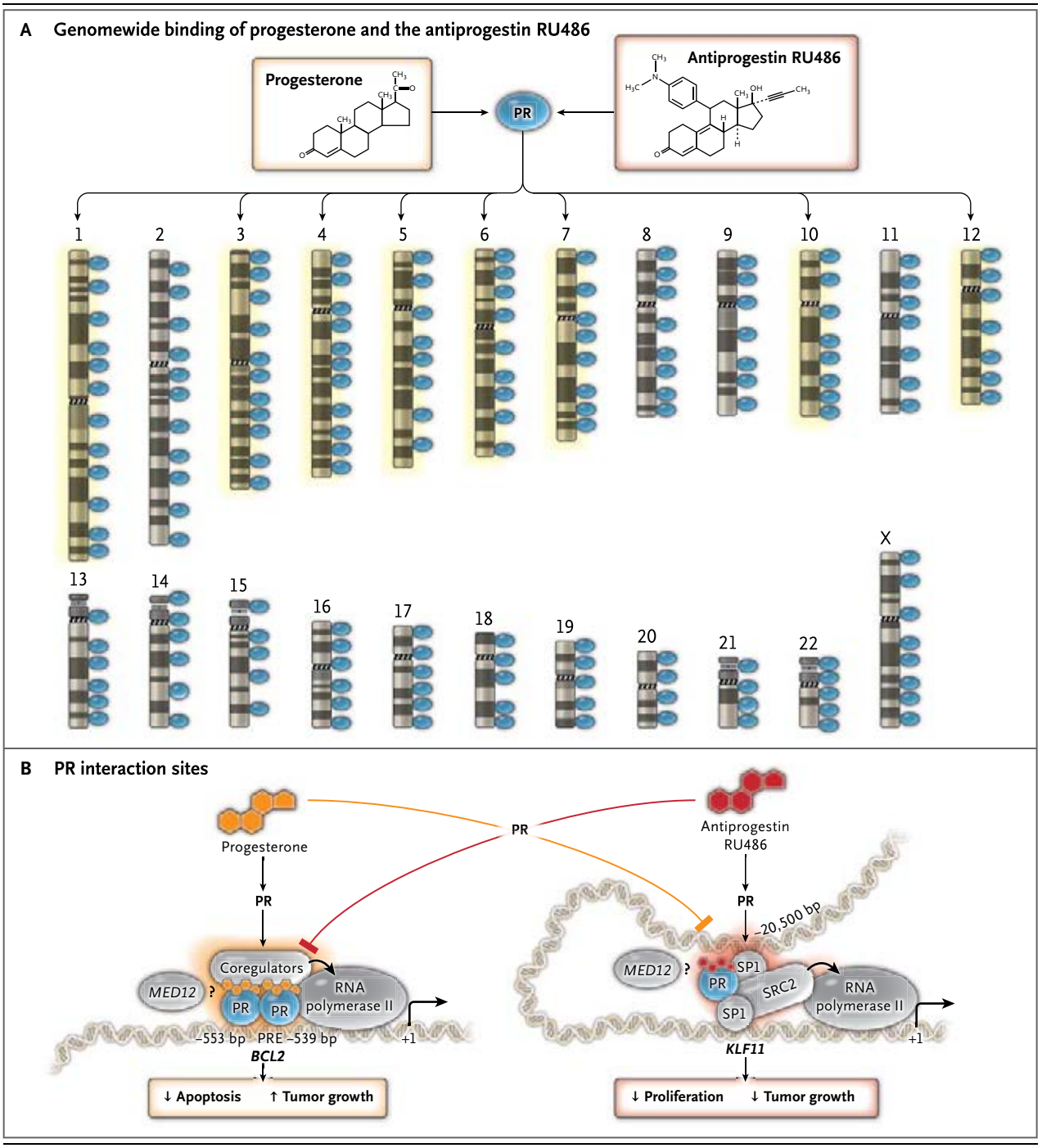
In fibroid cells, the antiprogestin RU486-bound progesterone receptor assembles a transcriptional complex that forms a bridge between a 20,500-bp distal DNA sequence and the transcription start site of the tumor-suppressor gene *KLF11*, leading to an increase in gene expression and protein levels (Fig. 5).[59] Once encoded, KLF11 effectively inhibits the proliferation of fibroid cells.[59] In contrast, progesterone-bound progesterone receptor maintains transcriptional repression of *KLF11* through the same regulatory DNA sequence; this transcriptional control occurs in addition to the epigenetic mechanism discussed above (i.e., hypermethylation of the *KLF11* transcription start site).[20,59] Progesterone, on the other hand, increases the level of the antiapoptotic protein BCL2 through the binding of progesterone receptor to a classical sequence immediately upstream of the BCL2 transcription start site, thereby inhibiting cell death in fibroid tissue (Fig. 5).[75]

In addition to the direct transcriptional effects mediated by nuclear progesterone receptor, the binding of progesterone to cytoplasmic progesterone receptors can rapidly activate the extranuclear phosphatidylinositol 3-kinase–AKT signaling pathway in uterine fibroid cells.[76] Consequently, treatment of leiomyoma cells with an AKT inhibitor reduces progesterone-induced proliferation and survival of fibroid cells, underscoring the capacity of the progesterone receptor to interact with cytoplasmic signaling pathways.[76]

During pregnancy, progesterone and its receptor are instrumental in the physiologic growth of myometrial tissue, which after delivery regresses almost to its original volume. This fact argues against the view that progesterone receptor exerts a primary tumor-initiating action. However, by signaling through its receptor, progesterone may play a central role in the clonal expansion of genetically or epigenetically altered fibroid stem cells into clinically detectable fibroids, and it may further the growth of these tumors by affecting both stem cells and differentiated fibroid cells.[31] Since the stem-cell population expresses much lower levels of progesterone receptor than the population of mature cells but serves as the key source of tissue growth, a paracrine signal originating from progesterone-receptor–rich differentiated cells may mediate the proliferative effects of progesterone on fibroid stem cells (Fig. 2).[33,34]

SUMMARY

During a woman's reproductive years, myometrial smooth-muscle cells undergo multiple cycles of growth followed by involution under the influence of ovarian hormones or the hormones of pregnancy. These cycles make stem cells vulnerable to the development of mutations. A point mutation affecting the function of *MED12*, a chromosomal rearrangement increasing the expression of *HMGA2*, or some other gene defect in a somatic stem cell in the myometrium may be the initiating event of tumorigenesis. This original, single genetic hit may alter key signaling pathways such as those involving β-catenin and TGF-β, which regulate cell proliferation, survival, and senescence and the formation of extracel-



lular matrix, leading to clonal expansion of the stem cells within the genetically normal myometrium. The majority of the cells in this expanding clone will differentiate and develop a phenotype similar to that of myometrial smooth-muscle cells but will also maintain the original mutation or chromosomal rearrangement and an abnormal epigenetic signature favoring further growth.

In this context, the inherent capability of myometrial tissue to respond to estrogen and progesterone for physiologic expansion during the luteal phase of the ovulatory cycle or preg-

Figure 5 (facing page). Mechanisms of Progesterone and Antiprogestin Action in Fibroid Cells.

Panel A shows the genomewide binding of PR (blue circles), which is bound by progesterone or the antiprogestin RU486. Each ligand acts as a principal regulator of gene expression and exerts broad biologic effects by inducing the binding of PR to thousands of sites across the genome and altering the expression of hundreds of genes at a time. The distribution of PR-binding sites across chromosomes (1 to 22 and X) is highly correlated with chromosome length and with the number of transcription start sites of genes in an individual chromosome. Panel B shows two target genes of PR, *BCL2* and *KLF11*; each has distinct promoter contexts. Progesterone induces the binding of PR as a homodimer to a classical progesterone response element (PRE) that lies approximately 500 bp upstream of the transcription start site (+1) of *BCL2*. This action enhances transcription by means of both unknown coregulators and RNA polymerase II, leading to increased levels of BCL2, which in turn reduce apoptosis and promote tumor growth. The antiprogestin RU486 inhibits BCL2 expression. The promoter region of another PR target, *KLF11*, a tumor-suppressor gene, lacks a classical PRE. The antiprogestin RU486 enhances PR binding to a site 20,500 bp upstream of the promoter region of *KLF11*. RU486-bound PR assembles an enhancer transcriptional complex containing specificity protein 1 (SP1), steroid receptor coactivator 2 (SRC2), and RNA polymerase II — all of which interact with both the transcription start site and the PR binding site. When RU486 is added to fibroid cells, it induces the production of KLF11, which suppresses cell proliferation and tumor growth. Progesterone inhibits KLF11 expression. The effects of the ubiquitous transcriptional regulator MED12 on these promoters are not known.

nancy may work to the advantage of fibroid-tumor growth. Such growth may be mediated by high levels of estrogen and progesterone receptors in normal myometrial cells or by the differentiated population of fibroid cells that send paracrine signals to the receptor-deficient fibroid stem cells for self-renewal. For unknown reasons, most uterine fibroids do not acquire further critical genetic hits and therefore remain benign. Many diverse molecular and cellular abnormalities may give rise to a uterine fibroid, an extraordinarily common phenotype. Thus, depending on their genetic and epigenetic makeup and the nature of the surrounding molecular and endocrine environment, these tumors vary in their potential for massive further growth, dormancy, and regression. The diverse mechanisms that favor tumorigenesis and the growth of uterine fibroids also provide the basis for their heterogeneous response to medical therapy.

A class of antiprogestins currently represents the most specific medical approach to targeting a defined mechanism in fibroids (Fig. 4).[69-72] In fact, antiprogestins induce amenorrhea and reduce tumor size in the majority of treated patients.[71,72] Targeting of pathways involving fibroid stem cells that primarily control tumor growth should lead to the development of new treatments.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

REFERENCES

1. Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. Am J Obstet Gynecol 2003;188:100-7.
2. Catherino WH, Parrott E, Segars J. Proceedings from the National Institute of Child Health and Human Development conference on the Uterine Fibroid Research Update Workshop. Fertil Steril 2011;95:9-12.
3. Marshall LM, Spiegelman D, Barbieri RL, et al. Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. Obstet Gynecol 1997;90:967-73.
4. Faerstein E, Szklo M, Rosenshein N. Risk factors for uterine leiomyoma: a practice-based case-control study. I. African-American heritage, reproductive history, body size, and smoking. Am J Epidemiol 2001;153:1-10.
5. Peddada SD, Laughlin SK, Miner K, et al. Growth of uterine leiomyomata among premenopausal black and white women. Proc Natl Acad Sci U S A 2008;105:19887-92.
6. Cardozo ER, Clark AD, Banks NK, Henne MB, Stegmann BJ, Segars JH. The estimated annual cost of uterine leiomyomata in the United States. Am J Obstet Gynecol 2012;206(3):211.e1-211.e9.
7. Sinclair DC, Mastroyannis A, Taylor HS. Leiomyoma simultaneously impair endometrial BMP-2-mediated decidualization and anticoagulant expression through secretion of TGF-beta3. J Clin Endocrinol Metab 2011;96:412-21.
8. Linder D, Gartler SM. Glucose-6-phosphate dehydrogenase mosaicism: utilization as a cell marker in the study of leiomyomas. Science 1965;150:67-9.
9. Parker WH, Fu YS, Berek JS. Uterine sarcoma in patients operated on for presumed leiomyoma and rapidly growing leiomyoma. Obstet Gynecol 1994;83:414-8.
10. Ishikawa H, Ishi K, Serna VA, Kakazu R, Bulun SE, Kurita T. Progesterone is essential for maintenance and growth of uterine leiomyoma. Endocrinology 2010;151:2433-42.
11. De Vivo A, Mancuso A, Giacobbe A, et al. Uterine myomas during pregnancy: a longitudinal sonographic study. Ultrasound Obstet Gynecol 2011;37:361-5.
12. Rosati P, Exacoustòs C, Mancuso S. Longitudinal evaluation of uterine myoma growth during pregnancy: a sonographic study. J Ultrasound Med 1992;11:511-5.
13. Laughlin SK, Herring AH, Savitz DA, et al. Pregnancy-related fibroid reduction. Fertil Steril 2010;94:2421-3.
14. Filicori M, Hall DA, Loughlin JS, Rivier J, Vale W, Crowley WF Jr. A conservative approach to the management of uterine leiomyoma: pituitary desensitization by a

luteinizing hormone-releasing hormone analogue. Am J Obstet Gynecol 1983;147:726-7.
15. Hodge JC, Morton CC. Genetic heterogeneity among uterine leiomyomata: insights into malignant progression. Hum Mol Genet 2007;16:Special Number 1:R7-R13.
16. Tomlinson IP, Alam NA, Rowan AJ, et al. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat Genet 2002;30:406-10.
17. Hodge JC, Kim TM, Dreyfuss JM, et al. 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 2012;21:2312-29.
18. Mehine M, Kaasinen E, Mäkinen N, et al. Characterization of uterine leiomyomas by whole-genome sequencing. N Engl J Med 2013;369:43-53.
19. Mäkinen N, Mehine M, Tolvanen J, et al. MED12, the mediator complex subunit 12 gene, is mutated at high frequency in uterine leiomyomas. Science 2011;334:252-5.
20. Navarro A, Yin P, Monsivais D, et al. Genome-wide DNA methylation indicates silencing of tumor suppressor genes in uterine leiomyoma. PLoS One 2012;7(3):e33284.
21. Mesquita FS, Dyer SN, Heinrich DA, Bulun SE, Marsh EE, Nowak RA. Reactive oxygen species mediate mitogenic growth factor signaling pathways in human leiomyoma smooth muscle cells. Biol Reprod 2010;82:341-51.
22. Mason HR, Lake AC, Wubben JE, Nowak RA, Castellot JJ Jr. The growth arrest-specific gene CCN5 is deficient in human leiomyomas and inhibits the proliferation and motility of cultured human uterine smooth muscle cells. Mol Hum Reprod 2004;10:181-7.
23. Laping NJ, Everitt JI, Frazier KS, et al. Tumor-specific efficacy of transforming growth factor-beta RI inhibition in Eker rats. Clin Cancer Res 2007;13:3087-99.
24. Gilden M, Malik M, Britten J, Delgado T, Levy G, Catherino WH. Leiomyoma fibrosis inhibited by liarozole, a retinoic acid metabolic blocking agent. Fertil Steril 2012;98:1557-62.
25. Norian JM, Owen CM, Taboas J, et al. Characterization of tissue biomechanics and mechanical signaling in uterine leiomyoma. Matrix Biol 2012;31:57-65.
26. Halder SK, Goodwin JS, Al-Hendy A. 1,25-Dihydroxyvitamin D3 reduces TGF-beta3-induced fibrosis-related gene expression in human uterine leiomyoma cells. J Clin Endocrinol Metab 2011;96(4):E754-E762.
27. Meadows KL, Andrews DM, Xu Z, et al. Genome-wide analysis of loss of heterozygosity and copy number amplification in uterine leiomyomas using the 100K single nucleotide polymorphism array. Exp Mol Pathol 2011;91:434-9.
28. Varghese BV, Koohestani F, McWilliams M, et al. Loss of the repressor REST in uterine fibroids promotes aberrant G protein-coupled receptor 10 expression and activates mammalian target of rapamycin pathway. Proc Natl Acad Sci U S A 2013;110:2187-92.
29. Arango NA, Szotek PP, Manganaro TF, Oliva E, Donahoe PK, Teixeira J. Conditional deletion of beta-catenin in the mesenchyme of the developing mouse uterus results in a switch to adipogenesis in the myometrium. Dev Biol 2005;288:276-83.
30. Szotek PP, Chang HL, Zhang L, et al. Adult mouse myometrial label-retaining cells divide in response to gonadotropin stimulation. Stem Cells 2007;25:1317-25.
31. Ono M, Maruyama T, Masuda H, et al. Side population in human uterine myometrium displays phenotypic and functional characteristics of myometrial stem cells. Proc Natl Acad Sci U S A 2007;104:18700-5.
32. Chang HL, Senaratne TN, Zhang L, et al. Uterine leiomyomas exhibit fewer stem/progenitor cell characteristics when compared with corresponding normal myometrium. Reproductive Sci 2010;17:158-67.
33. Ono M, Qiang W, Serna VA, et al. Role of stem cells in human uterine leiomyoma growth. PLoS One 2012;7(5):e36935.
34. Mas A, Cervelló I, Gil-Sanchis C, et al. Identification and characterization of the human leiomyoma side population as putative tumor-initiating cells. Fertil Steril 2012;98(3):741.e6-751.e6.
35. Tai CT, Lin WC, Chang WC, Chiu TH, Chen GT. Classical cadherin and catenin expression in normal myometrial tissues and uterine leiomyomas. Mol Reprod Dev 2003;64:172-8.
36. Tanwar PS, Lee HJ, Zhang L, et al. Constitutive activation of Beta-catenin in uterine stroma and smooth muscle leads to the development of mesenchymal tumors in mice. Biol Reprod 2009;81:545-52.
37. Mosimann C, Hausmann G, Basler K. Beta-catenin hits chromatin: regulation of Wnt target gene activation. Nat Rev Mol Cell Biol 2009;10:276-86.
38. Arici A, Sozen I. Transforming growth factor-beta3 is expressed at high levels in leiomyoma where it stimulates fibronectin expression and cell proliferation. Fertil Steril 2000;73:1006-11.
39. Walker CL, Stewart EA. Uterine fibroids: the elephant in the room. Science 2005;308:1589-92.
40. Cha PC, Takahashi A, Hosono N, et al. A genome-wide association study identifies three loci associated with susceptibility to uterine fibroids. Nat Genet 2011;43:447-50.
41. Hammond SM, Sharpless NE. HMGA2, microRNAs, and stem cell aging. Cell 2008;135:1013-6.
42. Markowski DN, Helmke BM, Belge G, et al. HMGA2 and p14Arf: major roles in cellular senescence of fibroids and therapeutic implications. Anticancer Res 2011;31:753-61.
43. Peng Y, Laser J, Shi G, et al. Antiproliferative effects by Let-7 repression of high-mobility group A2 in uterine leiomyoma. Mol Cancer Res 2008;6:663-73.
44. Pérot G, Croce S, Ribeiro A, et al. MED12 alterations in both human benign and malignant uterine soft tissue tumors. PLoS One 2012;7(6):e40015.
45. McGuire MM, Yatsenko A, Hoffner L, Jones M, Surti U, Rajkovic A. Whole exome sequencing in a random sample of North American women with leiomyomas identifies MED12 mutations in majority of uterine leiomyomas. PLoS One 2012;7(3):e33251.
46. Ravegnini G, Mariño-Enriquez A, Slater J, et al. MED12 mutations in leiomyosarcoma and extrauterine leiomyoma. Mod Pathol 2013;26:743-9.
47. Markowski DN, Bartnitzke S, Löning T, Drieschner N, Helmke BM, Bullerdiek J. MED12 mutations in uterine fibroids — their relationship to cytogenetic subgroups. Int J Cancer 2012;131:1528-36.
48. Guo X, Wang XF. A mediator lost in the war on cancer. Cell 2012;151:927-9.
49. Kim S, Xu X, Hecht A, Boyer TG. Mediator is a transducer of Wnt/beta-catenin signaling. J Biol Chem 2006;281:14066-75.
50. Rocha PP, Scholze M, Bleiss W, Schrewe H. Med12 is essential for early mouse development and for canonical Wnt and Wnt/PCP signaling. Development 2010;137:2723-31.
51. Lin X, Rinaldo L, Fazly AF, Xu X. Depletion of Med10 enhances Wnt and suppresses Nodal signaling during zebrafish embryogenesis. Dev Biol 2007;303:536-48.
52. Huang S, Hölzel M, Knijnenburg T, et al. MED12 controls the response to multiple cancer drugs through regulation of TGF-beta receptor signaling. Cell 2012;151:937-50.
53. Lee BS, Nowak RA. Human leiomyoma smooth muscle cells show increased expression of transforming growth factor-beta 3 (TGF beta 3) and altered responses to the antiproliferative effects of TGF beta. J Clin Endocrinol Metab 2001;86:913-20.
54. Catherino WH, Leppert PC, Stenmark MH, et al. Reduced dermatopontin expression is a molecular link between uterine leiomyomas and keloids. Genes Chromosomes Cancer 2004;40:204-17.
55. Dodge JE, Ramsahoye BH, Wo ZG, Okano M, Li E. De novo methylation of MMLV provirus in embryonic stem cells: CpG versus non-CpG methylation. Gene 2002;289:41-8.
56. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome

integrates intrinsic and environmental signals. Nat Genet 2003;33:Suppl:245-54.
57. Lister R, Pelizzola M, Dowen RH, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 2009;462:315-22.
58. Li S, Chiang TC, Richard-Davis G, Barrett JC, McLachlan JA. DNA hypomethylation and imbalanced expression of DNA methyltransferases (DNMT1, 3A, and 3B) in human uterine leiomyoma. Gynecol Oncol 2003;90:123-30.
59. Yin P, Lin Z, Reierstad S, et al. Transcription factor KLF11 integrates progesterone receptor signaling and proliferation in uterine leiomyoma cells. Cancer Res 2010;70:1722-30.
60. Marsh EE, Bulun SE. Steroid hormones and leiomyomas. Obstet Gynecol Clin North Am 2006;33:59-67.
61. Bulun SE, Simpson ER, Word RA. Expression of the CYP19 gene and its product aromatase cytochrome P450 in human uterine leiomyoma tissues and cells in culture. J Clin Endocrinol Metab 1994;78:736-43.
62. Imir AG, Lin Z, Yin P, et al. Aromatase expression in uterine leiomyomata is regulated primarily by proximal promoters I.3/II. J Clin Endocrinol Metab 2007;92:1979-82.
63. Ishikawa H, Fenkci V, Marsh EE, et al. CCAAT/enhancer binding protein beta regulates aromatase expression via multiple and novel cis-regulatory sequences in uterine leiomyoma. J Clin Endocrinol Metab 2008;93:981-91.
64. Ishikawa H, Reierstad S, Demura M, et al. High aromatase expression in uterine leiomyoma tissues of African-American women. J Clin Endocrinol Metab 2009;94:1752-6.
65. Sumitani H, Shozu M, Segawa T, et al. In situ estrogen synthesized by aromatase P450 in uterine leiomyoma cells promotes cell growth probably via an autocrine/intracrine mechanism. Endocrinology 2000;141:3852-61.
66. Parsanezhad ME, Azmoon M, Alborzi S, et al. A randomized, controlled clinical trial comparing the effects of aromatase inhibitor (letrozole) and gonadotropin-releasing hormone agonist (triptorelin) on uterine leiomyoma volume and hormonal status. Fertil Steril 2010;93:192-8.
67. Carr BR, Marshburn PB, Weatherall PT, et al. An evaluation of the effect of gonadotropin-releasing hormone analogs and medroxyprogesterone acetate on uterine leiomyomata volume by magnetic resonance imaging: a prospective, randomized, double blind, placebo-controlled, crossover trial. J Clin Endocrinol Metab 1993;76:1217-23.
68. Friedman AJ, Daly M, Juneau-Norcross M, et al. A prospective, randomized trial of gonadotropin-releasing hormone agonist plus estrogen-progestin or progestin "add-back" regimens for women with leiomyomata uteri. J Clin Endocrinol Metab 1993;76:1439-45.
69. Murphy AA, Kettel LM, Morales AJ, Roberts VJ, Yen SS. Regression of uterine leiomyomata in response to the antiprogesterone RU 486. J Clin Endocrinol Metab 1993;76:513-7.
70. Williams AR, Critchley HO, Osei J, et al. The effects of the selective progesterone receptor modulator asoprisnil on the morphology of uterine tissues after 3 months treatment in patients with symptomatic uterine leiomyomata. Hum Reprod 2007;22:1696-704.
71. Donnez J, Tomaszewski J, Vázquez F, et al. Ulipristal acetate versus leuprolide acetate for uterine fibroids. N Engl J Med 2012;366:421-32.
72. Donnez J, Tatarchuk TF, Bouchard P, et al. Ulipristal acetate versus placebo for fibroid treatment before surgery. N Engl J Med 2012;366:409-20.
73. Kim JJ, Sefton EC. The role of progesterone signaling in the pathogenesis of uterine leiomyoma. Mol Cell Endocrinol 2012;358:223-31.
74. Yin P, Roqueiro D, Huang L, et al. Genome-wide progesterone receptor binding: cell type-specific and shared mechanisms in T47D breast cancer cells and primary leiomyoma cells. PLoS One 2012;7(1):e29021.
75. Yin P, Lin Z, Cheng YH, et al. Progesterone receptor regulates Bcl-2 gene expression through direct binding to its promoter region in uterine leiomyoma cells. J Clin Endocrinol Metab 2007;92:4459-66.
76. Hoekstra AV, Sefton EC, Berry E, et al. Progestins activate the AKT pathway in leiomyoma cells and promote survival. J Clin Endocrinol Metab 2009;94:1768-74.