

REVIEW ARTICLE

MECHANISMS OF DISEASE

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

Uterine Fibroids

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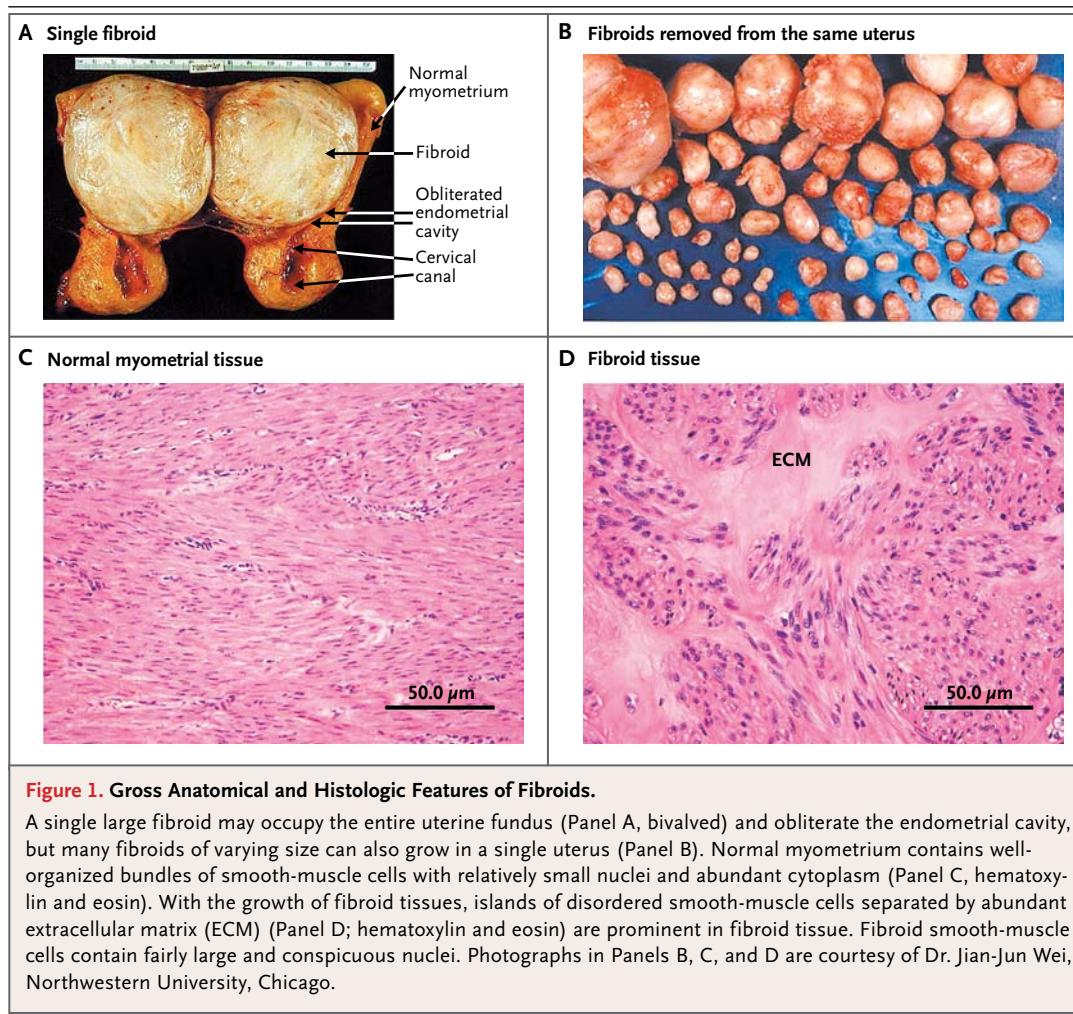
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UTERINE FIBROIDS (LEIOMYOMAS) REPRESENT THE MOST COMMON TUMOR in women. These lesions disrupt the functions of the uterus and cause excessive uterine bleeding, anemia, defective implantation of an embryo, recurrent pregnancy loss, preterm labor, obstruction 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



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 fumurate 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}

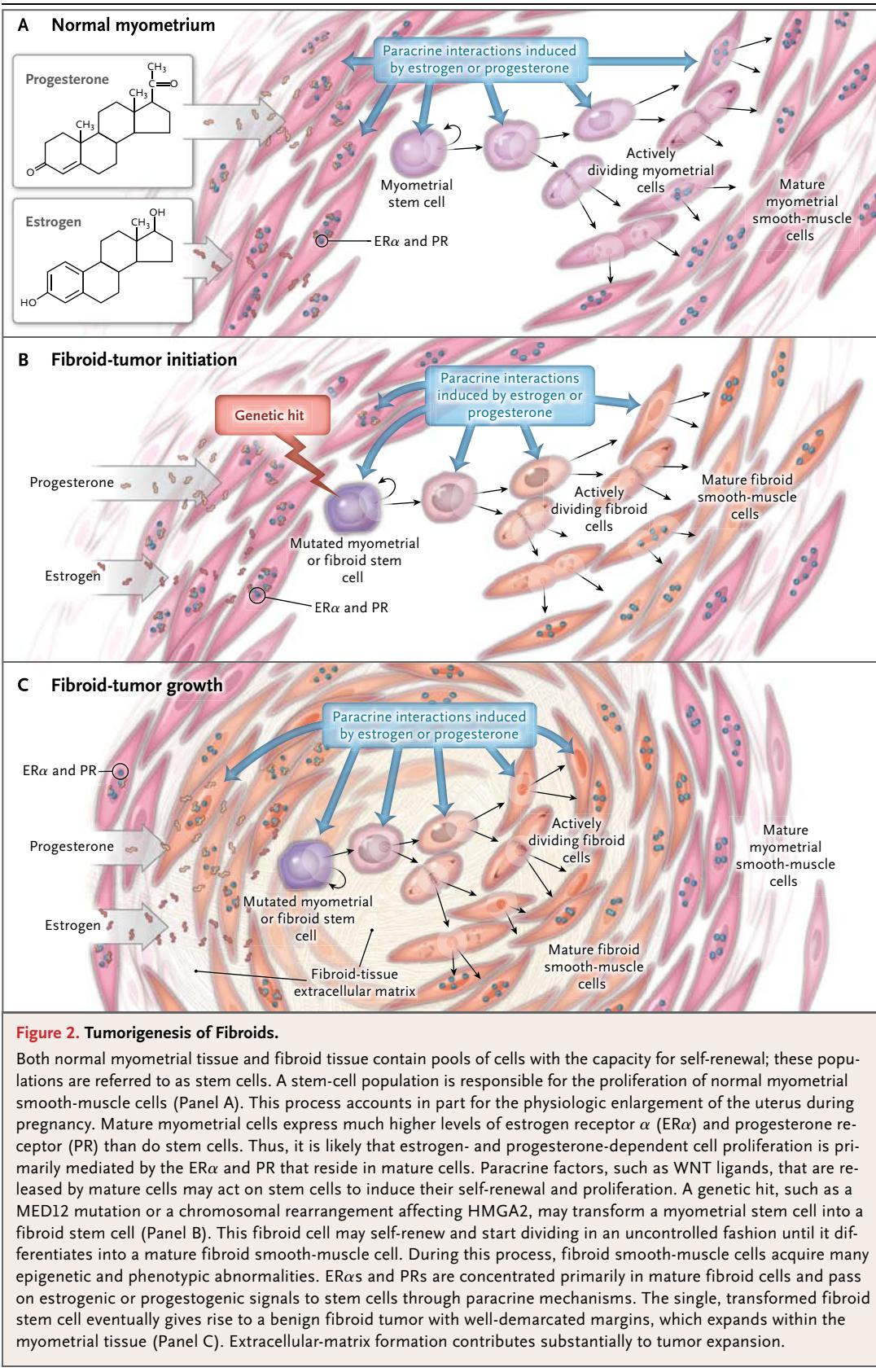
Signaling by the wingless-type MMTV integration site family (WNT)- β -catenin pathway seems to play a role in somatic stem-cell function in the myometrium and in uterine fibroid tissue. Overall, total β -catenin levels in the myometrium and fibroid tissue are similar.³⁵ But because the key effects of 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

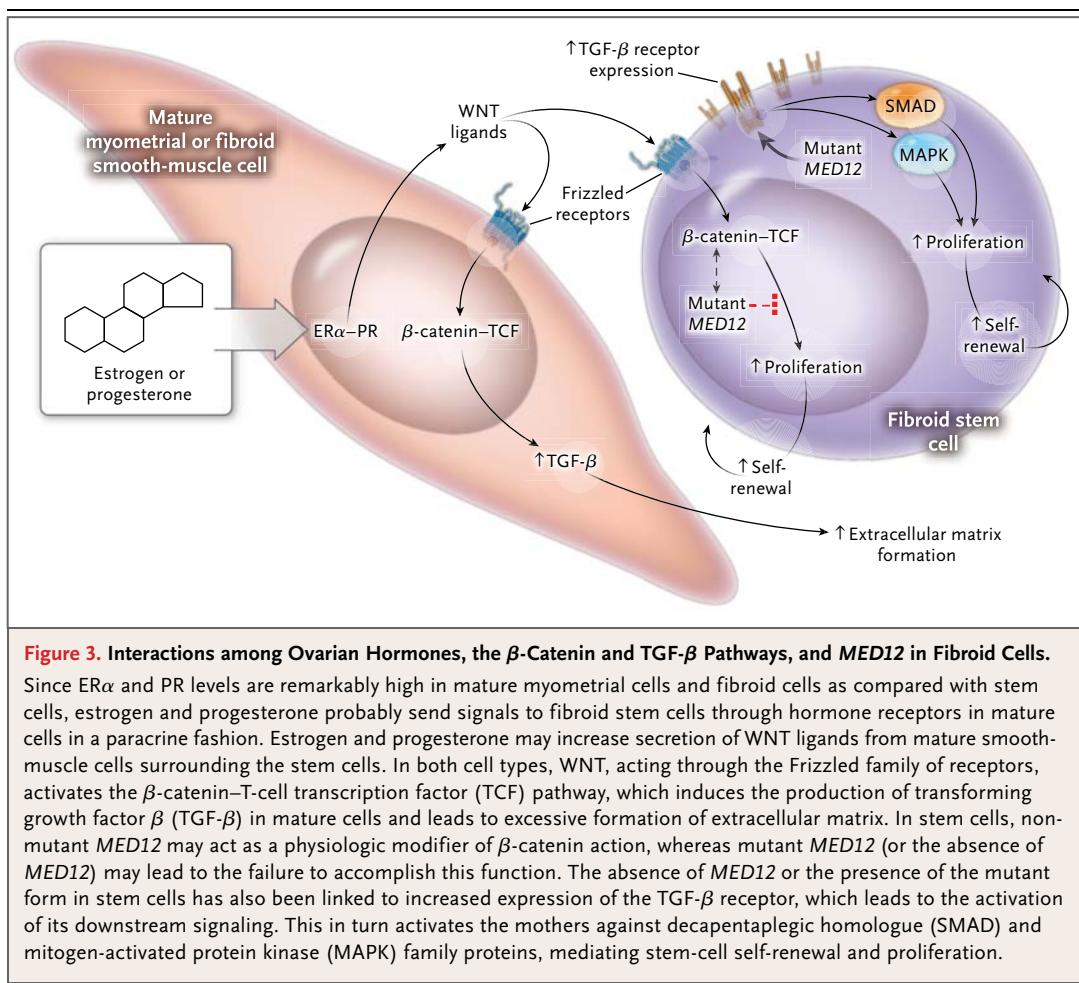
of β -catenin in uterine mesenchyme during embryonic development substantially reduces uterine size and replaces the uterus with adipocytes, disrupting entirely the normal myometrial differentiation or regeneration of smooth muscle. This observation suggests that β -catenin plays a key role in stem-cell 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 β 3 (TGF- β 3), which induces cell proliferation and the formation of extracellular matrix in human fibroid tissue.^{36,38} Fibroid-tissue-derived TGF- β 3 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





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 sup-

presses HMGA2.⁴³ Thus, alterations in the Let-7–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}

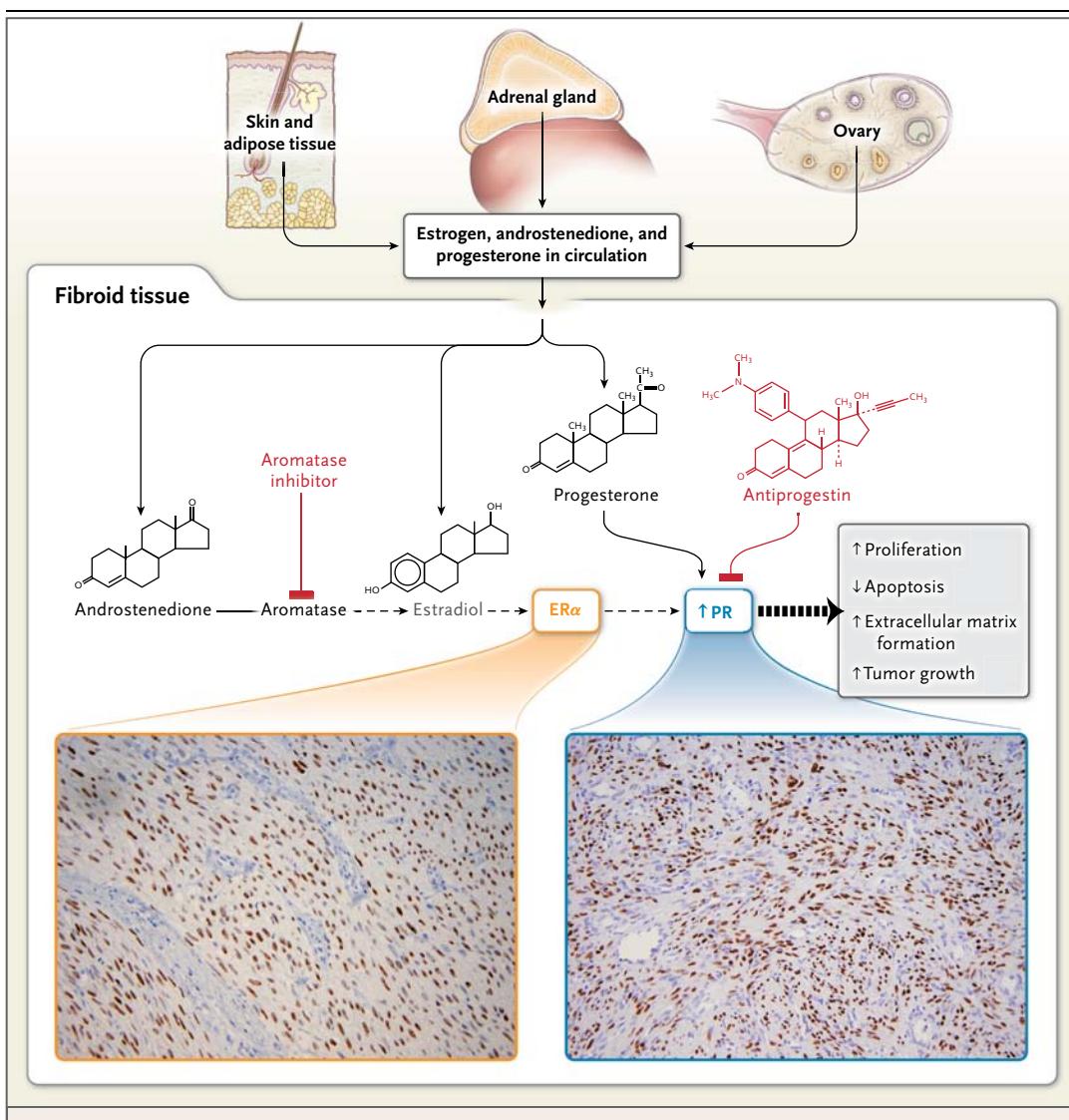
The aberrant expression of specific DNA methyltransferases in uterine fibroid tissue as compared with normal myometrial tissue prompted further research into DNA methylation in these tumors.⁵⁸ Genomewide profiling of DNA methylation and messenger RNA (mRNA) ex-

pression 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 ER α . PR is essential for the response of fibroid tissue to progesterone secreted by the ovaries. Progesterone and PR are indispensable to tumor growth, increasing cell proliferation and survival and enhancing extracellular-matrix formation. In the absence of progesterone and PR, estrogen and ER α are not sufficient for fibroid growth. Immunohistochemical staining in fibroid tissue (insets, brown) indicates nuclear localization of ER α 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 in-

dicated 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

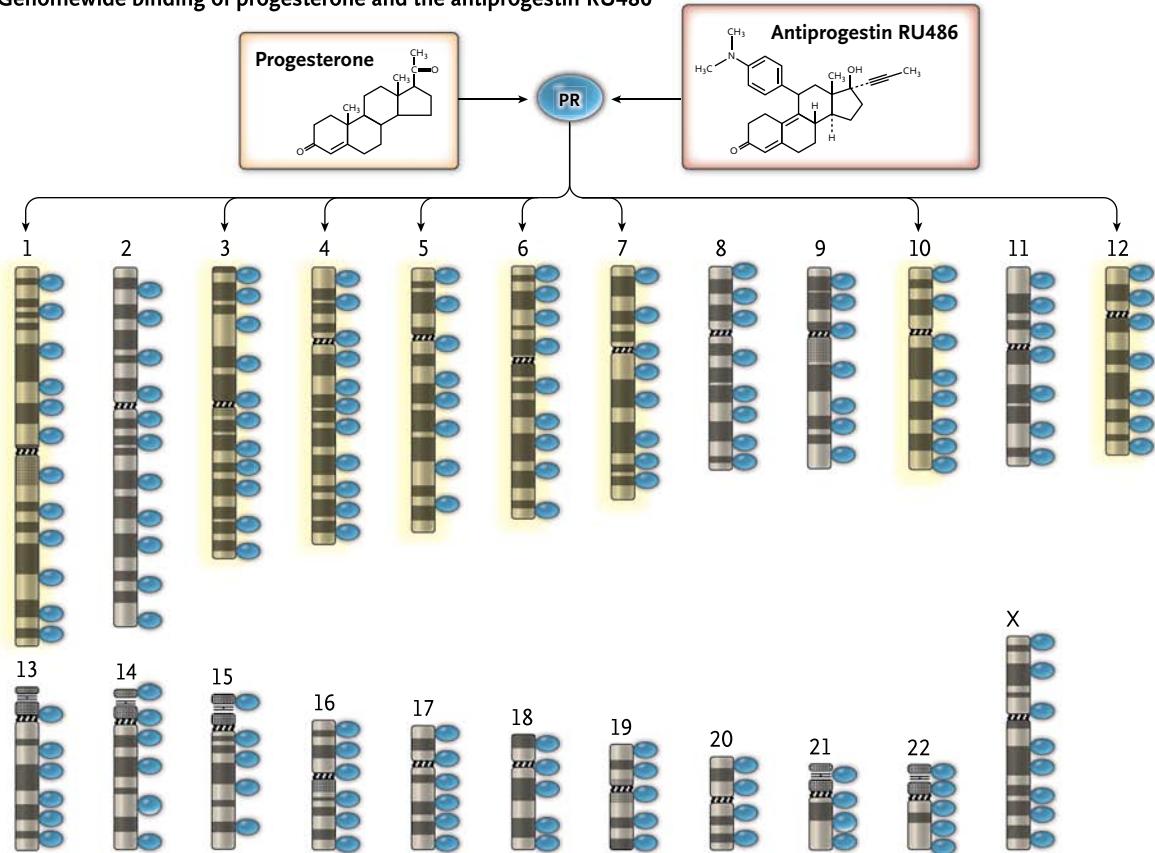
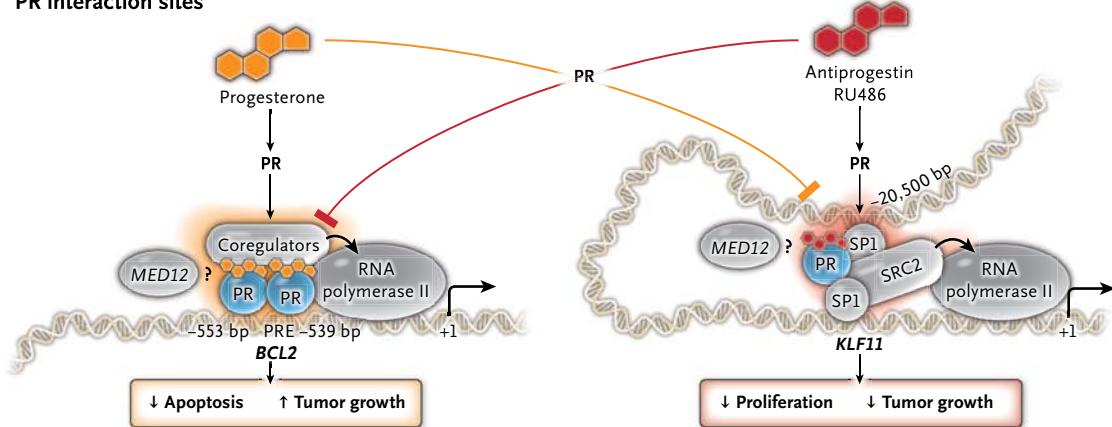
immediately upstream of the *BCL2* transcription start site, thereby inhibiting cell death in fibroid tissue (Fig. 5).⁷⁵

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 *HMG2A*, 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-

A Genomewide binding of progesterone and the antiprogestin RU486**B PR interaction sites**

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 genome-wide 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.

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