Review

Comprehensive Review of Uterine Fibroids: Developmental Origin, Pathogenesis, and Treatment

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AKT, protein kinase B; BMI, body mass index; DES, diethylstilbestrol; DSB, double-stranded break; ECM, extracellular matrix; EDC, endocrine-disrupting chemical; ER, estrogen receptor; EZH2, enhancer of zeste homolog 2; FH, fumarate hydratase; GnRH, gonadotropin-releasing hormone; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; HMG, high mobility group; IGF-1, insulin-like growth factor 1; IL, interleukin; MAPK, mitogen-activated protein kinase; MED12, RNA polymerase II transcriptional mediator complex subunit 12; MMSCs, myometrial stem cells; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide-3-kinase; RTI, reproductive tract infection; SPRM, selective progesterone receptor modulator; TAF, tumor-associated fibroblast; TAZ, transcriptional coactivator with PDZ-binding domain; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor-α; VDR, vitamin D receptor; YAP, Yes-associated protein.

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Abstract

Uterine fibroids are benign monoclonal neoplasms of the myometrium, representing the most common tumors in women worldwide. To date, no long-term or noninvasive treatment option exists for hormone-dependent uterine fibroids, due to the limited knowledge about the molecular mechanisms underlying the initiation and development of uterine fibroids. This paper comprehensively summarizes the recent research advances on uterine fibroids, focusing on risk factors, development origin, pathogenetic mechanisms, and treatment options. Additionally, we describe the current treatment interventions for uterine fibroids. Finally, future perspectives on uterine fibroids studies are summarized. Deeper mechanistic insights into tumor etiology and the complexity of uterine fibroids can contribute to the progress of newer targeted therapies.
**Key Words:** uterine fibroids, developmental origin, genetic instability, reprogramming, epigenetics pathways, novel treatment, future directions

**Graphical Abstract**

**ESSENTIAL POINTS**

1. Developmental exposure to EDCs in early life reprograms myometrial stem cells, thus increasing the risk of uterine fibroids development.
2. Several risk factors such as age, race, obesity, parity, hypertension, vitamin D deficiency, and diet in late life can trigger uterine fibroids pathogenesis.
3. Pathogenic exon 2 mutations in *MED12* promote uterine fibroids formation and disrupt CDK8/19 kinase activity.
4. Several vital pathways and mechanisms such as sex hormones, ECM, Wnt/β-catenin, TGF-β, growth factors, epigenetic and epitranscriptomic regulation, YAP/TAZ, Rho/ROCK, and DNA damage repair pathways contribute to the development of uterine fibroids.
5. Fertility therapy is highly needed for the treatment of patients with uterine fibroids.

Uterine fibroid lesions were initially known as the “uterine stone.” In the second century AD, they were called scleroses. The term *fibroid* was first introduced in the 1860s. Uterine fibroids are the most common pelvic tumors among women of reproductive age, affecting more than 70% of women worldwide, particularly women of color (1-3). Uterine fibroids are heterogeneous in composition and size among women and within the same individual, and vary in number between individuals (4-14). In addition, the fibroid pseudocapsule presents as a fibro-neurovascular structure surrounding a uterine fibroid, separating it from normal peripheral myometrium (15-18). Although benign, uterine fibroids are associated with significant morbidity; they are the primary indication for hysterectomy, and a major source...
of gynecologic and reproductive dysfunction, ranging from menorrhagia and pelvic pain to infertility, recurrent miscarriage, and preterm labor (19, 20). Accordingly, the annual USA health care costs associated with uterine fibroids have been estimated at ~$34 billion (21). Uterine fibroids thus represent significant societal health and financial burden.

Epidemiology, Risk/Protective Factors, Driver Mutations, and Tumor-Initiating Stem Cells

Risk Factors and Epidemiology

The prevalence of uterine fibroids is increasing in some populations, such as in African American women (22). However, its reported incidence is likely to be an underestimation, as many tumors are asymptomatic or slightly symptomatic and therefore remain undiagnosed (1). In addition, approximately only 25% to 30% of women report the clinical symptoms of uterine fibroids (5).

The most important and frequently reported risk factor for uterine fibroids is race, disproportionately impacting African American women (Figs. 1 and 2). Other risk factors include older age, premenopausal state, nonparity, family history of uterine fibroids, hypertension, food additives, and frequent consumption of soybean milk. On the other hand, protective factors for uterine fibroids include combined oral contraception or injectable medroxyprogesterone acetate in the depot form, smoking in women of low mass, and parity (1). Other important risk factors include obesity (23-25), vitamin D deficiency (26-28), excessive vitamin E levels (29), altered reproductive tract microbiome (30), exposure to endocrine-disrupting chemicals (eg, organophosphate esters and plasticizers) (31, 32), and various early-life adverse environmental exposures (33). Individual and environmental risk factors associated with tobacco smoking and alcohol abuse can also contribute to the formation of uterine fibroids (34, 35). More risk factors are associated with a higher probability of uterine fibroid formation and development (1, 23).

These points require some additional comments. Epidemiologists understand that they must study women from the community to eliminate bias and have a prospective study design with a large sample size and low loss to follow-up to enable the measurement of age-specific incidence and other risk factor-related pathogenesis of uterine fibroids (36). Improvement of awareness and education for uterine fibroids in the community will help to better understand the risk factors of this disease. Notably, data from uterine fibroid research in underrepresented groups are lacking (37). On the other hand, epidemiological studies may reflect both the natural and false effects of a selected factor on the investigated outcome. Findings may be subject to different explanations because they may occur due to random errors, biases, or confounding, which may produce false results. These factors need to be considered at both the design and analysis stage of a study to minimize them. Notably, the same instruments for health outcomes evaluation in exposed and unexposed groups should be applied to avoid misclassification or bias. Studies without including confounding variables from the onset or without matching by age, race, and other factors should always be treated with caution (38).

Age

Increasing age is a significant risk factor for uterine fibroids, especially among women at the premenopausal stage and those ≥ 40 years of age (24, 39, 40). For instance, 60% of African American women aged 35–49 years reported uterine fibroids, whereas 80% of those aged ≥ 50 have uterine fibroids. Among White women, 40% of those aged ≥ 35 years and 70% aged ≥ 50 years developed uterine fibroids (3). These tumors have not been detected in prepubertal girls, and only sporadic cases have been reported in adolescents. However, the factor(s) involved in their development at such an early age is unknown. Due to the slight difference in biochemical pathways, uterine fibroids in young women do not exhibit typical uterine fibroid biology. In several cases, adolescent patients had a translocation between chromosomes 12 and 14, which is a confirmed risk factor for uterine fibroids (41, 42). Women at the menopausal stage have shrunk uterine fibroid lesions and decreased sex hormones. Notably, the use of hormonal replacement therapy may cause these lesions to regrow and may induce the first clinical symptoms of uterine fibroids (43).

Race and ethnicity

Populations of different races/ethnicities vary in the risk of developing uterine fibroids. The United States Census recognized 5 racial categories (White or European; Black or African American; Asian American; American Indian/Alaska Native; and Native Hawaiian/Pacific Islander) as well as people of 2 or more races (https://www.census.gov/topics/population/race/about.html. Accessed July, 2021). In addition, the Census Bureau also classified Hispanic or Non-Hispanic as ethnicity. Medical records and self-report were used and demonstrated that Black women, the largest racial minority in the United States, are most likely out of any racial category to develop uterine fibroids (3, 5, 44-46). The severity of uterine fibroid-derived symptoms also tended to be greater among African American women (47). Uterine fibroids are 3 times more common in African American women and 2 times more common in Hispanic women compared with White women (3, 46). The more common occurrence of uterine fibroids in African American women may be attributed to higher concentrations of steroid hormones in African American women and...
may also be due to gene polymorphism, including the catechol-
O-methyltransferase (COMT) encoding gene (48). However,
the etiology of the increased incidence of uterine fibroids in
African American women has not been fully elucidated.
Additionally, the relationship between the higher incidence of
uterine fibroids and more severe manifestations of disease may
be due to vitamin D deficiency in African American women
(49, 50). African American women are diagnosed with vitamin
D deficiency at a rate of 5 to 10 times more than that of White
women. It is thought that the limited absorption of ultraviolet
(UV) radiation, which is essential for vitamin D metabolism,
may be the reasoning for this discovery (50).

Furthermore, the African American population experi-
ences higher levels of racial discrimination (51, 52) and
there are multiple ways by which perceived racism can af-
fect health (53). A positive association between self-reported

Figure 1. Developmental origin of fibroids from myometrial stem cells. Intrauterine and early-life adverse environmental exposure to endocrine-
disrupting chemicals may act as the early hit to induce normal myometrial stem cells’ reprogramming by hijacking epigenomic plasticity. The plastic-
tility of the developing epigenome is susceptible to epigenomic changes in myometrial stem cells following later-life adverse exposures, thereby
leading to mutations and their transformation into tumor-initiating stem cells. The development and growth of fibroids are mainly characterized by
abnormal cell proliferation, inhibited apoptosis, DNA instability, excessive deposition of ECM, and other critical biological pathways. Abbreviations:
ECM, extracellular matrix; MED12, RNA polymerase II transcriptional mediator complex subunit 12; ncRNAs, non-coding RNA.
experiences of racial discrimination and the incidence of uterine fibroids was demonstrated in a large follow-up study of the cohort of the Black Women’s Health Study (54). In this sense, Vines et al have found an association with the presence of uterine fibroids among the African American women in the high-stress intensity group (55).

Discrimination is thought to negatively influence physical wellbeing through the stress response (56, 57). The hypothesis that stress led to uterine fibroid pathogenesis could be explained by the fact that disturbance of the hypothalamic–pituitary–adrenal axis and the subsequent release of stress biomarkers such as cortisol and epinephrine (58) have been linked with increased uterine fibroid risk (59). In addition, stress also may provoke fluctuations in estrogen and progesterone hormone levels (60, 61), both important in uterine fibroid development. Furthermore, it is also biologically plausible that the higher uterine fibroid risk observed in African American women is associated with the systemic inflammation provoked by stress-related factors (Fig. 2) (62). To date, studies on the role of stress in uterine fibroid development among women of various race/ethnic groups are limited. In this sense, more studies that examine perceived racism as a chronic stressor linked to occurrence of uterine fibroids are needed to fully understand these dependencies.

**Obesity**

Obesity is directly related to increased energy consumption and reduced physical activity (63). Currently, obesity is the fifth leading cause of death (64). Several studies...
have found obesity as a significant risk factor for uterine fibroids development (23, 65), which has been attributed to the metabolic functions of adipose tissues. Adipose tissues produce and release various cytokines and growth factors involved in regulating diverse physiological and pathological processes, including immunity and inflammation (66). Adrenal androgens are mostly metabolized by aromatase in adipose tissues to estrogens (67-69). Obesity and particularly excess visceral fat may be complemented with the reduced production of the sex hormone–binding globulin (SHBG), which binds circulating hormones, disrupting the hormonal activity toward sensitive tissues, and thereby influencing the delicate hormonal balance in the body (70).

Each kilogram of excessive body weight is correlated with an increased risk of uterine fibroids development (71, 72). A study conducted in the United States found that women diagnosed with uterine fibroids are heavier than those without uterine fibroids (72). Moreover, an increase in the body mass index (BMI) by one unit (23), higher waist-to-hip ratios, and body fat percentage exceeding 30% (73) increase the risk for uterine fibroids. Abdominal visceral fat also enhances this risk (65). A recent meta-analysis of 22 studies, including 325,899 participants, and 19,593 cases, found a positive association between obesity and the risk or prevalence of fibroids (74).

Obesity is most prevalent among African Americans compared with other racial and ethnic populations in the United States, contributing to the higher risk of developing uterine fibroids in the African American population (25). Uterine fibroids occur more frequently in obese postmenopausal women and those who have undergone hormonal replacement therapy (75). Furthermore, obese women diagnosed with type 2 diabetes are more likely to develop uterine fibroids (75), and this observation has been related to elevated concentrations of insulin-like growth factor (IGF-1) (76). Insulin resistance plays a role in the development of uterine fibroids in obese women.

Parity
Main epidemiological studies demonstrated an inverse association between parity and uterine fibroids, suggestive of a protective effect (77). Nulliparous women are more commonly affected by uterine fibroids than multiparous women (44). Each subsequent child may lower the risk of this pathology (74). These study analyses were based on USA data, which need further investigation related to the difference in race and ethnicity in other countries. Steroid hormone exposure during pregnancy and dramatic remodeling of the uterine tissues after each pregnancy may be attributable to a decrease in uterine fibroid formation (77, 78).

Hypertension
There is a direct correlation between arterial hypertension and uterine fibroids (44, 79, 80). Increased diastolic blood pressure is associated with a higher risk of uterine fibroids, regardless of use of antihypertensive drugs (79). Women suffering from hypertension are 5 times more likely to develop uterine fibroids (81), and earlier diagnosis of hypertension is a significant factor. The formation of lesions is attributed to the chronic destruction of the myometrium due to increased blood flow and cytokines secreted by injured myometrial cells (79).

Vitamin D deficiency and diet
Vitamin D is a collective term for fat-soluble steroid compounds with pleiotropic solid influence in the human body (82, 83, 84). Vitamin D is synthesized in the human skin from 7-dehydrocholesterol upon exposure to sunlight. Then, it is transported by the vitamin D-binding protein to the liver and kidneys, where it is converted to 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)D] (83), respectively, and ultimately carried to the target tissues (85).

Age, race, health, and even clothing affect the rate at which vitamin D is produced in the skin (86). Endogenous vitamin D production from sun exposure is influenced by climate, namely, reduced and/or inefficient sunlight absorption may cause vitamin D deficiency (86). The synthesis of vitamin D decreases with age (82, 86). Of note, individuals with darker skin pigmentation and complexion need longer sun exposure to produce adequate amounts of vitamin D (87). Approximately 80% of African American women have vitamin D deficiency, compared with only 20% of Caucasian women (88). The higher risk of vitamin D deficiency in African Americans has been attributed to due to darker skin pigmentation and decreased access to solar radiation, resulting in increased risk for uterine fibroids (89).

Adequate vitamin D can also be ensured through diet or supplementation (90). The most stable form in circulating blood, 25(OH)D, is used to assess vitamin D levels in individuals (91). However, different organizations have different classifications for 25(OH)D levels. According to the Endocrine Society, vitamin D deficiency is defined as 25(OH)D serum concentrations ≤20 ng/mL; insufficient, between 21 and 29 ng/mL; and sufficient, ≥30 ng/mL (92). Meanwhile, the United States Institute of Medicine (IOM) defines the sufficient 25(OH)D serum level as ≥20 ng/mL (93). (94)

Some experts consider low 25(OH)D serum concentrations as a marker of poor health (90). Conversely, increased concentrations of vitamin D have been associated with reduced prolonged menstruation cycle (95), infertility, hyperandrogeism, insulin resistance, and polycystic ovary syndrome (PCOS) (96). Furthermore, abnormal vitamin D
levels tend to change the maternal-fetal vascular system and may cause abnormal pregnancy development, dysregulated metabolism, and disrupted placental function (97).

The role of vitamin D in the pathogenesis of uterine fibroids has been investigated (26). Three main studies demonstrated that vitamin D levels are much lower in the sera of uterine fibroid patients, suggesting the vitamin D may be linked to the pathogenesis of uterine fibroids. (98-100).

Lifestyle factors, such as diet and level of physical activity influence the formation of uterine fibroids. Women consuming more green vegetables, fruit, and fish than red meat are less commonly diagnosed with uterine fibroids (27, 49, 101). Of note, African American women consume lesser amounts of fruits, vegetables, vitamins, and minerals compared with White women (102, 103). Diets rich in citrus fruits markedly reduced the risk of uterine fibroids (104). (88, 105, 106).

Protective Factors

The use of oral and injectable contraceptives can reduce the risk of developing uterine fibroids (44). Hormonal contraception protected women from developing clinical symptoms of uterine fibroids (107). However, using oral contraceptives at adolescence may be considered a risk factor for developing symptomatic uterine fibroids later in life, whereas using them after adolescence reduces the risk (44, 108-110). Contraceptives increase estrogen and progesterone concentrations in the body, indicating that mechanisms other than hormonal levels are involved in the development of uterine fibroids (5).

Some substances of plant origin can prevent cell division and formation of fibrosis while modulating hypercritical pathways involved in the development of uterine fibroids (111). The use of phytochemicals in the prevention and treatment of uterine fibroids has been investigated and showed promising options (111-113). However, some substances that had been considered potentially helpful have been associated with adverse effects. For example, elevated vitamin E concentration in the serum may be an risk factor for uterine fibroids in Caucasian women. Vitamin E can function as a ligand for estrogen receptors (ERs) due to its structural determinants (29).

In addition, the consumption of milk and other dairy products may influence the development of uterine fibroids. An increased risk of uterine fibroids was associated with consumption of milk or soybeans (114). Other prospective cohort studies have yielded controversial results. One study reported no clear association with overall dairy consumption, whereas another study found that yogurt consumption and calcium intake from foods reduced the risk of uterine fibroid development (115). Moreover, some of the risk factors are described in the environmental exposure section.

Uterine Fibroids Driver Mutations

Within the past decade, the application of rapidly advanced genomic technologies, including high-throughput sequencing methodologies, has led to the identification of recurrent and largely mutually exclusive genetic alterations (so-called drivers) responsible for the formation of uterine fibroids. Among these, somatic mutations in the Xq13 gene encoding the RNA Polymerase II (Pol II) mediator subunit MED12 are the most prevalent, occurring in 45–90% cases of uterine fibroids depending upon patient ethnicity (8, 116-128). A proportionally smaller fraction of uterine fibroids has been attributed to genetic alterations leading to the overexpression of HMGA2, disruption of the COL4A5-COL4A6 locus, and biallelic loss of FH encoding the tricarboxylic acid (TCA) cycle enzyme fumarate hydratase (117, 129). Additionally, recurrent deletions and rearrangements involving chromosomes 6p21, 7q22, 22q, and 1p have been observed in patients with uterine fibroids. However, these mutations generally co-occur with other genetic alterations, suggesting that they represent secondary driver events restricted to a subpopulation of tumor cells (121, 130-133). Altogether, the identification of different fibroids driver mutations has permitted the genetic stratification of these tumors into at least 4 molecular subtypes (129, 134, 135). Interestingly, transcriptome-wide gene expression profiling studies of different uterine fibroid subtypes have revealed that distinct driver mutations are generally characterized by unique gene expression signatures, indicative of distinct pathways to tumorigenesis. This suggests that MED12 mutation–positive and MED12 mutation–negative uterine fibroids are likely unrelated by driver mutations occurring in a common MED12-dependent pathway (129, 134). There are 4 main driver mutations discovered in uterine fibroids.

MED12

High-frequency MED12 mutations have been observed in tumors from women of diverse racial and ethnic origins, including those of North American, European, African, Asian, and Middle Eastern descent, thus implicating MED12 as a dominant universal driver of uterine fibroids (8, 118-128). Nonetheless, data from a recent meta-analysis indicates that MED12 mutations occur more frequently in women of African as opposed to non-African descent (136). Regarding uterine fibroid–linked mutations in MED12, are all located within exons 1 or 2, and most are missense mutations with a smaller proportion corresponding to small in-frame deletions and insertions (118, 129, 137). Exon
2 mutations are far more frequent than those in exon 1, with the latter accounting for ~1% to 2% of pathogenic alterations reported in uterine fibroids (10, 129). Although missense mutations in exon 2 are distributed throughout the coding sequence, most are clustered in codons 36, 43, and 44, suggesting the importance of their corresponding and evolutionarily highly conserved amino acid residues (7, 8, 118, 121, 128). Notably, in addition to uterine fibroids, \textit{MED12} exon 2 mutations are also found at similarly high frequency (~80%) in breast fibroepithelial tumors, and to a lesser extent (~5%) of chronic lymphocytic leukemias (138-144).

In addition to their high frequency occurrence, several additional findings suggest that \textit{MED12} mutations are true drivers of fibrotic transformation. First, predominant monoallelic expression of mutant \textit{MED12} has been observed almost uniformly in \textit{MED12}-mutation-positive uterine fibroids, indicating a pathogenic requirement for a functionally altered \textit{MED12} allele (7, 129, 137). Second, targeted expression of a \textit{MED12} mutant transgene (c. 131G>A; p.G44D) in the uterine mesenchyme of mice was sufficient to induce uterine fibroid formation, providing direct genetic proof of disease causality (145).

Combined molecular and clinical analyses have been applied to identify relationships between \textit{MED12} mutation status and tumor characteristics as well as patient clinical variables. These analyses have consistently revealed that \textit{MED12} mutations are associated with smaller tumor size, conventional tumor histology, and increased tumor number within the uterus (7, 123, 146-152). While most of these studies have been underpowered to detect associations with additional clinical features, a comparatively larger analysis including 750 fibroid tumors from 244 hysterectomy patients confirmed these associations and additionally found \textit{MED12} mutations to be positively correlated with subserosal (compared to intramural) location and inversely correlated with parity (10). No associations were observed between \textit{MED12} mutations and patient infertility, smoke consumption, BMI, history of pelvic inflammatory disease and chlamydia, hypertension, thyroid disorder, diabetes, oral contraceptive use, or family history of uterine fibroids (10). The observation that \textit{MED12} mutation–positive uterine fibroids are associated with a subserosal location was subsequently confirmed in another large retrospective study that included 361 tumors from 234 myomectomy patients whose median age of 34 years also revealed that the \textit{MED12} mutation frequency in uterine fibroids from fertile-age women is comparable to that found in perimenopausal women (153). Altogether, these analyses support the relevance of \textit{MED12} driver mutations in the pathogenesis and clinical presentation of uterine fibroids.

The noted association between \textit{MED12} mutations and smaller tumor size has been variously ascribed to underlying study bias (ie, early clinical intervention in response to the combinatorial burden of multiple co-existing \textit{MED12}-mutant tumors) or inherent biological differences in the growth properties of \textit{MED12}-mutation–positive and \textit{MED12} mutation–negative tumors \textit{in situ}. While the underlying basis for this association remains unknown, the notion that \textit{MED12} mutation–positive and \textit{MED12} mutation–negative tumors might exhibit unique growth features is supported by studies showing a clear distinction in the ability of primary cells from either tumor type to survive monolayer culture in vitro. Thus, while primary cells from \textit{MED12} mutation–negative uterine fibroids were shown capable of survival and maintenance for many passages under normal culture conditions, those derived from \textit{MED12} mutation–positive tumors were shown to be rapidly lost within the first several passages (154). Interestingly, while passaging of cells was noted to accelerate the loss of \textit{MED12}-mutated cells from cultures, cell loss was nonetheless still observed in confluent cells absent passaging, revealing an apparent requirement for a niche-derived soluble factor(s) or matrix component(s) that is lacking in vitro (155). These novel findings reveal inherently unique growth requirements, and possible therapeutic vulnerabilities, for cells from \textit{MED12} mutation–positive uterine fibroids, and further suggest that alternative models will be required to overcome what currently constitutes a significant barrier to mechanistic studies concerning the molecular basis of \textit{MED12} in the pathogenesis of uterine fibroids.

An extenuating factor in rapid loss of \textit{MED12}-mutant cells from culture may relate to the recent observation that \textit{MED12} mutation–positive uterine fibroids, compared with \textit{MED12} mutation–negative tumors, exhibit apparently greater cellular heterogeneity. In this regard, prior studies have revealed that uterine fibroids, while clonally derived, are nonetheless heterogeneous in their cellular composition, consisting predominantly of smooth muscle cells and fibroblasts, along with smaller numbers of vascular smooth muscle cells, vascular endothelial cells, and immune cells (156, 157). Significantly, recent work has shown that \textit{MED12}-mutant tumors, compared with \textit{MED12}-WT (HMGA-overexpressing) tumors, harbor significantly more collagen-producing tumor-associated fibroblasts (TAFs) that also contribute significantly to excessive levels of extracellular matrix (ECM) observed in \textit{MED12}-mutant tumors (158). Notably, only smooth muscle cells, but not TAFs, carry \textit{MED12} mutations, suggesting antecedent divergence from a common progenitor before cell type–specific mutation acquisition or, alternatively, an extratumoral origin for TAFs. Interestingly, this work also showed that within \textit{MED12}-mutant tumors, smooth
muscle cells grow in response to progesterone, which has no effect on TAFs that instead grow in response to estrogen (158). The observation that MED12 mutation–positive uterine fibroids comprise similar ratios of smooth muscle cells and TAFs that respond differently to steroid hormones could explain the intriguing observation that estrogen alone can attenuate regression, but not promote growth, of progesterone-dependent MED12-mutant tumor xenografts (159). The high ratio of TAFs could also explain the rapid disappearance of MED12-mutant smooth muscle cells from primary cultures of uterine fibroids. Thus, growth-deficient MED12-mutant cells could be overwhelmed by TAFs, for which standard culture conditions were originally optimized. Ultimately, the number of heterogeneous cell types within MED12 mutation–positive uterine fibroids and the degree to which they are clonally related remains to be firmly established, and newer technologies, including single-cell RNA sequencing, could help to resolve these outstanding issues.

The molecular basis by which pathogenic mutations in MED12 drive uterine fibroid formation is presently unclear, but dysregulation of RNA Pol II–driven gene expression is implicated. Mediator is a conserved multiprotein interface found between gene-specific transcription factors and Pol II (137) and channels regulatory signals from activator and repressor proteins to affect changes in gene expression programs that control diverse physiological processes, including cell growth, homeostasis, development, and differentiation. Structurally, Mediator is comprised of a 26-subunit core that binds tightly to Pol II in the so-called holo-enzyme (137). MED12, MED13, CycC, and CDK8 (or its paralog CDK19) comprise a 4-subunit “kinase” module that variably associates with the core Mediator (137). Notably, the kinase module is a major ingress of signal transduction through the Mediator, and MED12-dependent CDK8 activation is required for the nuclear transduction of signals initiated by multiple oncogenic pathways, with which MED12 is biochemically and genetically linked (Fig. 3) (137). Furthermore, MED12 is a target of oncogenic mutation in colon, prostate, and renal cell carcinomas (119, 160, 161). However, these mutations predominantly occur in the MED12 C-terminus and thus lie distant from fibroids-linked mutations that cluster in the N-terminus, suggesting distinct tumorigenic mechanisms (162).

Uterine fibroid–linked mutations in MED12 are all located within exons 1 or 2, most of which are missense mutations, and a smaller proportion include in-frame deletions and insertions (118, 129, 137). Particularly, those occurring in exon 2 are far more frequent than those in exon 1, with the latter accounting for ~6% of pathogenic alterations reported in uterine fibroids (129). Although missense mutations in exon 2 are distributed throughout the coding sequence, most are clustered in codons 36, 43, and 44, suggesting the importance of their corresponding and evolutionary highly conserved amino acid residues (7, 8, 118, 121, 128).

Within the Mediator kinase module, MED12 is known to activate CycC-CDK8, and the mechanistic basis has recently been clarified (128, 163-167). Thus, MED12 binds directly to CDK8, leading to structural reconfiguration and stabilization of the CDK8 activation (T)-loop in a manner critically dependent upon MED12 residues recurrently mutated in uterine fibroids (167). These observations suggest that uterine fibroid driver mutations in MED12 could alter T-loop conformation and disrupt CDK8 kinase activity (Fig. 3) (167). Indeed, pathogenic exon 2 mutations in MED12 have been confirmed to disrupt CDK8/19 kinase activity both in vitro and in clinically relevant patients with uterine fibroids (128, 129, 165, 166). Collectively, these studies reveal a common molecular defect associated with uterine fibroid–linked mutations in MED12 and implicate the aberrant Mediator-associated CDK8/19 kinase activity in the pathogenesis of uterine fibroids. Mechanistically, Mediator kinase activity has been implicated in diverse cellular processes ranging from controlling transcription factor half-life and RNA Pol II activity to regulating chromatin chemistry and functional status (137, 168, 169). Accordingly, its disruption as a direct consequence of uterine fibroid mutations in MED12 could have broad implications for the dysregulation of gene expression programs that collectively contribute to tumor formation. Nonetheless, MED12 has also been shown to regulate transcription in a CDK8-independent manner (170-173), although this function is largely mediated through the MED12 C-terminus that lies spatially distant from N-terminal residues mutated in uterine fibroids (Fig. 3). Because MED12 mutations have been linked to pathways directly implicated in uterine fibroid pathology, including the Wnt/β-catenin, protein kinase B/mammalian target of rapamycin (AKT/mTOR), progesterone receptor, focal adhesion, extracellular matrix, angiogenic, and HIF1α pathways, among others (7, 134, 137, 174-176), the relative contribution of CDK8 to MED12-dependent regulation of these pathways and the extent to which altered Mediator kinase activity contributes to their dysregulation will be an important area of future investigation.

Finally, the molecular basis for the high-frequency occurrence of MED12 exon 2 mutations in uterine fibroids is not presently understood. Either of 2 alternative scenarios can be posited. First, high-frequency MED12 exon 2 mutations might simply reflect the selection of clustered mutations among disparate others arising randomly throughout the MED12 gene through errors of
replication, particularly if these mutations similarly impact an important biological function of MED12 in the myometrium. As described previously, all uterine fibroid driver mutations in MED12 disrupt Mediator-associated CDK8/19 kinase activity, and it is perhaps notable that Mediator kinase has been implicated in the control of stem cell plasticity and fate determination. For example, a developmentally programmed reduction in CDK8 expression is associated with naïve pluripotency during animal development in vivo, and chemical inhibition of CDK8/19 was recently shown sufficient to revert primed pluripotent stem cells to a naïve pluripotent state in vitro (177, 178). Furthermore, CDK8 has been implicated in cancer stem cell self-renewal and tumorigenicity in colon and brain cancer (179, 180). Finally, within the uterus, it was recently shown that Mediator kinase subunits are enriched in myometrial stem cells (MMSCs), and further, that chemical inhibition of CDK8/19 in MMSCs led to reduced phosphorylation of stem cell-enriched transcription factors and altered expression of myogenic genes. Thus, it seems possible that MED12 exon 2 mutations, through disruption of Mediator kinase activity, could provide a selective advantage to myometrial stem/progenitor cells by altering their growth and/or differentiative trajectory, leading to the formation of uterine fibroid stem cells that, in turn, seed and sustain monoclonal tumor growth.

An alternative hypothesis to explain the high-frequency occurrence of MED12 exon 2 mutations invokes a sequence- or structure-specific basis for clustered mutagenesis through error-prone repair of site-specific DNA damage. Replication fork arrest as occurs, for example, on repeat and satellite sequences or noncanonical B-DNA, is often processed through DNA double-strand break intermediates, which are prone to erroneous repair and punctual mutagenesis (or chromosomal rearrangements) (181). Accordingly, such error-prone sequences, should they reside in the vicinity of MED12 exon 2, might favor the occurrence of high-frequency somatic mutations found in uterine fibroids. However, the genomic sequence within and flanking MED12 exon 2 is characterized by neither particularly high GC content nor repeat motifs characteristic of replication-resistant DNA, perhaps arguing against replication-dependent site-specific mutagenesis as a basis for the high-frequency occurrence of MED12 mutations. Nonetheless, it was recently noted that this region does harbor a 16-bp sequence with significant homology to a putative terminator-based hairpin sequence within the tRNA gene cluster of Staphylococcus aureus, a common component of the uterine microbiota (182). On this basis, it was hypothesized that MED12 hot-spot mutations arise through site-specific mutagenesis brought on by replication-dependent processing of aberrant R-loops.
produced by insertion of *S. aureus* RNA with the homologous DNA sequence in MED12 (182). Although speculative, this intriguing hypothesis nonetheless does invoke a direct link between host and microbiome that together form a complex interrelationship prone to homeostatic disruption and the development of human disease (183, 184). While the genic basis for high-frequency MED12 mutations in uterine fibroids thus remains obscure, it is nonetheless clear that once incurred, these mutations interact with additional environmental components including hormonal, angiogenic, and growth regulatory factors to drive tumor progression.

**HMGA2**

The high mobility group A (HMGA) family includes related HMGA1 and HMAG2 non-histone chromosomal proteins that regulate transcription by altering chromatin structure. The HMGA non-histone proteins bind to the AT-rich enhancers or promoters’ minor groove and introduce structural alterations in chromatin. One of the most commonly observed cytogenetic abnormalities (8%-10%) in uterine fibroids is a translocation involving chromosomes 12 and 14, which disrupts a putative regulatory sequence typically 5’ of the HMGA2 gene (185, 186). In addition, the expression levels of HMGA2 are elevated in uterine fibroids compared to myometrium with 12q15 rearrangements (187, 188). Uterine fibroids with HMGA2 aberrations displayed significant upregulation of proto-oncogene pleomorphic adenoma gene 1 (PLAG1), suggesting that HMGA2 triggered the pathogenesis of uterine fibroids through PLAG1 activation (189).

**FH**

Mutations in fumarate hydratase (*FH*) on chromosome 1 in band q42 were found in uterine fibroids (190, 191). Heterozygous germline mutations in the *FH* gene caused a syndrome known as hereditary leiomyomatosis and renal cell carcinoma (HLRCC) (192). *FH* deficiency, accounting for up to 1.6% of uterine fibroids, alters the expression profiles of fibroids, most strikingly increasing the expression of genes involved in glycolysis (132) as well as activating nuclear factor erythroid 2 related factor 2 (NRF2) target genes (189).

**COL4A5/COL4A6**

Similar to the *FH* deficiency subtype, *COL4A5/COL4A6* deletions are a rare subtype constituting about 2% of uterine fibroids (193). Integrated data analysis reveals insulin receptor substrate-4 (*IRS4*), a gene located adjacent to *COL4A5*, as the most uniquely expressed gene in this uterine fibroid subtype (189).

Additionally, a small number of mutually exclusive drive mutations were recently identified. Germline mutations in SRCAP members YEATS4 and ZNHIT1 predispose women to uterine fibroids. The fibroids bearing these mutations exhibited defective deposition of the histone variant H2A.Z (194). Moreover, an integrative computational approach (decomposition and classification of genomic tensors) can discriminate normal and uterine fibroid subtype (195), suggesting that the inclusion of epigenetic features can help better understand the state and complexity of uterine fibroids.

**Conversion of Myometrial Stem Cells to Uterine Fibroid Stem Cells**

The human myometrium is the muscular wall of the uterus that is formed by an intricate network of smooth muscle fibers dispersed throughout an extracellular matrix of connective tissue. This process contributes to the normal tonicity of the uterus. Increasing evidence supports the hypothesis that uterine fibroids originate from stem cells in the myometrium, although the specific cell of origin has not yet been identified (196, 197). Stem cells derived from the myometrium and uterine fibroids have been isolated, and tumor-initiating cells in fibroids have been identified (198-201). Moreover, the markers used to enrich putative mesenchymal stem cells are similarly enriched for MMSCs from myometrium and uterine fibroids (202). Notably, *MED12* mutations are only found in uterine fibroid stem cells and not in MMSCs (203). In addition, distinct *MED12* mutations have been detected in different uterine fibroid tumors derived from the same uterus (7), indicating that the emergence of each mutation might be an independent event. The prevailing model for fibroid pathogenesis invokes the genetic transformation of a single MMSC into a tumor-initiating cell that seeds and sustains clonal tumor growth through endocrine, autocrine, and paracrine growth factors and hormone receptor signaling (204).

Several factors have been proposed as the origin of tumor-initiating cells, including genomic instability, inflammatory microenvironment, cell fusion, lateral gene transfer, and developmental environmental insult (205, 206). The adverse effect of developmental environmental insult may cause the deregulation of multiple developmental processes, including the disruption of stem cell niche, developmental reprogramming, and altered stem cell characteristics. Somatic stem/progenitor cells from various hormone-supported tissues remained susceptible to endocrine-disrupting chemicals (EDCs) (206, 207). In uterine fibroids, developmental exposure to EDCs impaired the biological characteristics of MMSCs in an Eker rat model with Tsc2 mutation. This model spontaneously develops uterine fibroids with 63% incidence. However,
Early life exposure to EDCs, such as diethylstilbestrol (DES), increased the penetrance of the Tsc2 mutation, resulting in 100% incidence (208).

The impact of environmental exposure to MMSCs that increase susceptibility to uterine fibroid development have been investigated using the same model. The more MMSCs in the DES-exposed myometrium have been observed than those exposed to vehicle. In addition, MMSCs from 5-month-old DES rats exhibited increased proliferation rates compared to MMSCs from age-matched control rats (206). These results suggest that developmental exposure to EDCs targets MMSCs and alters their characteristics, which may underlie reprogramming of epigenome and initiation of hormone-dependent uterine fibroid pathogenesis (Fig. 1).

Early and late epigenome and environment interactions can potentially impact uterine function and increase the risk of uterine fibroids (Fig. 1) by shaping the developing epigenome of target genes (209). This epigenomic reprogramming may remain transcriptionally and phenotypically silent until triggered by a later life event, such as exposure to risk factors. For example, during a critical developmental window of the liver, exposure to BPA induced epigenomic reprogramming at specific genes and chromatin states in the neonatal liver to accelerate acquiring an adult epigenetic signature. Although it persists until adulthood, much of this reprogramming remained transcriptionally silent until a later-life challenge with a Western-style diet high in fat, fructose, and cholesterol, which disrupted metabolic function and significantly elevated serum cholesterol and lipid levels (209). Further studies on stem cells from reproductive organs may contribute to a better understanding of the genome-environment interaction leading to reproductive diseases, including uterine fibroids.

The occurrence of MED12 driver mutations and how they interact synergistically with other implicated pathways in uterine fibroids remain largely unknown. Therefore, further investigation is highly needed to elucidate the mechanism of interplay between hormones, environments, DNA repair system, and other factors in the occurring of MED12 mutations.

Environmental Exposure and Pathogenesis of Uterine Fibroids

Direct, Intensive, and Adverse Environmental Exposure

Air pollution

Air pollution is one of the leading causes of death. Exposure to air pollutants affects vital cellular mechanisms and is intimately linked with the etiology of many chronic diseases such as chronic obstructive pulmonary disease and asthma (210-212). Particulate matter (PM) is a class of pollutants that comprises a complex combination of small-sized particles and gaseous components, such as organic chemicals, smoke, soot, sulfates, nitrates, acidic components, dust particles, and soil. The United States Environmental Protection Agency considers PM the pollutant category with the most significant impact factor on human health (213-215). Air pollution, including PM2.5, results in infertility, menstrual irregularity, and endometriosis (216). In addition, chronic exposure to PM2.5 is associated with the incidence of clinically symptomatic uterine fibroids (216). A 10-year cohort-based case-control study that included 11,028 Taiwanese women diagnosed with uterine fibroids suggested that exposure to PM2.5 and O3 may increase the risk of developing uterine fibroids (217). However, only limited research has investigated the relationship between air pollution and uterine fibroid development; therefore, more studies are needed to confirm these findings in other populations.

Alcohol consumption

Heavy alcohol consumption is a risk factor for uterine fibroids (35). The Nurses’ Health Study II revealed the positive association between current alcohol consumption and risk of uterine fibroids (35, 218). The Black Women Health Study concluded that uterine fibroids risk among African American women is positively correlated with past and current alcohol intake (219). In a study involving 133,000 female teachers and school administrators, drinking at least 20 g of alcohol per day was significantly associated with an increased risk for uterine fibroids (220). Another cross-section study on premenopausal Japanese women supported this causal risk factor for uterine fibroids and reported that mean alcohol intake is significantly higher among women with fibroids than those without (221). Moreover, a study of 1146 premenopausal African American and Caucasian women showed that current alcohol intake in Caucasian women is associated with an increased risk of uterine fibroids compared to African Americans and nondrinkers. Although no correlation between alcohol intake and uterine fibroid risk in African Americans was found, the relationship of current and past drinking history and uterine fibroid size was generally similar among African American and White women (35).

Although the underlying mechanism is largely unknown, several studies have proposed that alcohol intake increases the levels of steroid hormones in premenopausal women (222-224). Alcohol intake also altered the growth factors and cytokines, which play a critical role in uterine fibroid pathogenesis. Moreover, alcohol-induced DNA damage might be a contributor. Acetaldehyde, an endogenous and alcohol-derived metabolite, caused DNA damage, particularly double-stranded breaks, that, despite the activation of recombination repair, resulted in chromosomal rearrangements in stem cells (225). Other studies have reported
alcohol-induced mitochondrial DNA damage in lung, brain, and breast cancers (226-228). More studies are needed to explore alcohol-induced DNA damage in uterine fibroids.

Cigarette smoking
The effect of smoking on uterine fibroids remains controversial (229). An inverse correlation between smoking and uterine fibroids risk was reported (220, 230, 231). However, this association was not found in other case-control and prospective cohort studies (2, 232, 233). Early studies found that estrone and estradiol levels were reduced in smokers relative to nonsmokers, and cigarette smoking altered the hepatic metabolism of estrogen, thus resulting in lower circulating levels of activated estrogen (2). However, the components of cigarette smoke may also exert estrogen-related effects on the uterus to promote cell proliferation (2).

Developmental Exposures
Epidemiological studies and endocrine-disrupting chemical effects
Various niche factors act on stem cells during development to alter gene expression and induce their proliferation or differentiation for fetus development. During development and tissue maintenance, the highly plastic state of stem/progenitor cells permits the required flexibility for proper tissue formation and repair. Unfortunately, this plasticity also provides an opportunity for aberrant cellular reprogramming via epigenetic mechanisms due to inappropriate exposures to toxins (234). Developmental adverse exposure can lead to persistent, long-lasting effects and result in various diseases (235-237).

EDCs interfere with the body’s endocrine system to produce adverse developmental, reproductive, neurological, and immune effects (198, 238, 239). An increasing number of studies have shown that endocrine disruptors may pose a serious disease risk during development (240). According to epidemiological and experimental studies, EDCs increased the risk of tumorigenesis, especially in organs susceptible to endocrine regulation. For example, upon exposure to estrogen and progesterone, differentiated myometrial cells secreted wingless-type (WNT) ligands that induced the nuclear translocation of β-catenin in stem/progenitor cells from uterine fibroids. The activation of the β-catenin pathway ultimately enhanced the growth and proliferation of these stem/progenitor cells (241).

EDCs can exhibit nonmonotonic dose–response curves and produce a pathophysiologic effect even at low doses. Numerous EDCs can interact with nuclear receptors to exert their actions in target cells and tissues (242-244). For example, the binding of EDCs to nuclear receptors can alter hormonal functions by mimicking the naturally occurring hormones in the body, thereby blocking the binding of endogenous hormones, or by interfering with the production or regulation of hormones and/or their receptors. An EDC may interact with more than one receptor, and multiple EDCs can interact with the same receptor, highlighting the complexity of the response of animals and humans to environmental EDC exposures. Notably, EDCs exposure can increase the risk of uterine fibroids (Figs. 2 and 4). Two extensive prospective studies reported a positive association between developmental exposure to DES, a synthetic and nonsteroid estrogen, and uterine fibroids risk. (245, 246). In the Nurses’ Health Study II (n = 11 831), prenatal exposure to DES increased the risk of uterine fibroids by 13% in women older than 35 years (246), and exposure during the first trimester of gestation increases the risk by 21%. Large fibroids were more commonly found in those exposed to prenatal DES in the second National Institute of Environmental Health Sciences (NIEHS) uterine fibroid study. In a subset of the NIEHS sister study, the main factors associated with increased risk of uterine fibroids included DES exposure, maternal or gestational diabetes, and monozygotic twins, having risk ratios of 2.02, 1.54, and 1.94, respectively. However, another prospective cohort study, which employed medical records to document exposure, reported no association between prenatal DES exposure and uterine fibroids. Many other EDCs, including parabens, environmental phenois, alternate plasticizers, organophosphate esters, tributyltin, and phthalates, have been associated with uterine fibroids outcomes and their related processes. Phthalates have received increasing attention as they are tightly linked to uterine fibroid prevalence and severity (31, 247-249) (Figs. 1 and 2).

Experimental studies using animal models
Diverse animal species and techniques have been used for the in vivo investigation of uterine fibroid pathophysiology. These animal models include the xenotransplantation of human uterine fibroid tissues (250-252) or cells (253, 254) in mice, the implementation of genetically modified mice (Tsc2 knockout (255), GPR10 overexpression (256), β-catenin overexpression (257)), and the utilization of species that spontaneously develop uterine fibroids, such as the guinea pig (258), the potbellied pig (257), and the Eker rat. The latter carries a germline mutation in the tuberous sclerosis complex-2 (Tsc2) tumor suppressor gene and develop uterine fibroids with a frequency of about 65% by 16 months of age (259). Although the Eker rat model is the most widely used in vivo animal model to study uterine fibroids, this animal model has some limitations. For example, mutations in the Tsc gene have not been linked to the disease in humans. In addition, the developing uterine fibroids show relatively small amounts of collagenous connective tissue stroma (260), unlike the human uterine fibroids, which present a high amount of abnormally formed cross-linked collagen (261).
Figure 4. Estrogen receptor-mediated signaling pathways in the myometrium. The biosynthesis of natural E2 occurs in the ovary downstream the actions of the LH and the FSH, which are regulated by the GnRH. E2 mediates its biological response through several pathways, which can be classified as genomic and nongenomic. There are 3 main mechanisms of genomic regulation mediated by ER. Firstly, in the classical pathway, E2 ligands passively enter the cells by diffusion. ERα and ERβ are localized in the cytosol and are attached to the chaperon Hsp90, which is released after binding with estrogen. The estrogen-bound receptors form dimers that enter the nucleus and bind to the ERE, specific DNA sequences of the promoters of target genes affecting their transcription. Secondly, the nonclassical pathway involves binding the E2-bound ER to TFs that are already bound to the DNA. The third mechanism is hormone-independent. The ER can regulate E2 responses by activating the signaling of growth factors via the phosphorylation of different serine (118/167) residues on the receptor. In addition to upregulating gene expression, E2 exerts its nongenomic rapid biological actions by interaction with membrane receptors. GPER, a membrane-integrated 7-transmembrane receptor, activates heterotrimeric G-proteins after binding with estrogen to elicit various nongenomic responses, such as calcium signaling, PKC, and cAMP/PKA pathways. Bound-membrane ERs (ERα, ERβ) also activate cytosolic signalings, such as PI3K/Akt and MAPK. In addition, the activation of kinases results in the phosphorylation of specific transcription factors that regulate gene expression. EDCs are exogenous, manufactured chemicals, such as genistein, bisphenol A, and phthalates that mimic natural estrogen molecular and cellular responses, thereby altering the functions of the endocrine system. These chemicals are associated with the developmental origin of fibroids and their pathogenesis. Abbreviations: AC, adenylyl cyclase; AKT, protein kinase B; cAMP, cyclic AMP; CoA, coactivator; E2, estrogen; EDCs, endocrine-disrupting chemicals; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ERE, estrogen-responsive elements; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone;
Finally, Eker rats develop both benign and malignant smooth muscle tumors (260). However, studies in the Eker rat animal model provide a great opportunity to reveal links between early-life exposure to EDCs and the origin and development of uterine fibroids. Upon neonatal exposure to EDCs, Eker rats developed increased susceptibility to spontaneous uterine fibroids, multiplicity, and tumor size with age (208, 262-264), whereas those without the Tsc2 mutation did not develop any tumors. These studies suggest that developmental exposure to EDCs increases the penetrance of the Tsc2 mutation (208). In addition, the window of susceptibility to environmental exposures coincided with critical periods of myometrial development (208). Exposure to DES during postnatal day (PND) 3–5 or 10–12 increased tumor incidence from 63% to 95% and 100%, respectively, in Eker rats carrying germline TSC2 mutation. During this time, estrogen protection of the developing uterus is disrupted (265) as DES and other xenoestrogens do not bind circulating steroid hormone–binding proteins, such as alpha-feto protein A. A later exposure at PND 17–19 did not result in increased uterine fibroid incidence. Overall, developmental exposure to EDCs during a critical time window of uterus development increases uterine fibroid risk later in life (Figs 1 & 2).

Molecular mechanism underlying developmental EDC exposure–induced risks of uterine fibroids

During development, various niche factors act on stem cells to alter gene expression, therefore altering the signaling pathway and modulating its biological characteristics for the development of the fetus. The adverse developmental exposure can lead to persistent, life-long effects and result various diseases via pathological reprogramming (208, 209, 234, 240, 266).

Early-life exposures to 3 EDCs (ie, DES, genistein, and BPA) have been investigated to detect their effect on estrogen signaling, which plays a role in triggering fibroids formation in an animal model (267-269). All 3 EDCs act as ER ligands and induce ER-mediated gene transcription. However, only DES and genistein induced nongenomic ER signaling to activate phosphoinositide-3-kinase (PI3K)/AKT in the developing uterus. The histone methyltransferase enhancer of zeste homolog 2 (EZH2) is phosphorylated by activated PI3K/AKT signaling to repress EZH2 activity and reduce the levels of histone 3 lysine 27 trimethyl (H3K27me3). Significantly, decreased H3K27 methylation via developmental exposure correlated with the promoting effect of xenoestrogens on uterine fibroids.

In addition to EZH2, altered DNA methylation patterns due to environmental exposure have been reported in animal studies. Neonatal exposure induced the reprogramming of DNA methylation in animals exposed to DES during PND 1–5 compared with PND 17 (prepubertal), 21, and 30 (postpuberty) (270). Furthermore, neonatal DES exposure reprogrammed LTF, an estrogen-responsive gene. At PND 21 and 30, the promoter upstream of the estrogen response element was demethylated in animals exposed to DES during PND 1–5. Importantly, this postpubertal DES-induced demethylation was dependent on ovarian hormones, as evidenced by the absence of this demethylation in DES-exposed ovarioctomized mice (270). Another animal study showed that neonatal DES exposure-induced metabolic changes last until adulthood, suggesting a permanent effect on energy metabolism in the uterus (271). Thus, developmental exposure to EDCs causes uterine diseases via epigenomic reprogramming. However, studies on the mechanism of epigenetic reprogramming by EDCs and its influence on fibroids development are limited. Additional mechanistic studies to elucidate the epigenetic biomarkers/signatures specific to EDCs can contribute to the development of precision medicine.

The reprogramming of MMSCs, the cell origin of uterine fibroids, was recently identified following early-life exposure in the Eker rat (PND 10-12) to EDC. MMSCs isolated from prefibroid-stage tissue were analyzed using omics methods and showed altered biological pathways, including estrogen signaling (272) and inflammatory pathways (273, 274). The reprogramming of estrogen pathways is driven by activated mixed-lineage leukemia protein-1 (275). In addition, DNA hypomethylation is involved in regulating estrogen and estrogen-responsive genes in MMSCs (274, 276). In summary, EDC exposure epigenetically targeted MMSCs, imparting a hormonal imprint on key signaling pathways, thus resulting in an increased risk of uterine fibroids in a hormone-dependent manner (274) (Figs. 1, 2, and 4).

Due to some limitations using animal models, the use of 3-dimensional (3D) models has attracted more attention in uterine fibroids research (277-279), particularly using myometrial stem cells instead of differentiated myometrial cells (280). The 3D model provides a more biomimetic cell culture environment than 2D substrates, with the advantage of more closely mimicking in vivo tissue architecture. MMSC-material interactions in 3D with topographical cues may provide an effective means to regulate many fibroid-related biological events, including differentiation, epigenetic state, or cell reprogramming, and rapidly advance our understanding of how the

Figure 4: continued

GPER, G-protein coupled estrogen receptor 1; Hsp90, heat shock protein 90; IGFR, insulin-like growth factor 1 receptor; IP3, inositol triphosphate; LH, luteinizing hormone; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mER, membrane-bound estrogen receptor; mTOR, mammalian target of rapamycin; PI3K, phosphoinositol-3-kinase; PKA, protein kinase A; PKC, protein kinase; PLC, phospholipase C; Raf, Rapidly Accelerated Fibrosarcoma Kinase; Ras, Ras GTPase; TFs, transcription factors.
environment impacts risk for this disease as well as the tumor process via conversion of MMSCs to tumor-initiating cells.

Notably, so far, very few studies have attempted to disentangle the effects of early-life exposures concomitantly with late-life exposure on the pathogenesis of uterine fibroids. Thus, mechanistic insights into fibroid pathogenesis through the integration of risk exposures, genetic, epigenome, and MMSC biology will better understand the onset of fibroids.

Key Pathways Contributing to Uterine Fibroids Formation

Estrogen and Progesterone

Classically, uterine fibroids were considered estrogen-dependent tumors, based on their association with reproductive age \( (281, 282) \). The estrogen signaling pathway as a major impactful pathway in uterine fibroids comprises genomic (direct and indirect effects of gene expression) and nongenomic factors, including the Ras-Raf-MEK (MAPK/ERK kinase)-mitogen-activated protein kinase (MAPK) and PI3K-phosphatidylinositol-3,4,5-trisphosphate (PIP3)-Akt-mTOR pathways (the Ras-Raf-MEK-MAPK and PI3K-PIP3-Akt-mTOR pathways, respectively) \( (283) \). Figure 4 and 5.

Several aberrations in ERs and signaling pathways are implicated in uterine fibroid pathobiology \( (283) \). Recently, another role for estrogen has been identified, ie, estrogen-induced, progesterone receptor expression and allowing progesterone receptor ligands to act on their target cells \( (284) \). Uterine fibroid cells have been shown to increase the expression of progesterone receptors in response to estradiol \( (285) \). Progesterone and progesterone receptors play a key role in uterine fibroid growth and development \( (286) \).
Table 1. Inflammatory factors involved in uterine fibroids pathophysiology

| Factor   | Biological relevance                                      | Results in UF studies                                                                                           | References |
|----------|-----------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|------------|
| IL-8     | Chemoattracts neutrophils                                | Lower levels of IL-8 (IHC/ELISA) and its receptor (IHC) in UF than in the surrounding myometrium                 | (436)      |
| IL-4     | Induces the differentiation of naïve helper T cells into Th2 cells | UF progenitor cells had higher mRNA levels (qRT-PCR) and secreted higher levels of these factors (ELISA) than myometrial progenitor cells | (437)      |
| IL-5     | Promote the growth, differentiation, and activation of eosinophils |                                                                                                                  |            |
| IL-10    | Anti-inflammatory cytokine                              |                                                                                                                  |            |
| IL-13    | Proinflammatory cytokine responsible for Th2 responses  |                                                                                                                  |            |
| IL-6     | Proinflammatory cytokine                                | UF progenitor cells had lower mRNA (qRT-PCR) and secreted levels of these cytokines (ELISA) than myometrial progenitor cells | (438)      |
| IL-12    | Promotes the development of Th1 responses               |                                                                                                                  |            |
| IL-17A   | Proinflammatory cytokine                                |                                                                                                                  |            |
| IL-33    | Important in innate and adaptive immunity and contributes to tissue homeostasis | Serum IL-33 (ELISA) levels were elevated in women with UFs and positively correlated with the number, volume, and mass of UFs | (353, 439) |
| GM-CSF   | Hematopoietic growth factor                             | Smooth muscle cells isolated from UF patients expressed higher GM-CSF mRNA (RT-PCR) and protein (IHC) levels than myometrial smooth muscle cells | (357)      |
| G-CSF    | Hematopoietic growth factor                             | Higher levels in the conditioned media of UF stem cells than those of the adjacent myometrium                     | (357)      |
| TNF-α    | Pleiotropic cytokine that plays a major role in the cell cycle and controls apoptosis | Increased protein levels (IHC-TMA and WB) in UFs compared with adjacent myometrium tissues                         | (440)      |
| CXCL12   | Chemoattracts lymphocytes and macrophages; plays an important role in angiogenesis | TNF-α serum levels (ELISA) were elevated in women with clinically symptomatic UF                                 | (441)      |
| MCP-1    | Chemoattracts monocytes/macrophages                      | Increased CXCL12 and decreased CXCR4, its receptor, mRNA levels (qRT-PCR) in the UF and myometrium of women with UF compared with those without. Increased CXCL12 protein secretion (ELISA) in cultured UF cells compared to adjacent and normal myometrial | (442)      |
| MIP-1α (CCL3) | Chemoattractant for monocytes/macrophages   | Lower mRNA levels (Northern blot) in UF tissues compared to the adjacent myometrium                               | (443)      |
| MIP-1β (CCL4) | Chemoattractant for monocytes/macrophages       | Lower mRNA levels (RT-PCR) in UF tissues than the adjacent myometrium                                          | (444)      |
| CCR5     | Chemokine receptor                                       |                                                                                                                  |            |
| Eotaxin  | Potent chemoattractant for eosinophils                   |                                                                                                                  |            |
| INF-γ    | Proinflammatory cytokine                                |                                                                                                                  |            |
| NFκB     | Transcription factor with a key role in regulating the immune response |                                                                                                                  |            |
| IL-1β    | Proinflammatory properties                              |                                                                                                                  |            |
| TSLP     | Involved in the maturation of T cells                    |                                                                                                                  |            |

Abbreviations: CCR5, C-C chemokine receptor type 5; CXCL12, C-X-C Motif Chemokine Ligand 12; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; INF-γ, interferon gamma; MCP-1, monocyte chemoattractant protei n-alpha; MIP-1αβ, macrophage inflammatory protein 1 alpha/beta; NFκB, nuclear factor kappa B; PD-L1, programmed death-ligand 1; SCs, stem cells; TMA, tissue microarray; TNF-α, tumor necrosis factor alpha; TSLP, thymic stromal lymphopoietin; UF, uterine fibroid.
Increased proliferation of uterine fibroid cells in vitro was observed upon exposure to both estradiol and progesterone (288). Finally, a uterine fibroid xenograft animal model showed that steroids, including estradiol and progesterone, are required for tumor growth (289), supported by selective progesterone receptor modulators (SPRMs) (290).

**Extracellular Matrix and Growth Factors**

Excessive ECM accumulation and aberrant remodeling are crucial for fibrotic diseases, including uterine fibroids (Fig. 5). Uterine fibroid cells are characterized by abundant disorganized ECM deposition, which contributes to the formation of the bulk structure of the tumor. The large amounts of glycosaminoglycans and highly cross-linked interstitial collagens present in uterine fibroids (291) underlie the increased stiffness of the ECM. It is proposed that this ECM-rich rigid structure is the cause of the abnormal bleeding and pelvic pain (292). Moreover, ECM stiffness greatly impacts how cells sense physical forces and translate them into biochemical and biological responses, a molecular process collectively known as mechanotransduction. A clear example of proteins that respond to mechanical signals is β-catenin (293, 294), a protein known to be involved in the pathobiology of uterine fibroids (294). It has been proposed that uterine fibroids can be divided into 4 stages based on the extracellular collagenous matrix content (295, 296). Excessive collagen production by the transformed myocyte and its accumulation in the ECM results in decreased microvessel density, followed by myocyte death due to extreme deprivation of nutrients and oxygen (297). At the same time, changes in the ECM stiffness may activate mechanotransduction pathways that contribute to the myocyte phenotypic transformation. Interestingly, recent studies have linked mechanotransduction, nuclear rupture, and subsequent DNA damage in other diseases (298, 299). Unraveling the interactions among different mechanobiology pathways in the uterine fibroid’s context would eventually help to comprehend better the origin and development of these tumors.

ECM accumulation is also affected by growth factors [transforming growth factor [TGF]-β, activin-A, and platelet-derived growth factor], cytokines (tumor necrosis factor-α [TNF-α]), steroid hormones (estrogen and progesterone) (300), and microRNAs (particularly the miR-29 family, including miR-200c and miR-93/106b) (301-303). Interestingly, ECM acts as a reservoir of profibrotic growth factors and enhances their activity by increasing their stability and prolonging signaling duration. Therefore, a better understanding of ECM composition and metabolism in uterine fibroids is critical for developing new therapeutics for uterine fibroids. At present, several classes of drugs, including gonadotropin-releasing hormone (GnRH) agonist (leuprolide acetate), GnRH antagonists, SPRMs (eg, ulipristal acetate), and natural compounds (vitamin D), targeting the ECM have been studied as treatment options for uterine fibroids (303, 304). In this sense, a local collagenase injection from *Clostridium histolyticum*, which specifically cleavage interstitial collagens, has been proposed as an alternative treatment for uterine fibroids. Notably, a phase I clinical trial (NCT02889848) has been completed, and it demonstrated the safety and tolerability of this treatment. Furthermore, direct injection of collagenase from *C. histolyticum* significantly reduced the stiffness of uterine fibroid tissue (305), which is fundamental to continued tumor growth throughout the activation of mechanotransduction pathways. Therefore, such mechanotransduction pathways may be disturbed after the reduction of fibroid stiffness, leading to ECM remodeling and finally to reduced fibroid size.

**DNA Damage and Repair**

**DNA damage in the uterus**

Women are exposed to several exogenous (eg, EDCs) and endogenous factors that can impart pathophysiological alterations in internal organs, including the uterus. Endogenous factors include regular menses and hormonal changes that induce DNA damage through oxidative stress. Fluctuations in circulating estrogen and progesterone levels during the menstrual cycle can lead to increased tumor susceptibility in women, including breast cancer. Additionally, DNA damage and repair, and apoptosis occur cyclically in the normal myometrium during the follicular phase. Smooth muscle cells proliferate in the luteal phase, which may be a vulnerable period for DNA damage. These damages need to be properly repaired; otherwise, the hormonal imbalance can lead to diseases. For example, repeated incidents of damage to myometrial cells affect DNA repair activity, which could predispose the uterine environment to chronic inflammation, thereby creating an ideal environment for uterine fibroid development. However, the biological mechanisms involved in uterine fibroid progression remain unknown. This section will discuss studies on DNA lesions in uterine fibroids (306-308) (Fig. 5).

**Defective DNA damage response pathways**

Although the mechanistic basis underlying genomic instability in uterine fibroids remains to be established, defects in DNA damage response and repair gene expression programs are heavily involved. Several DNA repair genes in uterine fibroid tumors are downregulated compared with adjacent matched myometrium from the same
women, suggesting that impaired DNA repair capacity is linked to the genomic integrity and subsequent initiation/propagation of uterine fibroids. In addition, the expression of DNA repair-related genes RAD51 and BRCA1, which are involved in double-stranded break (DSB) homologous recombination (HR), were deregulated in uterine fibroid tumors (309, 310).

Interestingly, human uterine fibroid stem cells have accumulated DNA damage and reduced DNA repair gene expression and signaling, suggesting that human fibroid stem cells have impaired DNA repair capacity compared with normal myometrium stem cells. This compromised DNA repair system may contribute to promote mutagenesis, as well as the growth and propagation of uterine fibroids (311). In addition, DNA damage was significantly increased in uterine fibroids relative to MMSCs, as shown by increased mean percentage DNA in the tail of alkaline comet assay. Moreover, uterine fibroid stem cells had decreased expression of total DNA repair-related proteins belonging to DSB repair, specifically HR, and differential phosphorylation in comparison to adjacent MMSCs, indicating altered DNA damage response and increased DNA damage as evidenced by increased phosphorylation of histone H2A.X at serine 139 (ie, γ-H2AX) as a response to DNA DSB formation (311).

A germline mutation in Tsc2 predisposes to uterine fibroids in Eker rats, which is attributed to “second hits” in the normal allele of this gene. The risk for developing these tumors is significantly increased by early-life exposure to EDCs, suggesting that the early drivers for these tumors modulate increased uterine fibroid penetrance. Analyses of DNA repair capacity using gene and protein expression and DNA repair function in MMSCs derived from adult rats exposed to DES during uterine development were conducted. Adult MMSCs isolated from developmentally exposed rats showed decreased DNA end-joining ability, increased DNA damage, and impaired ability to repair DNA double-strand breaks compared with those from age-matched, vehicle-exposed rats, suggesting that early-life developmental EDC exposure alters the power of MMSCs to repair and reverse DNA damage, thereby providing a driver for the acquisition of mutations that may promote the development of these tumors in adulthood (312).

Other types of DNA repair, including nucleotide excision repair, which removes bulky DNA adducts caused by polycyclic aromatic hydrocarbons, and base-excision repair of oxidative DNA damage (313), should be explored in the context of uterine fibroids (Fig. 5).

Wnt/β-Catenin Signaling Pathway

The Wnt/β-catenin signaling pathway is involved in various physiological events, including development, tissue renewal, cell proliferation and differentiation, and several types of tumorigenesis (314, 315). This pathway has also been recently investigated in uterine fibroid formation (241, 316, 317) (Fig. 5). However, contrasting results on the β-catenin expression in uterine fibroids have been reported: 1 study detected upregulated expression in uterine fibroids (318), whereas others detected no difference between uterine fibroids and myometrium (319). Recently, the mislocalization of β-catenin has been causally linked to uterine fibroids phenotype, wherein fibroids expressed higher levels of nuclear β-catenin than normal myometrium tissues. Moreover, estrogen activated β-catenin nuclear translocation and enhanced β-catenin responsive gene expression in human uterine fibroids cells via the ER (320).

Thus, a paracrine role for the WNT/β-catenin pathway that enables mature myometrium or fibroid cells to send mitogenic signals to neighboring tissue stem cells in response to estrogen and progesterone has been proposed, thus leading to the growth of uterine fibroids (241).

The use of vitamin D3 has been associated with the inhibition of the Wnt/β-catenin pathway and decreased uterine fibroid cell proliferation (321, 322). β-catenin inhibitors, such as ICG-001, cordycepin, and XAV939, and histone deacetylase (HDAC) inhibitors (HDACi), including apicidin and HDACi VIII, exhibited antiproliferative effects on uterine fibroid cells by suppressing the β-catenin signaling pathway. Additionally, HDACi exposure induced cell cycle arrest and apoptosis of uterine fibroid cells, thus representing a promising epigenetic-based therapy for uterine fibroids (320). Recently, the MED12 somatic mutation has been revealed to modulate oncogenic Wnt4/β-catenin and mTOR signaling pathways in uterine fibroids (174).

Compounds targeting Wnt/β-catenin signaling and other vital pathways are summarized in Table 2.

YAP/TAZ Pathway

ECM accumulation and aberrant cell proliferation are essential components in uterine fibroid pathogenesis. An important regulatory mechanism that connects mechanical stimuli to cellular proliferation is the Hippo pathway, including the effector proteins Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding domain (TAZ) as main mediators. YAP/TAZ has been involved in fibrotic diseases such as lung fibrosis (323) as well as in hormone-regulated organs such as ovary (324) with aberrant activation that resulted in tumorigenesis (325). A recent study has shown that YAP/TAZ nuclear localization in situ and confluent cells was higher in uterine fibroid cells compared with normal myometrium and associated tissue stiffness was higher in uterine...
fibroids compared with normal myometrium. Moreover, exposing fibroid cells to verteporfin (a YAP inhibitor) reduced cell survival and reduced fibronectin deposition (326). Furthermore, a recent study showed that the antifibrotic drug nintedanib inhibited YAP and produced antifibrotic effects (327). The same study showed that in vivo injection of collagenase into uterine fibroids led to a reduction in Hippo/YAP signaling and crucial genes and pathways involved in fibroid growth (327) (Table 2). More studies are encouraged to further explore the role of the Hippo/YAP/TAZ pathway in fibroid pathogenesis with subsequent identification of novel targeted therapeutic strategies.

### Rho/ROCK Signaling Pathway

Studies showed that mechanotransduction activates the Ras homolog family (Rho), which interacts with its downstream target Rho-kinase (ROCK) and activates the ERK/p38 MAPK-signaling cascade, and resulting in increased cell proliferation, decreased apoptosis, and upregulation of genes involved in ECM composition and remodeling (303). RhoA expression was found to be higher in uterine fibroid compared with myometrial cells (328). Interestingly, inhibition of integrin-β1 caused a decrease in active RhoA expression in uterine fibroid cells (301), while Fasudil, a ROCK inhibitor, relaxed the contraction of uterine fibroid cells in 3D collagen gels (278). It might be interesting to pursue potential antifibrotic effects of these pathway inhibitors in further studies.

### Epigenetic Regulation

Tumorigenesis cannot be exclusively explained by genetic changes, as epigenetic processes are also involved (329, 330). All epigenetics involved in regulation of gene activities, including DNA methylation, histone modifications, non-coding RNA, and heterochromatinization, are disturbed in the pathogenomics of uterine fibroids (331, 332).

#### Table 2. Compounds targeting key pathways involved in uterine fibroids pathogenesis

| Compound                  | Family            | Molecular effects                                      | References |
|---------------------------|-------------------|--------------------------------------------------------|------------|
| Leuprolide acetate, Goserelin | GnRH agonist      | ECM and TGF-β inhibition, tumor shrinkage              | (445-448) |
| Ulipristal                | SPRM              | Apoptosis induction, proliferation inhibition, ECM inhibition | (290)      |
| Vitamin D                 | Natural compound  | DNA repair induction, Wnt/β-catenin pathway inhibition, TGF-β-induced inhibition of ECM production, anti-inflammatory | (370, 395) |
| EGCG (green tea)          | Natural compound  | COMT suppression, apoptosis induction, proliferation inhibition | (449, 450) |
| Tranilast                 | Anti-allergic compound | ECM inhibition, inhibit proliferation, cell cycle arrest, induction of miR-29c and miR-200c expression | (451-453) |
| Curcumin                  | Natural compound  | Inhibition of ECM production, inhibition of UF proliferation, anti-inflammatory effect | (454, 455) |
| 2-methoxyestradiol        | Estradiol metabolite | P38K/Akt/mTOR inhibition, ECM inhibition | (456) |
| Letrozole                  | Aromatase inhibitor | Apoptosis induction, inhibition of proliferation | (457) |
| All-trans retinoic acid   | Active metabolite of vitamin A | TGF-β-induced inhibition of ECM production | (458) |
| Methyl jasmonate          | Natural compound  | Wnt/β-catenin pathway inhibition, apoptosis induction, inhibition of proliferation | (459) |
| Apicidin                  | Class I HDAC inhibitor | Inhibition of Wnt/β-catenin pathway, apoptosis induction, inhibition of proliferation | (320) |
| HDACi VIII                | HDAC 6 inhibitor  | Inhibition of Wnt/β-catenin pathway, apoptosis induction, inhibition of proliferation | (320) |
| ICG-001                   | β-catenin inhibitor | Inhibition of Wnt/β-catenin pathway, inhibition of proliferation | (320) |
| Resveratrol               | Natural compound  | Inhibition of ECM production, apoptosis induction | (460) |
| Collagenase               | Proteolytic enzyme | ECM degradation | (431, 461) |
| Simvastatin               | Statin drug       | ECM, apoptosis, ER-α palmitoylation and degradation | (462-466) |
| Verteporfin               | YAP inhibitor     | Anti-proliferation, -fibrosis and -mechanotransduction | (461) |
| Nintedanib                | YAP inhibitor     | Anti-fibrotic effect | (461) |
| 5-Aza                     | Hypomethylation drug | Fibroid stem cell differentiation and PGR signaling | (467, 468) |

Abbreviations: Akt, protein kinase B; COMT, Catechol-O-methyltransferase; ECM, extracellular matrix; EGCG, epigallocatechin gallate; GnRH, gonadotropin-releasing hormone; HDAC, histone deacetylase; miR, microRNA; mTOR, mechanistic target of rapamycin; PI3K, phosphoinositide 3-kinase; SPRM, selective progesterone receptor modulator; TGF-β, transforming growth factor beta; PGR, progesterone receptor; UF, uterine fibroids.
Numerous data demonstrated aberrant alterations of genome methylation/demethylation in uterine fibroid cells (175, 333). In addition, unsupervised clustering of results from DNA methylation analysis can segregate normal myometrium from uterine fibroids and classify uterine fibroids into subtypes according to MED12 mutation or the activation of HMGA2 or HMGA1. Moreover, HOXA13 encoding the class of transcription factors called homeobox genes was identified to be hypomethylated and upregulated in fibroids compared to myometria. Abnormal HOXA13 expression was considered an essential factor contributing to the homeotic transformation into a more cervical phenotype which was linked to the development of uterine fibroids (135). MicroRNAs play a significant role in the epigenetic control of uterine fibroid development. Significant alteration in the synthesis profile of regulatory microRNAs of the families let7, miR-21, miR-93, miR-106b, miR-29, and miR-200 has been observed (334, 335). More recently, studies on the role of long non-coding RNAs in the pathogenesis of uterine fibroids have been investigated, further demonstrating the critical role of RNA network in uterine fibroids (332, 336-338). Epigenetic changes in the uterine fibroid genome can activate critical transduction signaling pathways, such as Wnt/β-catenin and Wnt/MAPK (320, 339). More studies focusing on the epigenetic regulation of pathways involved in uterine fibroid pathogenesis can provide potential therapies against this disease.

Inflammation

Inflammation is a protective response from the immune system against foreign agents and involves immune cells and the release of molecular mediators. However, prolonged inflammatory status leaves the body in a constant state of alert and subsequently transforms into chronic inflammation. Chronic inflammation contributes to various conditions, including gynecologic diseases and cancer (340, 341).

Tumor-extrinsic chronic systemic inflammation is caused by many factors, including obesity, tobacco smoking, autoimmune diseases, environmental chemical exposure, vitamin D deficiency, and excessive alcohol consumption (342, 343). In addition, a normal cell can be transformed into a tumorous cell when repeatedly subjected to DNA damage during prolonged inflammatory status, thus triggering tumor-initiating mutations via impaired DNA repair pathways (Fig. 2). Consequently, these genetic events may induce the proliferation, recruitment, and activation of inflammatory cells and increase the production of reactive oxygen species and cytokines, contributing to tumor-intrinsic inflammation (344).

Some epidemiological studies have explored the relationship between uterine fibroids and self-reported diagnosis of reproductive tract infection (RTIs) and inflammation (233, 345, 346). History of pelvic inflammatory disease and chlamydia infection, and intrauterine device use have been positive associated with uterine fibroid risk (233). In contrast, no association between uterine fibroids and self-reported diagnosis of pelvic inflammatory disease in African American and Caucasian women was found. However, a positive correlation with a self-reported history of chlamydia infection in Caucasian women and trichomonas, syphilis, and bacterial vaginosis in African American women has been reported (345). There is no significant association between self-reported RTIs and uterine fibroids. However, adverse associations of chlamydia infection and pelvic inflammatory disease with multiple fibroids have been reported (346). More studies using serology and biochemical characterization of past infections are needed to clarify the associations between RTIs and uterine fibroids. Interestingly, multiple associations between the higher occurrence of uterine fibroid inflammatory mediator gene polymorphisms, including interleukin (IL)-1β (347, 348), IL-4 (349), TNF-α (349, 350), IL-6 (351), IL-10 (348), and IL-12b receptor (352), have been reported.

A chronically active inflammatory immune system is suggested to be involved in uterine fibroid formation (341, 353-355). It is hypothesized that in the uterus of a woman with a chronically inflammatory immune profile (due to chronic low-grade systemic inflammation) suffering a series of insults, including intrauterine infection, injury, and menses, the immune system response is exacerbated, thereby directly or indirectly inducing cell proliferation and fibrosis, which are implicated in the formation and growth of uterine fibroids (354). Numerous studies using molecular strategies and focusing on inflammatory mediators in the context of uterine fibroids have been conducted. They detected the expression of a significant number of cytokines and chemokines with proinflammatory and probiotic features in uterine fibroids (Table 1). Future research employing high-throughput methods and bioinformatic analysis should be conducted.

The characterization of infiltrating and circulating immune cells in women with uterine fibroids has received increasing attention. More tissue-CD68-positive macrophages inside the uterine fibroids and in the surrounding myometrium have been found than in distant myometrium tissues (at least 1.5 cm from the border of fibroids). However, no differences in the number of CD45-positive leukocytes and MCT-positive mast cells have been observed (355). A recent study in a Chinese population demonstrated that the numbers of circulating CD4+CD8+ T cells, regulatory T (Treg, CD4+) cells, and T follicular helper (Tfh) cells was notably increased in patients with uterine fibroids relative to the healthy controls, whereas the numbers of...
natural killer (NK) and delta gamma (γδ) T cells (CD4-CD8–) was reduced (356). Furthermore, the levels of immune check-point PD-L1 proteins were increased in uterine fibroids compared to adjacent myometrial tissues (357). Interestingly, uterine fibroids demonstrated decreased PD-L1 expression and cytotoxic T-cell infiltration compared with malignant leiomyosarcomas, indicating that the immune system behavior spectrum varies among different uterine smooth muscle tumors (358).

The expression of growth factors and cytokines is partly regulated by ovarian steroid hormones (359), critical in uterine fibroids formation and growth (197). The relationship between uterine fibroid development and early-life exposure to xenoestrogen in the context of inflammation was recently revealed in an Eker rat animal model (360). The immune cells and secreted cytokines and chemokines may influence uterine fibroid development and the tumor microenvironment. The myometrial and systemic immune profiles of women with fibroids should be investigated better to understand the role of inflammation linking to epigenome (33) in uterine fibroid pathogenesis.

Role of Vitamin D Pathway

Vitamin D forms a complex with a specific receptor called the vitamin D receptor (VDR) to mediate its pleiotropic functions via steroid transcriptional mechanisms (361). VDR modulates the expression of various genes in a tissue-specific manner. Vitamin D exhibits antiproliferative and pro-apoptotic activities and induces cell differentiation in different diseases (82, 362), including musculoskeletal, infectious, cardiovascular, metabolic, autoimmune, neurocognitive diseases, and cancers. The role and mechanism of vitamin D action in uterine fibroids have been investigated in the past decade. In 2011, the antifibrotic effect of vitamin D was reported, showing that vitamin D suppressed the expression of FEN1, an enzyme involved in DNA damage repair, in a concentration-dependent manner (321). In 2018, low serum vitamin D levels in animals were associated with increased expression of sex steroid receptors and proliferation-related genes, fibrosis, and enhanced inflammation. Vitamin D-deficient diet enhanced DNA damage in murine myometrium (368). Importantly, vitamin D also exhibits anti-inflammatory functions, and the inflammation-induced pathology of uterine fibroids has been described (369). Vitamin D suppressed the uterine fibroid phenotype by targeting different DNA repair networks (370). Recently it was reported that long-lasting and high-dose treatment with vitamin D induced significant lesion volume reduction through reduced cell proliferation via the inhibition of TGF-β3 expression and inducing apoptosis (371). Italian researchers found that vitamin D in women acted as an antiproliferative compound against small uterine fibroids (372) by arresting cell growth and inhibiting the Wnt/β-catenin pathway (322). Vitamin D effectively reduces proliferation and extracellular matrix formation in different molecular subtypes of uterine fibroids (371). In the first randomized study trial, the supplementation of 50 000 IU of vitamin D for 12 weeks inhibited lesion growth, whereas the volume of uterine fibroids in the placebo group increased (373).

Overall, the relationship between vitamin D and uterine fibroid metabolism has been characterized. Recently, our research team proposed a preliminary clinical instruction of 25(OH)D measurements and vitamin D supplementation that may be useful for clinicians involved in treating patients with uterine fibroids (374).

Role of the Microbiome

Foreign microorganisms colonizing our body include bacteria, yeasts, fungi, protozoa, archaea, and viruses; these are collectively referred to as the “microbiome” (375). The cells comprising the microbiome and the host are considered as a single ecological and biological unit, the so-called human halobiont.

Intestinal estrogen metabolism

Gut microbes shape the complex micro-ecological system of the human body (376, 377). Many human
intestinal microbes influence the physiological functions of the host and have profound effects on the synthesis and secretion of hormones, trace elements, growth factors, and immune system function. The intestinal flora and the host have a mutually beneficial relationship and are interdependent (377). The intestinal flora can be modified via hormone interaction both in vitro and in vivo, thus affecting the body’s biological equilibrium (378). Recently, whether the intestinal flora can control the levels of estrogen and its metabolites has been explored. The enzyme glucuronidase in the intestinal flora improved estrogen reabsorption (377, 379, 380). Several bacteria in the intestinal flora can metabolize estrogen, and they are referred to as the estrobolome. High bacterial enzyme activity of the estrobolome elevates the level of free estrogen in the enterohepatic circulation by promoting an endogenous hormonal environment, leading to an increase in hormone levels which may have a direct and indirect impact on the risk of uterine fibroids (381).

The abundance of the intestinal flora closely correlates to systemic estrogen. Many bacteria at the family and species levels control estrogen content, particularly Clostridium and Pneumococcus exhibit the most significant effect on estrogen metabolism (377, 382). Reduced estrogen levels have been related to decreased intestinal flora diversity, including the phylum Bacteroidetes, and increased abundance of the phylum Firmicutes and species diversity of the phylum Proteobacteria (377, 383). In addition, it has been shown that elevated levels of estrogen and its metabolites have related to the abundance of several taxa in the class Clostridia, including the order Clostridiales and the family Ruminococcaceae (384).

Wang et al have studied the effect of hysterectomy on the intestinal flora diversity, ie, the number of different species present, in patients with uterine fibroids (377). The top 3 dominant bacteria, Bacteroidetes, Proteobacteria, and Firmicutes, were the same in preoperative and postoperative patients at the phylum level. However, the enrichment of Bacteroidetes in postoperative patients was significantly lower than in preoperative patients, whereas the abundance of Proteobacteria was significantly higher. Although at the class level, Gammaproteobacteria, Bacteroidia, and Clostridia predominated in both groups, the author found a statistically higher abundance of Gammaproteobacteria (Proteobacteria) in patients after the surgery. Specifically, at the order level, the higher average abundance observed was in Enterobacteriales (377). Wang’s study reported that estrogen levels in the body could alter the intestinal flora; however, the underlying mechanism of this regulation remains unknown (377). Deeper analysis may help to understand whether gut microbiota could influence the risk of uterine fibroids through effects on endogenous estrogens.

Female reproductive tract sterility and uterine fibroid microbiome

For many years, the female reproductive tract was considered a sterile organ (30, 385, 386). Culture-based technologies were usually utilized to detect bacterial resistance; however, they have limited applications as many microorganisms are difficult to culture in vitro. With the launch of the Human Microbiome Project in 2007, modern sequencing technologies using taxonomy-associated marker genes, such as the 16s rRNA gene or whole-genome sequences, have been used to distinguish bacteria at the species level (387). In addition, body sites that were historically supposed to be sterile are colonized by microorganisms (386, 388). Specifically, the Human Microbiome Project reported that the uterine cavity harbors a unique microbiome (30, 386, 389). However, the specific function of the uterine microbiome remains unknown (385, 389).

A pilot study evaluated the distribution of bacteria in African American women with uterine fibroids. The authors evaluated the bacterial diversity on the endometrium, adjacent myometrium, and uterine fibroids and compared them with samples from healthy controls. A greater diversity was found in the uterus fibroid patient samples compared with controls. In addition, uterine fibroid patients showed a significantly increased abundance of Bacteroidetes, Firmicutes, and Actinobacteria in the endometrium. At the general level, uterine fibroid tissues were significantly enriched in Clostridium, Allobaculum, Anaerostipes, Odoribacter, Turicibacter, Oscillibacter, Ruminococcus, and Coprococcus, which are commonly associated with the gut environment. These results suggest that the systemic distribution of the gut bacteria extends to the uterus of patients with uterine fibroids, following dysbiosis or gut-barrier impairment (390). Further studies are needed to better understand whether the uterine microbiome play a role in the pathogenesis of uterine fibroids.

Conducting investigations using human tissue is critical for deciphering many normal and pathogenic processes and developing disease diagnosis techniques and future therapies. However, it could be a challenge to state the significance of the findings since uterine fibroid samples are obtained at a specific time point, at which limited information about biological samples is available. For example, whether the uterine fibroids are growing or shrinking at the time of sample collection is a relevant question. In addition, the heterogeneity of uterine fibroids also represents structural properties and collagen content changes (391), genetic (9), epigenetic (194, 392), and cell type variations. Furthermore, due to the paracrine and mechanical effect of
Uterine fibroids, the manner of collecting the myometrium is a significant factor. Notably, the biology of myometrium from the uterus with fibroids or without fibroids differs (393, 394). For these reasons and more, uterine fibroid studies need to be carefully designed and should take these factors into consideration. When working with samples, it is crucial to consider multiple factors, including race, age, BMI, menstrual cycle phase, driver mutations, to draw concise conclusions.

Integration of Multiple Pathways in Uterine Fibroids

It is important to emphasize that during the initiation and development of uterine fibroids, many biological events coincide, and multiple abnormal pathways interact, contributing to the pathogenesis of uterine fibroids. For instance, estrogen signaling activated the β-catenin pathway via the estrogen/receptor axis, therefore induced β-catenin nuclear translocation and enhanced β-catenin responsive gene expression in human uterine fibroids cells. Vitamin D3 has been associated with inhibiting the Wnt/β-catenin, proinflammatory pathways, and ECM deposition in uterine fibroid cells (395) and restored the DNA repair capacity in both uterine fibroid cells and at-risk MMSCs (370, 396). Diet-induced vitamin D deficiency triggered inflammation and DNA damage in murine myometrium (368). Moreover, VDR-knockdown in normal human myometrial cells increased DNA damage and inhibited DNA repair capacity (370). These studies suggested the interaction of the vitamin D3/receptor pathway with β-catenin and DNA repair pathway. Activator protein 1 (AP1) is a family of transcription factors consisting of FOS and JUN members that form homodimers or heterodimers involved in many biological processes, including differentiation, proliferation, apoptosis, and fibrotic diseases. Recent studies demonstrated that API signaling promotes ECM deposition (397), and ECM activated β-catenin signaling in uterine fibroids (317). In addition, uterine fibroids expressed higher Class I HDAC enzyme levels than normal myometrial tissues, associated with activated β-catenin signaling. (320). Notably, MED12 mutations have been linked to multiple pathways directly implicated in uterine fibroid pathology as described (7, 134, 137, 174, 175). Considering that diverse pathways play a role in the pathogenesis of uterine fibroids (Fig. 5), additional studies will be necessary to identify the molecular mechanism underlying the interaction network and signaling in uterine fibroids.

Malignant Transformation of Uterine Fibroids

Uterine leiomyosarcoma is a rare, aggressive, malignant tumor, which originates from the smooth muscle layer in the uterus and shares many common clinical grounds with uterine fibroids (398). Approximately 6 out of every 1,000,000 women are diagnosed with uterine leiomyosarcoma yearly. Unfortunately, its prognosis is poor, with the lowest survival rates among soft-tissue sarcomas. Uterine leiomyosarcoma represents ~1% of all uterine malignancies, and the average age of patients ranges from 40 to 50 years. Uterine leiomyosarcoma mainly metastasizes to the lungs, liver, brain, kidney, and bones (399).

Characteristics Common Between Uterine Fibroids and Leiomyosarcoma

Notably, uterine leiomyosarcomas and uterine fibroids share several common clinicopathological features that complicate their differential diagnosis. First, both tumors arise from the myometrium as focal masses within the uterine wall. Second, abnormal uterine bleeding, pelvic pain/pressure, and a pelvic mass are the primary presenting signs and symptoms for both uterine leiomyosarcoma and uterine fibroids, making it difficult to differentiate between them (400-403). Third, uterine leiomyosarcoma and fibroids share morphological and molecular characteristics that cannot be differentiated through current clinical diagnostic tests. Finally, it is said that the Black women are at higher risk for both uterine fibroids and leiomyosarcoma (404, 405). Because suspected uterine fibroids are often conservatively managed or with minimally invasive treatments, the misdiagnosis of leiomyosarcoma for a benign uterine fibroid could potentially result in significant treatment delays, increasing patient morbidity and mortality (406).

Malignant Transformation of Uterine Fibroids to Leiomyosarcomas

It has been debated whether uterine fibroids and uterine leiomyosarcoma are part of a disease continuum. Genetic studies have demonstrated that uterine leiomyosarcomas arise de novo and may be unrelated to benign fibroids. Uterine leiomyosarcoma typically has complex karyotypes and aneuploids, while uterine fibroids have characteristic rearrangements, many of which are shared by other benign neoplasms. Intermediate forms between these 2 patterns had not been described. However, recent microarray data identified a rare subset of uterine fibroids with deletions of chromosome 1 that have transcriptional profiles that cluster with those of uterine leiomyosarcoma, suggesting that some rare leiomyosarcomas may arise from a specific subset of uterine fibroids (407). Moreover, the observation that up to 4% to 30% of uterine leiomyosarcomas harbor exon 2 mutations in the established fibroids driver gene MED12 suggests that a subset of malignant leiomyosarcomas may derive from benign uterine fibroids (119, 408, 409). The discrepancy in the frequency of uterine fibroids and uterine leiomyosarcoma lies in the fact that only rare histological and karyotypic variants of fibroids may be responsive.
to malignant progression (410). Notably, a large number of random chromosomal aberrations in uterine leiomyosarcoma suggests that genomic instability is involved in the pathogenesis of these tumors, and such instability hinders efforts to identify the primary change(s) that may now be discovered through studies of variant uterine fibroids (411).

Novel therapeutic advances for the aggressive type of uterine cancer leiomyosarcoma are hindered by the overall lack of knowledge about the functional consequences of the complex genomic and pathway alterations found in patients and limited characterization of tumor biology. The employment of new models together with advanced technologies such as single-cell sequencing, mass cytometry, and other high-throughput approaches will ultimately help to characterize the mechanisms of its pathogenesis, resistance to conventional treatments and develop novel options for treating the patients with this aggressive disease.

![Figure 6. Future research prospects. Studies elucidating the interplay among genes, epigenome, and epitranscriptome in the context of stem cell biology, microbiome, and the interaction between the endometrium and uterine fibroids can advance the knowledge on the pathogenesis of uterine fibroids and are expected to contribute to developing novel therapeutic approaches for the treatment of patients with uterine fibroids.](https://academic.oup.com/endrev/advance-article/doi/10.1210/endrev/bnab039/6422392)
Future Perspectives

Despite substantial progress in our understanding of the pathobiology of uterine fibroids, several gaps need to be addressed (Fig. 6).

Transcriptome and Epigenome Analysis

The early-life environment dramatically affects the functions of developing organs and increases disease susceptibility across the lifespan. In a rat model, early-life exposure to DES affected several signaling pathways, including estrogen signaling, and reprogrammed histone mark H3K4me3 and DNA methylation patterns in MMSCs derived from the developing myometrium, thus accelerating the acquisition of an adult epigenomic signature. Furthermore, this epigenomic reprogramming persisted long after the initial exposure. These findings demonstrate the importance of the epigenome, that is, early interactions between the gene and environment reprogram the epigenome, and interactions in adulthood accelerate or activate restricted epigenetic reprogramming to increase genome instability and initiate fibroids pathogenesis. Omics studies also demonstrated that uterine fibroids contain disrupted epigenome linked with genetic stability (135, 412). Notably, the myometrium of uterine fibroids patients has a distinct transcriptomic pattern compared with non-diseased myometrium (394), and cells from diseased myometrium responded differently to progesterone (413). These studies suggest that adjacent myometrium from the uterus with uterine fibroids should not be considered as normal tissue.

To further elucidate the role of interaction among the genes, epigenome, and environment in process of uterine fibroid pathogenesis, the following issues should be addressed. First, the genetic landscapes of normal, at-risk, and uterine fibroids stem cells should be characterized using genome-wide omics methods to better understand the genome, epigenome, and epitranscriptome. Second, the other insults that cause the abnormal reprogramming of MMSCs should be identified. Lastly, the interaction between early hits in early life and late hits in adult life should be explored in the context of uterine fibroid development.

Recently, the rapid development of single-cell multomics approaches is transforming our understanding of disease initiation and progression. Therefore, they can be utilized to assess the transcriptome, epigenome, spatial profiling, and proteome of uterine fibroids, contributing to increased knowledge on the cell state, ontogeny, epigenome state, phenotype, and function of uterine fibroids (Fig. 6).

Eptitranscriptomics

The epitranscriptome refers to the complete ensemble of chemical modifications affecting the RNA transcripts (coding and non-coding RNAs), and epitranscriptomics is an emerging field in molecular medicine with vast potential. To date, more than 160 different chemical modifications in RNA have been identified in living organisms. N6-methyladenosine (m^6A) is the most pervasive, abundant, and conserved in eukaryotic mRNAs, occurring in ~25% of transcripts genome-wide, and it is enriched near stop codons, 5' and 3'-untranslated regions, and within long internal exons (414, 415). m^6A is co-transcriptionally incorporated by so-called writers, including the METTL3-METTL14 core methyltransferase complex and associated proteins, such as RBM15, WTPA, and VIRMA, that confer target mRNA specificity, eliminated by demethylases FTO and ALKBH5 (erasers), and recognized by readers, including the YTH protein family (Fig. 6). m^6A-bound readers ultimately determine the posttranscriptional fate of methylated mRNAs by modulating cellular activities that control RNA stability, processing, and translation. m^6A is thus a pervasive regulator of gene expression and a key determinant of cell fate and function. Accordingly, the disruption of its homeostasis has been implicated in several pathological conditions, including cancer. Thus far, epitranscriptomic studies on uterine fibroids are mainly unknown. However, several key m^6A regulators were recently revealed to be dysregulated in uterine fibroids, suggesting that epitranscriptomics play an important role in uterine fibroid pathogenesis (416, 417). However, more studies are needed to explore the function of the RNA methylation machinery and m^6A in uterine fibroid development. Recently, a potent and selective catalytic inhibitor of METTL3 was developed and can reduce acute myeloid leukemia growth and increase differentiation and apoptosis, suggesting that targeting METTL3 as a potential therapeutic strategy against diseases such as acute myeloid leukemia. Therefore, investigations on epitranscriptomic targeting uterine fibroids should be conducted.

Endometrium Receptivity and Heavy Menstrual Bleeding

Uterine fibroids cause female reproductive disorders, including heavy menstrual bleeding and poor receptivity and implantation, leading to infertility and affecting millions of women globally. These disorders indicate endometrial dysfunctions and are related to the presence of adjacent uterine fibroids. Remarkably, the degree of dysfunction is associated with the location and size of the uterine fibroids.

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(418). However, how fibroids affect endometrial functions remains unclear.

Uterine fibroid-caused expansion in the endometrial surface area generally results in more significant menstrual bleeding and transformations in the shape of uterine cells, thus eventually affecting gene expression and function. Furthermore, recent studies demonstrated that uterine fibroids could actively influence the adjacent endometrium and the entire uterine cavity (419). Therefore, elucidating the mechanism underlying the effect of uterine fibroids on the endometrium is necessary. In addition, the impact of uterine fibroids on endometrium function via exosome is a promising area to be explored (266, 420) (Fig. 6).

**Mycobiome**

The impact of alterations in the endometrial microbiome on uterine fibroid pathogenesis has not been investigated. The paradigm that the uterus is a sterile environment remains highly controversial. The vagina contains trillions of bacterial cells, whereas the uterus and the Fallopian tubes are generally considered sterile. As alterations in the gut microbiota can lead to several pathologies, such as inflammation, autoimmune diseases, and obesity, it is proposed that the uterine microbiota may be altered in patients with uterine fibroids. A diverse microbiota has been detected in fibroids relative to other uterine tissues and those from healthy controls. In addition, uterine fibroids may associate with local and systemic inflammation that may promote the translocation of the gut microbiota into the endometrium. Future studies should explore the composition and identify the role of the microbiome in uterine fibroids. How bioactive metabolites from the microbiome provide the constituents of the fibroids microenvironment and affect the surrounding tissues should also be investigated (Fig. 6).

**Leiomyosarcoma**

Although the current standard practice has been used to differentiate aggressive leiomyosarcoma from benign uterine fibroids, further studies are needed to explore early diagnosis tools to distinguish these 2 uterine tumors. Currently, several approaches are employed in basic research, which may lead to future clinical practice options.

**Promoter-based imaging system**

Uterine leiomyosarcoma is most often discovered by chance when a woman has a hysterectomy performed for uterine fibroids. To differentiate uterine leiomyosarcomas from fibroids with imaging, a molecular bio-imaging probe for noninvasive differentiation approach was recently established in a preclinical animal model (398). The diagnostic strategy was to use the cancer-specific enhanced survivin expression in malignant vs normal/benign cells to test its promoter driving potential of a downstream reporter gene that will detect cancer cells once activated. Because of solid promoter activity, tumor specificity, and capacity for clinical translation, survivin promoter-driven biological signal may represent a practical, new system to facilitate early leiomyosarcomas diagnosis. One study reported that adenovirus (Ad-SUR-LUC) was injected into the animals and paired the imaging reporter gene with a complementary imaging agent in a system that can be used to measure bioluminescence driven by the surviving promoter. This approach could distinguish leiomyosarcoma lesions from normal uterine tissue or benign uterine fibroid lesions with good accuracy. In this regard, this system can impact the management of suspicious uterine masses, a current major challenge in clinical gynecology.

**Genetic and transcriptome profiling**

In recent years, high-throughput sequencing technologies have provided unprecedented opportunities to depict the development of diseases at multiple molecular levels. The integration and analysis of these multi-omics datasets allow us to yield a better understanding and a clearer picture of the under-studied systems (421, 422). Through integrated comparative genomic and transcriptomic analysis, differential genetic targets between uterine leiomyosarcoma and fibroids have been identified (423, 424). In leiomyosarcomas, genetic mutation burden exhibited higher copy number variations, single nucleotide variants, small insertions/deletions, and gene fusions compared with uterine fibroids. Comparative genomic hybridization (CGH) array analysis reveals a chromosomal and genomic complexity starting from uterine fibroids, with few chromosomal alterations, to uterine leiomyosarcoma, which harbors many intrachromosomal damages, and chromothripsis as evidence of their genomic complexity (116, 424, 425). In addition, a differential transcriptomic profile was observed for uterine leiomyosarcomas (423). These novel genetic and transcriptional targets may be potential diagnostic markers to differentiate uterine leiomyosarcoma from fibroids (423, 426).

**Aberrant specific pathways**

The Hedgehog pathway is one of the key regulators involved in many biological events. Malfunction of this pathway is associated with various diseases, including several types of female cancers (427). An initial study demonstrated that elevated expression of SMO and GLI 1, the key members of the Hedgehog pathway, was observed in leiomyosarcoma relative to normal myometrium and uterine fibroid tumors. In addition, overexpression of Hedgehog proteins was correlated with poor prognosis in
leiomyosarcomas patients (428). A subsequent study demonstrated that the expression profile of Hedgehog signaling pathway markers and the response to Hedgehog pharmacological inhibition differed between leiomyosarcoma cells and fibroids cells (429). The expression of crucial Hedgehog members SMO and GLIs 1, 2, and 3 was upregulated in leiomyosarcoma cells, increasing the nuclear levels of GLI proteins. Treatment with LDE225 (SMO inhibitor) and Gant61 (GLI inhibitor) resulted in a significant reduction in GLI protein levels in leiomyosarcoma concomitantly with a decrease in leiomyosarcoma cell proliferation, migration, and invasion (429).

Additionally, the expression of DNMT members (1, 3a, and 3b) was upregulated in leiomyosarcoma compared to normal uterine smooth muscle cells. Treatment of leiomyosarcoma cells with DNMT inhibitor (5-Aza-dC) decreased the expression of SMO and GLI1 as well as nuclear translocation of GLI1 and 2 concomitantly with a decrease in leiomyosarcoma cell proliferation, migration, and increase in leiomyosarcoma cell apoptosis (429). These studies showed that the Hedgehog pathway and DNA methylation network and their interaction might play an essential role in uterine leiomyosarcoma development.

Targeted Therapeutics

Targeted therapy exclusively focuses on uterine fibroid cells to shrink tumor lesions with minimal insult to surrounding tissues, not interfering with systemic hormones or fertility. Targeted therapies have been proposed via the local injection of collagenase from Clostridium histolyticum and gene therapy to deliver designed viral vectors. Collagenase can dissolve disorganized extracellular collagen fibers in uterine fibroids, and proof-of-principle studies have significantly reduced uterine fibroid size (430, 431). Moreover, a phase I clinical trial with 15 women demonstrated the safety and tolerability of collagenase derived from C. histolyticum (NCT02889848). Localized targeted strategy via modified adenovirus vectors (432, 433) resulted in reduced tumor size and showed the absence of adenovirus in surrounding tissues, uterine fibroid-targeting specificity, and good safety profile. Adenovirus expressing dominantly negative ERs has also been used, and inhibition of fibroid growth in nude mice was observed (434). Magnetic nanoparticles can enhance the effectiveness of gene therapy against both differentiated human fibroid cells and tumor-initiating stem cells (435). Using localized nonsurgical adenovirus-based alternative for the treatment of uterine fibroids, the combination of viral-based gene delivery and nanotechnology led to more efficient targeting of fibroid tumors, the lower viral dose required, and consequently, an overall safer profile (435). Novel targeted therapies against uterine fibroids with better efficacy profile are needed, especially for African American women.

In summary, considerable progress has been made in the past decade to study the interplay of steroid hormones, risk factors, stem cells, genetics, and epigenetics that contribute to the developmental origin and pathogenesis of uterine fibroids. Deeper mechanistic insights into uterine fibroids’ etiology and complexity will lead to long-term, fertility-friendly, and effective drugs for preventing patients with this common tumor.

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