Genetics and Genomics of Uterine Fibroids

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Hiroshi Ishikawa and Makio Shozu

Abstract

Uterine fibroids are benign smooth muscle tumors of monoclonal origin that arise from the uterus. African-American women have a higher risk of developing the disease than do Caucasian women, and a family history of uterine fibroids is a risk factor for their development. The relative risk for uterine fibroids is significantly higher in monozygotic twins than in dizygotic twins, suggesting a correlation of the disease susceptibility with the patient's genetic background. Chromosomal abnormalities are observed in approximately 40% of cases, where nonrandom and tumor-specific chromosomal abnormalities caused by chromosomal rearrangements affect alterations in the driver genes of uterine fibroids, such as high-mobility group AT-hook 2 (*HMGA2*) overexpression. Hereditary leiomyomatosis and renal cell cancer are caused by biallelic inactivation of the fumarase hydratase (*FH*) gene. Alport syndrome associated with diffuse leiomyomatosis is caused by deletions of collagen type IV alpha 5 chain (*COL4A5*) and alpha 6 chain (*COL4A6*). Somatic alterations of these genes are also observed in non-syndromic uterine fibroids. Whole-genome sequencing (WGS) revealed that approximately 70% of uterine fibroids have somatic mutations of Mediator complex 12 (*MED12*), which is the most frequently observed driver gene alteration in these tumors. Through WGS, uterine fibroids have been categorized into at least four subgroups according to the types of driver gene alterations: *MED12* mutation, *HMGA2* overexpression, biallelic *FH* inactivation, and *COL4A5* and *COL4A6* deletions. Each alteration is mutually exclusive in the fibroid nodule. In addition, the role of microRNAs in the development of uterine fibroids is extensively examined.

H. Ishikawa, M.D., Ph.D. (✉) · M. Shozu, M.D, Ph.D.
Department of Reproductive Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan
e-mail: ishikawa@chiba-u.jp

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2.1 Introduction

Uterine fibroids, also called uterine leiomyomas, are benign smooth muscle tumors that arise from the uterus. Uterine fibroids show sex-steroid-dependent growth, and typically become symptomatic during the reproductive age. Epidemiological studies indicate that the susceptibility to uterine fibroids depends on the ethnicity of the woman. Approximately 40% of uterine fibroids have an abnormal karyotype, and gene alterations associated with chromosomal rearrangements are also related to the disease's pathogenesis. Furthermore, driver gene alterations are frequently observed in karyotypically normal fibroids. Women with hereditary syndromes who have a germ-line mutation of some specific genes have multiple uterine fibroids, implicating the important role of genetic factors in the disease's development. This chapter focuses on the genetic alterations and genomic variations and their significance in the pathophysiology of uterine fibroids.

2.2 Genetic Backgrounds

Studies have suggested an ethnic difference in the susceptibility to uterine fibroids. Epidemiological studies have shown that African-American women have a significantly higher (two to three-fold) relative risk for uterine fibroids than do Caucasian women in the United States [1]. In addition, fibroids in African-American women are diagnosed at an earlier age and are more symptomatic and larger than those in Caucasian women [2]. Although the difference in fibroid prevalence between African-American and ethnicities other than Caucasian (e.g., Hispanics and Asians) is still controversial, its high prevalence in African-American women suggests a correlation with the patient's genetic background.

A family history of uterine fibroids is another risk factor for their development. A case-control study revealed that both a maternal history of uterine fibroids and reduced parity are significant risk factors for the disease in Caucasian women [3]. First-degree relatives of an affected proband have a 2.2–2.5-fold higher risk of developing uterine fibroids, and the odds ratio increases to 5.7 after selecting for early onset cases [4, 5].

Twin cohort studies further support the relationship between genetic background and uterine fibroid susceptibility. In a Finnish study of monozygotic and dizygotic twin pairs, the relative risk for the disease was significantly higher in the monozygotic twins [6]. The relative risk of hysterectomy due to uterine fibroids was also higher in monozygotic twins than in dizygotic twins in a United Kingdom twin study [7]. Monozygotic twins are identical in terms of genetic background compared with dizygotic twins. These data again suggest the role of genetic factors in the development of uterine fibroids.

2.3 Clonality

Clonality analysis has shown that a uterine fibroid is a monoclonal tumor, where each fibroid nodule is derived from a single progenitor myocyte due to somatic mutation; therefore, each fibroid results from an independent clonal event. Within the same uterus, each fibroid nodule shows a specific inactivation pattern of X chromosome-linked genes, such as glucose-6-phosphate dehydrogenase (*G6PD*), phosphoglycerate kinase (*PGK*), and androgen receptor (*AR*) genes [8–11]. The methylation status of these genes is different among fibroid nodules. Thus, different nodules in multiple fibroids are of different cytogenetic origins [12].

2.4 Genome-Wide Association Study

Genome-wide association study (GWAS) is a powerful tool for identifying common genetic variants associated with specific disorders. A case-control GWAS of Japanese women revealed significant associations between chromosomal loci (at the chromosome 10q24.33, 22q13.1, and 11p15.5 regions) and uterine fibroids [13]. Another GWAS on a US and Australian cohort of Caucasian women revealed one single nucleotide polymorphism (SNP), located on chromosome 17q25.3, to be significantly associated with uterine fibroid risk [14]. This locus was located near the fatty acid synthase (*FASN*) gene, and FAS protein levels were significantly upregulated in fibroid tissue compared with those in matched myometrial tissue. This implicates *FASN* as a candidate gene in the predisposition to uterine fibroids in Caucasian women.

On the other hand, the same SNP was not associated with uterine fibroid risk in African-American women. A GWAS in the Black Women's Health Study, consisting of a cohort of 59,000 African-American women, failed to replicate GWAS findings on uterine fibroids in Japanese women [15]. There might be multiple loci in the genome with relatively small effects that contribute to the increased risk of uterine fibroids in African-American women, since ethnicity may be associated with a genetic predisposition to these tumors.

2.5 Chromosomal Rearrangements

Approximately 40% of uterine fibroids have nonrandom and tumor-specific chromosome abnormalities, including deletion of portions of 7q or trisomy 12, or rearrangements of 12q14-15, 6p21, or 10q22 [16]. Rearrangements of chromosomes X, 1, 3, and 13 have also been identified in fibroid nodules [4, 17].

In addition to the simple chromosomal aberrations leading to the affected single gene mutations, complex chromosomal rearrangements (CCRs), which lead to simultaneous multiple chromosomal rearrangements, have been identified in uterine fibroids [18, 19]. Whole-genome sequencing (WGS) of each fibroid nodule revealed that CCRs resembling chromothripsis (a single genomic event that results in focal losses and rearrangements in multiple genomic regions) are a major cause of

chromosomal abnormalities in uterine fibroids [20]. The CCR may allow tumor-promoting genetic changes, which can impair the control of cell-cell checkpoints and the repair of DNA double-strand breaks, such as translocations of the high-mobility group AT-hook 2 (*HMGA2*) and DNA repair protein RAD51 homolog B (*RAD51B*) loci [21].

One of the well-known chromosome rearrangements observed in women with uterine fibroids is t(12;14)(q14-q15; q23-q24), involving the overexpression of *HMGA2* [22]. The presence of t(12;14) is often associated with fibroids of larger size than those with either normal karyotypes or interstitial 7q22 deletions. In fact, *HMGA2* overexpression is observed in large fibroids [23]. This translocation allows fusion transcripts of *RAD51B* and *HMGA2*. *RAD51B*, a member of the RAD51 recombination gene family, is located on chromosome 14q24 and is the most frequent translocation partner of *HMGA* rearrangements. *RAD51B* encodes a protein involved in DNA double-strand break repair by homologous recombination [24, 25].

In other subgroups of uterine fibroids, rearrangements of 6p21 have been observed that lead to an overexpression of *HMGA1*, another high-mobility group AT-hook gene [26]. Thus, aberrant expression of HMG family genes due to chromosomal rearrangements may contribute to the pathogenesis of these tumors [27]. Deletion and translocation of chromosome 7 (i.e., del(7)(q22q32) and t(1;7)(q42;q22)) are other frequently observed chromosomal rearrangements in uterine fibroids [16, 28, 29]. The fact that del(7) is often observed as a sole change indicates that the loss of a tumor suppressor gene may be the most likely pathogenic mechanism in this subgroup, with deletion of the expression of specific genes, including the proliferation inhibitor HMG-box transcription factor 1 (*HBP1*), and the mitosis integrity-maintenance tumor suppressor RAD50 interactor 1 (*RINT1*) [16]. Rearrangement of 10q22 allows disruption of a histone acetyltransferase gene, monocytic leukemia zinc finger protein-related factor (*MORF*), in uterine fibroids. Similarly, rearrangement of 17q21 allows disruption of another gene with histone acetyltransferase activity, lysine acetyltransferase 2A (*KAT2A*) [30]. These chromosomal rearrangements observed in each fibroid nodule lead to aberrant expression of specific genes and are related to the pathophysiology of uterine fibroids.

2.6 Syndrome-Associated Fibroids

Women with hereditary syndromes caused by germ-line mutations of specific genes tend to have multiple uterine fibroids or leiomyomatosis. Hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome is an autosomal dominant inherited tumor predisposition syndrome characterized by multiple cutaneous fibroids, uterine fibroids, and renal cell cancer [31]. Women with HLRCC have a heterozygous germline mutation of fumarate hydratase (*FH*) at 1q43. This fumarase enzyme catalyzes the hydration of fumarate to L-malate in the tricarboxylic acid cycle. Biallelic inactivation of *FH* through loss of heterozygosity or an inactivating mutation in the wild-type allele causes a driver alteration of fibroids. Uterine fibroids are present in almost all women with HLRCC. The fibroid nodules become multiple and large, and most

women experience heavy menstruation and pelvic pain [32]. Compared with sporadic fibroids, HLRCC-associated fibroids are detected in younger women, where the mean age at diagnosis is ~30 years [31]. In a comprehensive series of HLRCC-associated uterine fibroids, 7.8% of the fibroids had somatic Mediator complex 12 (*MED12*) mutations. However, these fibroids have different immunoreactivities for 2-succinyl cysteine that affect the accumulation of fumarate compared with *FH*-altered fibroids; therefore, fibroids with *MED12* mutations are distinct from the syndrome-associated fibroids with biallelic inactivation of the *FH* gene [33].

Alport syndrome (AS), a hereditary syndrome characterizing progressive renal failure with hematuria, eye disorder, and high-tone sensorineural hearing loss, arises from mutations in genes coding for basement membrane type IV collagen. Diffuse leiomyomatosis is observed in the esophagus, tracheobronchial tree, and genital reproductive tract in women with diffuse leiomyomatosis-associated AS, a rare subtype of AS due to germ-line mutations in collagen type IV alpha 5 chain (*COL4A5*) and alpha 6 chain (*COL4A6*) [34, 35].

2.7 Genetic Driver Alterations of Uterine Fibroids

As stated above, uterine fibroids are monoclonal tumors, where each fibroid nodule has a distinct character of single myocyte origin. Chromosomal rearrangements cause specific driver gene alterations of uterine fibroids, where each alteration independently occurs in each fibroid nodule. WGS has revealed that the major somatic gene alterations related to fibroid formation are *MED12* mutations and *HMGA2* overexpression. Other alterations are biallelic inactivation of *FH* and deletions in *COL4A5* and *COL4A6*. These alteration events occur in an independent manner and are mutually exclusive in uterine fibroids, with some exceptions in syndrome-associated fibroids [33, 36, 37].

2.7.1 *MED12* Mutations

Somatic mutations of *MED12* in uterine fibroids were initially reported by Mäkinen et al. in 2011 [38], where surprisingly they occurred in approximately 70% of fibroids in Caucasian women, as revealed by WGS. Since then, different researchers worldwide have identified this mutation in 50–70% of uterine fibroids beyond ethnic and country differentials [39, 40].

MED12 is located on chromosome Xq13 and encodes a 250-kDa protein that is involved in transcriptional regulation of the RNA polymerase II complex. The MED12 protein is a component of a subcomplex of the large Mediator complex, namely, the cyclin-dependent kinase 8 (CDK8) module composed of CDK8, cyclin-C (CCNC), MED12, and MED13 [41]. In *MED12* mutation-negative uterine fibroids, no somatic mutations in the coding regions of *CDK8*, *CCNC*, or *MED13* have been observed, suggesting that mutations in other CDK8 submodule genes do not contribute to the disease pathogenesis [42].

There is a hot spot of *MED12* mutations on exon 2 in uterine fibroids, the most common being c.131G > A. Other types of point mutations have been identified on exon 2 and intron 1 [43, 44]. The region of the gene that is most frequently evolutionarily conserved is located on exon 2 and at the intron 1–exon 2 junction. Both missense and in-frame insertion-deletion mutations were observed, with a notable predominance of single-base substitutions in codon 44 [44].

The frequency of *MED12* mutations in histopathological variants of uterine fibroids and uterine leiomyosarcoma has been demonstrated. Typical fibroids have a high mutation frequency of *MED12*, whereas this is less frequently observed in histopathological fibroid variants, including cellular leiomyoma and smooth muscle tumor of uncertain malignant potential [45–47]. *MED12* mutations in leiomyosarcoma are rare, and the most common variant c.130G > A in exon2, which is observed in typical fibroids, has never been identified in this fibroid variant [43, 48], indicating the genetic heterogeneity of uterine smooth muscle tumors.

Because of the high prevalence of *MED12* mutations in uterine fibroids, the mode of action of this gene in the disease's development and pathogenesis has been extensively studied. Recently, the role of *Med12* mutation in fibroid development was identified using a mouse model. The common *MED12* variant c.131G > A can drive tumor formation alone in a gain-of-function manner and cause genomic instability. Whereas conditional loss of function of *Med12* did not lead to uterine fibroids in mice, expression of the *Med12* c.131G > A variant on a background of conditional *Med12* knockout did [49]. In these mice, 80% of the uteri contained lesions consistent with fibroids, including extracellular matrix (ECM) deposits, fibroblast and macrophage infiltrations, and disorganized muscle fiber arrangement. Moreover, the *Med12* c.131G > A variant caused uterine fibroids in mice with a wild-type background, where approximately 50% of the uteri from these mice developed fibroid-like lesions consisting of ECM deposition and disorganized smooth muscle fiber arrangement. The authors concluded that the *Med12* missense c.131G > A variant acts as a gain-of-function mutation and is related to genomic instability in the fibroid-like lesions, with copy number gains and losses.

The role of *Med12* in fibroid cell proliferation through direct interaction with the Wnt/β-catenin and associated signaling pathways has been reported [50]. The proliferation of *Med12* knockdown immortalized uterine fibroid cells was significantly inhibited compared with that of scrambled control cells. Silencing of *Med12* in these cells showed significantly reduced levels of Wnt4 and β-catenin proteins, cell cycle-associated proteins, and transforming growth factor-β-regulated fibrosis-related proteins, indicating that *Med12* plays a crucial role in fibroid cell proliferation via the Wnt/β-catenin signaling pathway.

2.7.2 *HMGA2* Overexpression

Overexpression of *HMGA2* is found in 7.5–10% of uterine fibroids. *HMGA2* is located at 12q14.3, and chromosomal rearrangements involving 12q14-15 result in *HMGA2* overexpression in affected uterine fibroids. Expression of the *HMGA2* transcript is significantly upregulated in fibroid tissue with 12q14-15

rearrangements, compared with that in normal karyotype fibroids [23, 51]. *HMGA2* is a member of the high-mobility group gene family and encodes nonhistone components of chromatin that act as architectural factors to influence diverse cellular processes, including differentiation, death, growth, and proliferation.

Although little is known about the underlying mechanisms through which *HMGA2* overexpression leads to fibroid development, the overexpression of this gene is associated with large fibroid size, suggesting that HMGA2 promotes fibroid growth [52]. Expression of both *HMGA2* and fibroblast growth factor 2 (*FGF2*) has a significant positive correlation with the affected chromosomal rearrangements in uterine fibroids [53]. Stimulation of myometrial tissue by *FGF1*, a strong inducer of *HMGA2*, leads to an increase of *HMGA2* and *FGF2*, suggesting that overexpression of *HMGA2* upregulates *FGF2* expression in fibroid tissue.

HMGA2 is a predicted target of let-7 microRNAs (let-7s), which are significantly dysregulated in uterine fibroids [54]. High levels of let-7 and low levels of *HMGA2* expression in small fibroids and low levels of let-7 and high levels of *HMGA2* expression in large fibroids have been elucidated. Furthermore, exogenous let-7s directly repressed *HMGA2* transcripts in cultured fibroid cells, suggesting that let-7-mediated repression of *HMGA2* may play an important role in fibroid growth [55].

A recent WGS study revealed uniquely expressed genes in the *HMGA2*-overexpressing fibroids [37]. *HMGA2* itself, insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*), and cyclin D2 (*CCND2*) were the top three most uniquely expressed genes. Expression of the proto-oncogene pleomorphic adenoma gene 1 (*PLAG1*) was significantly upregulated in fibroids with *HMGA2* aberrations, suggesting that HMGA2 promotes fibroid tumorigenesis through PLAG1 activation.

2.7.3 Biallelic *FH* Inactivation

FH is an enzyme that catalyzes the reversible hydration/dehydration of fumarate to L-malate in the tricarboxylic acid cycle. Germ-line mutations in the *FH* gene encoding fumarase, at chromosome 1q43, cause biallelic *FH* inactivation in HLRCC, and women with this condition have multiple uterine fibroids. Although FH deficiency through biallelic inactivation of *FH* also occurs in non-HLRCC uterine fibroids, the frequency of *FH* deficiency for sporadic uterine fibroids is less than 2% [56–58]. *FH*-deficient uterine fibroids are often soft and amorphous, resembling a fibrothecoma. Histologically, they lack cellular packeting and distinct collagenous zones and show chain-like or palisading nuclear arrangements, prominent staghorn or slit-like blood vessels, oval nuclei with no or at most mild atypia, small eosinophilic nucleoli, and a low mitotic rate [57]. Thus, *FH*-deficient uterine fibroids occur less frequently and consist of a distinct subgroup of non-syndromic uterine leiomyomas.

2.7.4 Alterations of *COL4A5* and *COL4A6*

WGS also revealed aberrations of *COL4A5* and *COL4A6* on chromosome Xq22 in a small-numbered but distinct group of uterine fibroids [20, 37]. Both genes are

responsible for type IV collagen synthesis. Insulin receptor substrate-4 (*IRS4*), a gene located adjacent to *COL4A5*, is the most uniquely expressed gene in these fibroids. Deletions of *COL4A5* and *COL4A6* are observed in diffuse leiomyomatosis-associated AS, a rare variant of AS characterized by renal dysfunction and leiomyomatosis in the gastrointestinal, respiratory, and reproductive organs [59, 60].

2.8 Role of MicroRNAs in the Pathogenesis of Uterine Fibroids

MicroRNAs (miRNAs) are noncoding, stable, single-stranded RNAs consisting of 20–25 base pairs. These RNAs regulate the expression of multiple genes through posttranscriptional regulation, mainly through gene silencing. Differential and aberrant miRNA expression in uterine fibroids has been reported.

Microarray-based miRNA expression analysis using multiple myometrial tissue revealed that 45 miRNAs were significantly up- or down-regulated in uterine fibroids compared with the matched myometrium [54]. The authors compared miRNA expression profiles of uterine fibroids in women of different ethnicity and tumors of different size: African-American, Caucasian, and others and large, medium, and small tumors, respectively. Five dysregulated miRNAs were identified: the let-7 family, miR-21, miR-23b, miR-29b, and miR-197. *HMGA2* is one of the target genes of the let-7 family. The same research group further investigated the role of let-7 family miRNAs in *HMGA2* expression and fibroid cell proliferation and found that the let-7 miRNAs directly repress the dominant *HMGA2* transcript [55].

Another microarray-based miRNA analysis revealed that 46 miRNAs were differentially expressed in uterine fibroids compared with normal myometrium [61]. They reported the 20 most differentially expressed miRNAs, of which miR-29 species (miR-29a, miR-29b, and miR-29c) were significantly downregulated in the fibroid tissue compared with myometrial tissue. Overexpression of the miR-29 family in fibroid cells results in downregulation of the major fibrillar collagens, whereas downregulation of the miR-29 species results in increased expression of collagen type III, indicating that the miR-29 family plays a crucial role in ECM collagen deposition in uterine fibroids [62].

The role of miR-29b in uterine fibroid pathogenesis has been examined using a fibroid xenograft model [63]. Restoring miR-29b into the fibroid xenograft inhibited ECM accumulation, and 17β-estradiol and progesterone downregulated miR-29b and upregulated the mRNAs for multiple collagens. This suggests that ECM deposition in uterine fibroids is regulated by sex steroids via the downregulation of miR-29b.

The mechanism underlying the aberrant expression of miR-29c in uterine fibroid has been further clarified [64]. Expression of *COL3A1* and DNA methyltransferase type 3A (*DNMT3A*), both of which are target genes of miR-29c, was increased in uterine fibroids, and an inverse correlation between miR-29c and its target gene expression was observed. Overexpression of miR-29c by the transfection of pre-miR-29c inhibited the expression of *COL3A1* and *DNMT3A* in leiomyoma smooth muscle cells, whereas knockdown of miR-29c had the opposite effect. The suppression of

miR-29c for its target gene expression was primarily mediated by transcription factor SP1, nuclear factor-kappa B signaling, and epigenetic modification.

The role of miR-21 upregulation for the apoptosis of immortalized uterine fibroid cells has been clarified [65]. Fibroid tissues express significantly higher levels of miR-21 than does the normal myometrium. Silencing of miR-21 in the fibroid cells increases both the cleavage of caspase-3 and the phosphorylation of elongation factor-2, suggesting that miR-21 may contribute to the regulation of apoptosis and translation in uterine fibroids. Furthermore, the roles of other dysregulated miRNAs, including miR-197, miR-200c, and miR-15b, in the pathogenesis of uterine fibroids have been elucidated [66–68].

It is obvious that dysregulated miRNAs play crucial roles in the pathophysiology of uterine fibroids, and specific miRNAs have specific roles through modification of their specific target genes. However, most of the miRNAs regulate multiple target genes in a complicated manner. Therefore, the targeting of miRNAs for uterine fibroid treatment should be further clarified.

Conclusions

Genetic backgrounds affect the susceptibility of women to uterine fibroids, with genetic abnormalities being the pathological cause of the disease. Uterine fibroids are of monoclonal origin, where each fibroid nodule has a mutually exclusive driver gene alteration pattern that occurs in an independent manner. Chromosomal rearrangements occur in approximately 40% of uterine fibroids, which may cause the driver gene mutations. Several miRNAs also play roles in the disease's pathology.

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