



Epigenetics and Uterine Fibroids

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Ryo Maekawa and Norihiro Sugino

Abstract

The pathogenesis of uterine fibroids, the most common benign tumor in women, remains unclear. Environmental factors such as obesity, hypertension, and early menarche place women at greater risk for uterine fibroids. Epigenetic processes such as DNA methylation, histone modification, and microRNA expression play key roles in regulating gene expression and have been shown to be affected by environmental and other factors. Thus, uterine fibroids may be associated with epigenetic abnormalities caused by unfavorable environmental factors.

Several reports have investigated the epigenetic profiles of uterine fibroid and normal myometrium. The profiles of DNA methylation in the myometrium with and without fibroids were quite similar while those in fibroids were distinct. In uterine fibroids, the biological relevance of the aberrantly methylated and expressed genes was cancer process. Some of these genes include *IRS1*, which is related to tumor transformation, and others such as *GSTM5*, *KLF11*, *DLEC1*, and *KRT19*, which have tumor-suppressive roles. Some microRNAs including *miR-21*, *mir-200*, and *let-7* were found to be dysregulated in uterine fibroids and associated with the growth and the accumulation of extracellular matrix of uterine fibroids via aberrant expression of the target genes. Many estrogen receptor (ER) alpha-target genes, which were associated with apoptosis and collagen production, had aberrant DNA methylation in the promoter, which contributes to an abnormal response to estrogen. Moreover, some recent reports have demonstrated that several microRNAs which are dysregulated in uterine fibroids aberrantly mediate the actions of estrogen and progesterone.

Epigenetic abnormalities and their related transcriptional aberration have been associated with tumorigenic or tumor-suppressive roles, which may trigger the transformation of a single cell into a tumor stem cell that will eventually

R. Maekawa, M.D., Ph.D. (✉) · N. Sugino, M.D., Ph.D.
Department of Obstetrics and Gynecology, Yamaguchi University
School of Medicine, Ube, Yamaguchi, Japan

develop into a monoclonal uterine fibroid tumor. After menarche, the epigenetically dysregulated responses to estrogen and progesterone contribute to the growth of uterine fibroids.

Keywords

Uterine fibroids · Epigenetics · DNA methylation · microRNA · Estrogen receptor

5.1 Introduction

Uterine fibroids are the most common uterine tumors in reproductive-age women with a prevalence of about 25% [1] and frequently cause serious gynecological problems such as pelvic pain, menorrhagia, dysmenorrhea, infertility, and recurrent pregnancy loss [1, 2]. In addition, uterine fibroids are the most common indication for hysterectomy.

Risk factors for uterine fibroids include African descent, high body mass index, meat consumption, early menarche, and hypertension. Several studies have suggested an association between an increased risk of uterine fibroids and infection of the uterus and pelvic inflammatory diseases such as chlamydia infection [3]. On the other hand, factors that lower the risk include use of hormonal contraception, smoking, giving birth, and consumption of green vegetables [1]. These findings suggest that not only genetic but also environmental factors are involved in the development of uterine fibroids. Uterine fibroids often show multifocal tumorigenesis with various sizes from the corresponding myometrium. Therefore, smooth muscle cells of normal myometrium in a uterus with uterine fibroids may have already acquired the potential to develop into uterine fibroids in the future. Uterine fibroids develop only after menarche, and their growth depends on estrogen and progesterone [4], suggesting that they are caused by aberrant responses to these ovarian steroids.

Epigenetics refers to heritable changes in gene activity without changes in DNA sequences [5]. It has become clear that epigenetic regulation including DNA methylation, histone modification, and noncoding RNAs play key roles in gene expression. Epigenetic status has been shown to be affected by environmental and other factors [6–8]. Environmental exposure during development can alter susceptibility to adult diseases later in life [9], suggesting that environmental factors are associated with the pathogenesis of uterine fibroids by inducing epigenetic changes.

5.2 Epigenetics

5.2.1 DNA Methylation

DNA methylation mainly occurs at the 5-position of cytosine in a CpG dinucleotide forming 5-methylcytosine, and when it occurs in the gene promoter regions causes gene silencing [5]. DNA methylation is specific to each cell type and has been used

to characterize abnormal cells [5]. DNA methylation is involved in genomic imprinting, X-chromosomal inactivation (XCI), aging, and mutagenesis [5]. Maintaining a specific DNA methylation profile in a cell is necessary for cellular integrity, and alterations in DNA methylation may have serious health consequences. Silencing caused by aberrant DNA methylation is well known in various cancers, especially in tumor suppressor genes [5].

DNA methylation at the CpG dinucleotides is a postreplication event catalyzed by DNA methyltransferases (DNMTs) [5, 10] that add a methyl group to the cytosine ring to form methylcytosine. This established normal methylation patterns during embryogenesis and reproduces these patterns during replication of adult cells [5, 10]. DNA methylation appears to be established by a complex interplay of DNA methyltransferases [5, 10]. DNMT1 has a role in maintaining DNA methylation patterns during DNA replication, while DNMT3A and DNMT3B act as *de novo* methyltransferases in establishing methylation patterns.

5.2.2 Histone Modifications

Histone modifications affect chromatin structures and are critical for the interaction of transcriptional factors with response elements in the promoters [11]. Histone modifications such as acetylation of histones H3 and H4 or trimethylation of lysine 4 on histone H3 (H3K4me3) activate transcription by loosening the chromatin structure and allowing the recruitment of transcriptional factors to their response elements [12]. On the other hand, histone modifications such as trimethylation of lysines 9 and 27 on histone H3 (H3K9me and H3K27me3) inactivate transcription by condensing the chromatin [12]. Histone proteins are modified in several ways at their N-terminus, such as by acetylation, methylation, phosphorylation, ubiquitylation, and sumoylation [12]. The enzymes involved in these modifications include histone acetyltransferases (HATs) and deacetylases (HDACs), lysine methyltransferases and demethylases, arginine methyltransferases and demethylases and phosphatases, and lysine ubiquitinases and deubiquitinases [12, 13]. A few reports have shown histone modification enzymes were dysregulated in uterine fibroids [14, 15], but so far there is no evidence that they are directly associated with the pathogenesis or development of uterine fibroids. The possibility that further studies may find such an association can't be ruled out.

5.2.3 Noncoding RNAs

Noncoding RNAs (ncRNAs) have an important role in modulating gene and protein expression. They are classified into short ncRNAs, such as microRNAs, and long ncRNAs (lncRNAs). MicroRNAs, which have lengths of 21–23 nt, participate in transcriptional and posttranscriptional regulation of gene expression [16]. MicroRNAs function by binding to complementary sequences within mRNA molecules, usually, but not exclusively, resulting in gene silencing via translational repression or target degradation [16]. They have been implicated in multiple

biological events including numerous diseases [16]. lncRNAs are transcripts of greater than 200 nucleotides that may function as guides, scaffolds, and decoys and thus have the potential to regulate gene expression and spatial localization within the cell [17]. They are involved in several cellular processes, including chromosome dosage compensation, imprinting, epigenetic regulation, cell cycle control, nuclear and cytoplasmic trafficking, transcription, translation, splicing, and cell differentiation [17]. Recent studies have shown that lncRNAs act as both tumor suppressor genes and oncogenes [18, 19] and have implicated their aberrant expression in carcinogenesis [17].

5.3 Aberrant DNA Methylation in Uterine Fibroids

5.3.1 Aberrant Expression of DNA Methyltransferases (DNMTs)

The expressions of DNMT1, DNMT3A, and DNMT3B in human uterine fibroids have been found to differ from their expressions in the adjacent myometrium [20, 21]. In samples from African-American, Caucasian, and Hispanic women, the mRNA expressions of DNMT3a and DNMT3b were lower in uterine fibroids than in the myometrium, while the expression of DNMT1 in uterine fibroids was higher than in the myometrium [20]. On the other hand, we reported that, in Japanese women, DNMT1 and DNMT3a mRNA expression levels were higher in uterine fibroids than in the myometrium, whereas there was no significant difference in DNMT3b mRNA expression between uterine fibroids and the myometrium [21]. The increased DNMT1 expression that was found in both studies may reflect an elevated proliferative activity of uterine fibroid cells because DNMT1 is responsible for copying methylation patterns following DNA synthesis [5, 10]. However, the two studies differed in their findings on the relative expressions of DNMT3a and DNMT3b. The reason for the discrepancies is unclear but may be due to race-dependent differences.

5.3.2 Genome-Wide DNA Methylation Profiles of Uterine Fibroids

We previously examined the genome-wide DNA methylation patterns among uterine fibroids and myometrium [22]. In a hierarchical clustering and principal component analysis, the uterine fibroids clustered separately from the myometrium (Fig. 5.1a, b) [22]. However, a principal component analysis of the mRNA expression patterns could not separate the uterine fibroids from the myometrium (Fig. 5.1c, d) [22]. These findings indicate that the DNA methylation profile of uterine fibroids is much more distinct than the mRNA expression profile. Therefore, DNA methylation analyses appear to be well-suited for screening and detecting the frequently dysregulated genes in uterine fibroid cases that are involved in pathogenesis and development.

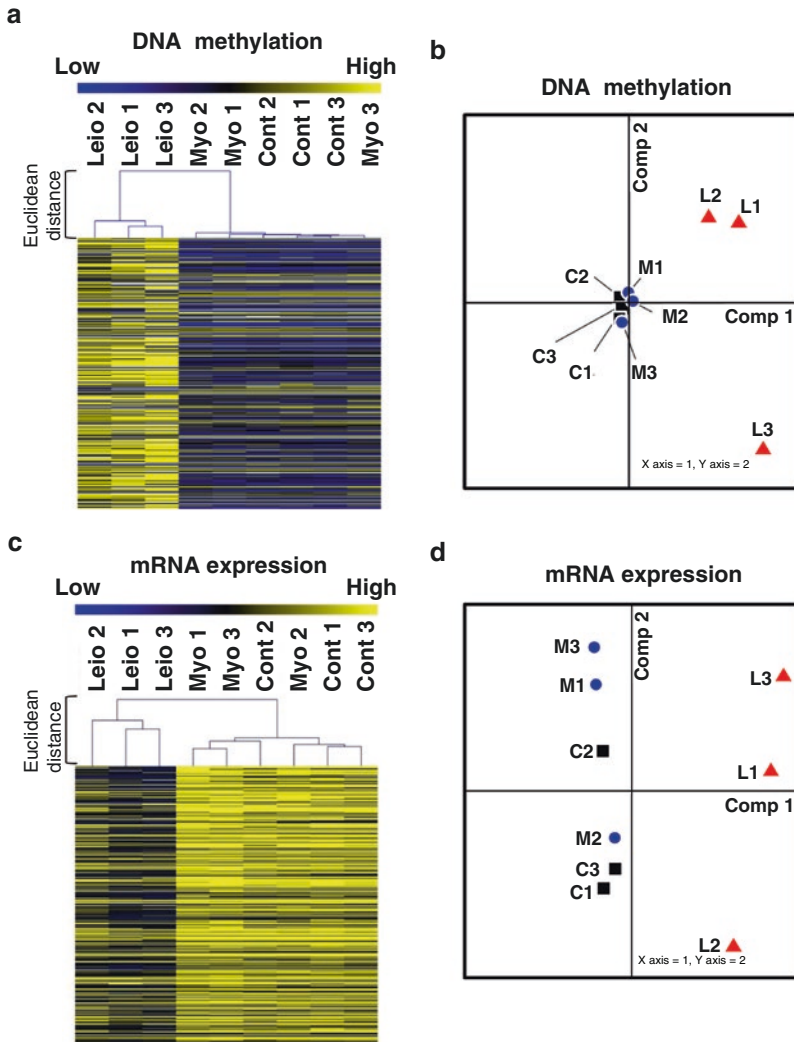


Fig. 5.1 DNA methylation profiling and mRNA expression profiling of fibroids and myometrium with and without fibroids. Fibroids (L1, L2, and L3), myometrium with fibroids (M1, M2, and M3), and myometrium without fibroids (C1, C2, and C3) were compared using hierarchical clustering analyses and principal component analyses. **(a)** Hierarchical clustering analyses according to DNA methylation profiles. The heat map in the hierarchical clustering analysis indicates DNA methylation levels from unmethylated (blue) to completely methylated (yellow). Distances of DNA methylation pattern (Euclidean distances) were calculated by MultiExperiment Viewer. **(b)** Principal component analyses according to DNA methylation profiles. Horizontal and vertical axes show principal components 1 and 2, respectively. The principal components were analyzed with MultiExperiment Viewer. **(c)** Hierarchical clustering analyses according to mRNA expression profiles. The heat map in hierarchical clustering analysis indicates mRNA expression levels from low (blue) to high (yellow). Distances of mRNA expression pattern (Euclidean distances) are shown on the left side. **(d)** Principal component analyses according to mRNA expression profiles. Horizontal and vertical axes show principal components 1 and 2, respectively

Uterine fibroids often show multifocal tumorigenesis with tumors of various sizes from the myometrium, suggesting the possibility that the adjacent myometrium, which looks normal, has the potential to develop into uterine fibroids in the future, i.e., already has aberrant DNA methylation. However, the DNA methylation profiles of the myometrium adjacent to the uterine fibroids and the normal uterine myometrium were quite similar [22]. This result suggests two possibilities. One possibility is that adjacent myometrium with a uterine fibroid nodule does not acquire the potential in DNA methylation levels to develop into uterine fibroid. The other possibility is that cells with aberrant DNA methylation are present in the adjacent myometrium but are too few to detect. In other words, aberrant DNA methylation may occur only in a limited number of cells. In a flow cytometry analysis of myometrial and fibroid tissues, side populations of cells were found to act as tissue stem cells and have the potential to differentiate and proliferate [23, 24]. In addition, each fibroid nodule was found to have monoclonal cell features [25], indicating that each fibroid nodule is derived from just one affected cell as the origin of the tumor. Taken together, these findings suggest that tumor stem cells with aberrant DNA methylation are produced in the myometrium and, after menarche, develop into fibroid nodules by exposure to estrogen and progesterone concomitant with the aberrant response to these steroids.

Uterine fibroids are classified as intramural, submucosal, and subserosal based on their location and, in more than half the cases, are multifocal fibroids. However, the DNA methylation profiles were not specific to tumors of each location, solitary and multifocal tumors (see Chap. 6 for details) [26].

5.3.3 Aberrantly Methylated Genes in Uterine Fibroids

Several genome-wide studies including our own have demonstrated differences between the DNA methylation profiles of uterine fibroids and the myometrium [14, 21, 22, 27]. Using an Infinium HumanMethylation450k BeadChip (Illumina, San Diego, USA) and samples from Japanese women, we found that 478 genes were hypomethylated, and 1014 genes were hypermethylated in uterine fibroids compared to the myometrium [22]. Subsequent analyses showed that the mRNA expressions of 120 of these genes were altered [22]. According to the pathway analyses, the most specific pathway was cancer process in both hypermethylated-downregulated and hypomethylated-upregulated genes [22]. Three of the hypomethylated-upregulated genes related to cancer process were COL4A1 and COL4A2, which are reported to be upregulated in uterine fibroids [28, 29] and insulin receptor substrate 1 (IRS1). COL4A1 and COL4A2 are related to collagen metabolism and extracellular matrix formation, and when disrupted, they may contribute to the increase of fibroid volume by altering collagen deposition and extracellular matrix patterning [1]. IRS1 was initially characterized as a cytosolic adaptor protein involved in insulin receptor (INSR) and insulin-like growth factor 1 receptor (IGF1R) signaling. More recently, it has been shown to be involved in proliferation and transformation of cancer cells [30, 31]. In terms of hypermethylated-downregulated genes, the cancer process category included glutathione S-transferase mu 5 (GSTM5), which is an antioxidant

enzyme that protects cells against reactive oxygen species. Downregulation of *GSTM5* is known to be involved in cancer development [32].

Using an Infinium HumanMethylation27k BeadChip (Illumina) and samples from African-American women, Navarro et al. identified 55 genes in which promoter methylation and mRNA expression differed between uterine fibroids and the myometrium [27]. Pathway analysis of these genes identified cancer process as the most specific pathway in uterine fibroids. Three of these 55 genes (Kruppel-like transcription factor 11 (*KLF11*), deleted in lung and esophageal cancer 1 (*DLEC1*) and Keratin19 (*KRT19*)), were hypermethylated-downregulated in uterine fibroids and are known to be tumor suppressor genes.

These results indicated that aberrant DNA methylation has an important role in transformation, proliferation, and collagen deposition of uterine fibroids. A number of differences were found between our study of Japanese women and Navarro et al.'s study of African-American women [22, 27]. These discrepancies may be due to differences in microarray platforms and race.

5.3.4 Aberrantly Enriched DNA Hypomethylation in X Chromosome in Uterine Fibroids

We previously reported a greater level of DNA hypomethylation on the X chromosome in uterine fibroids than in the adjacent normal myometrium [14, 22]. In women, breast cancers, ovarian cancers, and cervical cancers have been reported to have aberrant DNA hypomethylation on the X chromosome due to loss of the inactive X chromosome or aberrant replication of the active X chromosome [33]. Our analysis of the X chromosome genotypes demonstrated that these events do not occur in uterine fibroids [22], which suggested that X-inactivation machineries are disturbed in uterine fibroids. Therefore, we examined the mechanism of enriched DNA hypomethylation in the X chromosome in uterine fibroids [34]. Hypomethylation was not enriched in the imprinted genes, suggesting that dysfunction of polycomb repressive complexes was not involved. Analysis of the expression of X chromosome inactivation (XCI)-related genes revealed that the expressions of *XIST* and *SATB1* were downregulated in 36% and 46% of uterine fibroids, respectively. *XIST* is an lncRNA and initiates X chromosome inactivation [17]. *SATB1* is involved in tethering the inactive X chromosome to the repressive core compartment for gene silencing [34]. This raises the possibility that the aberration of XCI-related genes such as *XIST* or *SATB1* is involved in aberrant hypomethylation on the X chromosome in a certain population of the patients with uterine fibroids, although the mechanism is unknown.

5.3.5 Estrogen Receptor Alpha and Its Target Genes

The growth of uterine fibroids depends on estrogen [4]. The biological effect of estrogen is mediated by estrogen receptor (ER), which is a transcription factor. We previously reported that ER-alpha was upregulated in uterine fibroids by

Table 5.1 Estrogen receptor alpha-target genes with aberrant DNA methylation in the promoter and aberrant mRNA expression

Gene symbol	Gene name	mRNA expression ^a
<i>Hypomethylated ERα target genes</i>		
COL4A1	Collagen, type IV, alpha 1	↑
COL6A3	Collagen, type VI, alpha 3	↑
RPL39	Ribosomal protein L39	↑
ZMAT3	Zinc finger, matrin-type 3	↑
OXTR	Oxytocin receptor	↓
<i>Hypermethylated ERα target genes</i>		
BCAN	Brevican	↑
KIF5C	Kinesin family member 5C	↑
NPTX2	Neuronal pentraxin II	↑
TFAP2C	Transcription factor AP-2 gamma	↑
SCIN	Scinderin	↑
THBS2	Thrombospondin 2	↑
OCIAD2	OCIA domain containing 2	↓
CRHBP	Corticotropin-releasing hormone binding protein	↓
NUAK1	NUAK family, SNF1-like kinase, 1	↓
CCDC68	Coiled-coil domain containing 68	↓
NR5A2	Nuclear receptor subfamily 5, group A, member 2	↓
ELTD1	EGF, latrophilin, and seven transmembrane domain containing 1	↓
FAM162B	Family with sequence similarity 162, member B	↓
IGF2BP3	Insulin-like growth factor 2 mRNA binding protein 3	↓
DAPK1	Death-associated protein kinase 1	↓
GSTM5	Glutathione S-transferase mu 5	↓
EZR	Ezrin	↓

^aMicroarray mRNA expression statuses of leiomyoma relative to adjacent myometrium are shown

aberrant DNA hypomethylation [35, 36]. On the target genes of ER-alpha, the DNA methylation status of the gene promoter region affects the response to estrogen [36]. These findings raise the possibility that aberrant DNA methylation of the promoter of ER-alpha-target genes causes the aberrant responses to estrogen in uterine fibroids. Thus, we investigated the ER-alpha-target genes with aberrant DNA methylation and mRNA expression. Of 120 genes that were aberrantly methylated and expressed in uterine fibroids compared to the adjacent myometrium, 22 genes had the consensus sequences of ER response element (ERE) in the promoter region (Table 5.1) [22]. In addition to COL4A1 and GSTM5, death-associated protein kinase 1 (DAPK1) and novel kinase family 1 (NUAK1) were included in the genes that were hypermethylated and transcriptionally downregulated. DAPK1 is a tumor suppressor gene and has been shown to be associated with apoptosis [37–39]. NUAK1 is known to possess tumor-suppressive roles through the control of cellular senescence [40, 41]. As a result of aberrant response to estrogen, these genes might be overexpressed and involved in the growth of uterine fibroids.

5.4 Noncoding RNAs and Uterine Fibroids

A number of genome-wide studies using microarray or deep sequencing have analyzed the expression statuses of miRNAs in uterine fibroids (Table 5.2) [42–47] and their target genes (Table 5.3) [42, 44, 45, 48–52]. Since each miRNA is predicted to have a broad range of target genes, even an alteration of a single miRNA could have a significant impact on the outcome of diverse biological functions regulated by the products of these genes (Table 5.3) [42, 44, 45, 48–52]. One genome-wide study reported that 13% of aberrantly expressed mRNAs in uterine fibroids were associated with dysregulated microRNAs [42]. Furthermore, several studies demonstrated that dysregulated microRNAs were inversely correlated with their targets at the protein level (Table 5.2) [42, 44, 45, 48–52].

5.4.1 Aberrantly Expressed MicroRNAs in Uterine Fibroids

Mir-21 has been frequently reported to be upregulated in uterine fibroids (Table 5.2) [43, 45–47, 52]. Since miR-21 has been shown to be overexpressed in the vast majority of tumors including malignant tumors, it is thought that miR-21 is a key regulator of cell proliferation, survival, and tumorigenesis and acts as an anti-apoptotic factor [53–57]. The oncogenic role of miR-21 in breast cancer is thought to be partially mediated through Bcl-2 [53, 57], which is regulated by progesterone

Table 5.2 MicroRNAs dysregulated in uterine fibroids

Authors	Expression status	Number of dysregulated miRNAs	Highly dysregulated miRNAs
Wang (2007) [45]	Upregulated	24	let-7 family, miR-21, miR-23b, miR-27a, and miR-30a
	Downregulated	21	miR-29b, miR-32, miR-144, miR-197, and miR-212
Marsh (2008) [46]	Upregulated	19	miR-21, miR-125b, miR-34a, and miR-323
	Downregulated	27	miR-139
Pan (2008) [47]	Upregulated	Not shown	miR-20a, miR-21, and miR-26a
	Downregulated	Not shown	–
Georgieva (2012) [43]	Upregulated	>50	miR-21, miR-200a/b, miR-363, miR-490, and miR-137
	Downregulated		miR-217 and miR-4792
Chuang (2012) [49, 50]	Downregulated	–	miR-200 family and miR-93
Fitzgerald (2012) [52]	Upregulated	–	miR-21
Qiang (2014) [51]	Downregulated	–	miR-29b

Table 5.3 Target genes of microRNAs and lncRNAs

microRNAs	Expression in uterine fibroids	Targets	Class of expression	Targets expression	Status	Reference
miR-21	Upregulated	PLAG1 ^a , PPAR α , TIMP3 ^a , MAPK10 ^a , BRD1 ^b , and SKI ^b	mRNA	Downregulation	Predicted	Wang (2007) [45] ^a , Zavadii (2010) [42] ^b
let-7	Upregulated	HMG2 ^a and Ki67 ^b	Protein	Downregulation	Confirmed	Wang (2007) [45] ^a and Zavadii (2010) [42] ^b
miR-194-1	Upregulated	EGFR	Protein	Downregulation	Predicted	Zavadii (2010) [42]
miR-199a-2	Downregulated	TGF- α	Protein	Upregulation	Predicted	Zavadii (2010) [42]
miR-200a/200b	Downregulated	MAF, CTBP2, BCL2, CITED2, LASS6, PHF21A, TSC22D1, ATXN1, JUN, and NFIB	mRNA	Upregulation	Predicted	Zavadii (2010) [42]
miR-200a	Downregulated	CYP1B1 and CTBP2	mRNA	Upregulation	Confirmed	Zavadii (2010) [42]
miR-200c	Downregulated	ZEB1, ZEB2, TIMP2, FBLN5, VEGFA, and IL8	mRNA and/or protein	Upregulation	Confirmed	Chuang (2014) [48] Chuang (2012) [50]
miR-93	Downregulated	F3, IL8, CGF, and PAI-1	mRNA	Upregulation	Confirmed	Chuang (2012) [49]
miR-29b	Downregulated	COL1A1, COL2A1, COL4A1 and COL7A1	mRNA	Upregulation	Predicted	Wang (2007) [45]
Long noncoding RNAs (lncRNAs) <i>Intergenic10</i>	Expression in uterine fibroids Upregulated	Targets ADAM12	Class of experiment mRNA	Targets expression Upregulation	Status Confirmed	Reference Guo (2014) [44]

and plays a key role in regulating apoptosis in uterine fibroids [1]. These facts suggest that the upregulation of miR-21 concomitant with altered expression of their target gene such as Bcl-2 contribute to the growth of uterine fibroids, although the mechanism in uterine fibroids is unknown.

The expressions of let-7 family microRNAs were found to be significantly higher in uterine fibroids (Table 5.2), and high mobility group A2 (HMGA2) genes were identified as their target genes (Table 5.3) [42, 45]. HMGA2, which is associated with 12q15 anomalies, is frequently dysregulated in uterine fibroids (see Chap. 6 for details) [25] and is strongly associated with the pathogenesis and development of uterine fibroids [58]. The expression of let-7 was found to be higher in small uterine fibroids than in large uterine fibroids, and the expression of HMGA2 protein was found to be lower in small uterine fibroids than in large uterine fibroids [45]. The latter finding is consistent with a previous report that overexpression of HMGA2 was associated with large tumor size [59]. These facts suggest that let-7 family microRNAs are associated with the size of uterine fibroids via downregulation of HMGA2.

Mir-200 family microRNAs have been shown to be downregulated in uterine fibroids [42, 48, 50]. The predicted target genes of miR-200a/c (e.g., RAS, WNT, and TGF-beta) have regulatory functions in cell cycle control, angiogenesis, matrix remodeling, and cancer-related signaling. Loss of miR-200 family microRNAs is associated with aggressive tumor phenotypes in ovarian cancer [60]. In fact, transfection of miR-200c in cultured uterine fibroid cells was associated with a reduction of cell viability and proliferation [42, 50]. Therefore, the decreased expression of miR-200a/c may significantly increase uterine fibroid growth [42, 50]. Loss of miR-200 family microRNAs is also associated with epithelial and mesenchymal transition (EMT) [60]. Zavadil et al. overexpressed miR-200a in cultured uterine fibroid cells. Overexpression of miR-200a in cultured uterine fibroid cells suppressed CYP1B1 and CTBP2, which are associated with EMT, and caused the fibroblastoid morphology to revert to a more pronounced epithelial phenotype (Table 5.3) [42]. Transfection of miR-200c repressed the mRNA and/or protein levels of other EMT-associated genes (ZEB1, ZEB2, TIMP2, FBLN5, and VEGF), increased the expression of E-cadherin, and reduced the expression of vimentin (Table 5.3) [50]. Overexpression of miR-200c repressed the expression IL8, an inflammatory mediator, through a mechanism involving suppression of IKK β and alteration of NF κ B activity (Table 5.3) [48]. Downregulation of miR-93 also has been associated with the increase of inflammatory mediators (IL8 and F3, CTGF, and PAI-1) in uterine fibroids (Table 5.3) [49]. These findings suggest that decreases of miR-200c and miR-93 in uterine fibroids are involved in the development of uterine fibroids.

Recently, Qiang et al. reported that downregulation of miR-29b is essential for pathogenesis of uterine fibroids [51]. Using subrenal uterine fibroid xenograft models, they found that restoring miR-29b inhibited the accumulation of extracellular matrix (ECM) and the development of solid tumors. In fact, they detected many collagen genes that were predicted targets of miR-29b in uterine fibroid cells (Table 5.3) [45]. Other reports showed that downregulation of miR-29b caused upregulation of collagen genes in uterine fibroids [51]. These facts indicate that downregulation of miR-29b is involved in the growth of uterine fibroids.

5.4.2 Steroid Hormone and MicroRNAs in Uterine Fibroids

Uterine fibroids grow in response to estrogen and progesterone, and the actions are mediated by their receptors. Recently, several microRNAs were found to be associated with the actions of these hormones. Pan et al. found that estradiol and medroxyprogesterone acetate regulated the expression of miR-21 in myometrial and uterine fibroid cells [47]. Qiang et al. reported 17 β -estradiol and progesterone downregulated miR-29b with upregulation of several collagens in uterine fibroid xenografts [51]. These facts suggest that ovarian steroid actions on the target genes are mediated in part through the regulation of miRNAs in uterine fibroids. Such a regulatory mechanism may control the expression of target genes which are necessary for the growth of uterine fibroids and the response to ovarian steroids.

5.4.3 Chromosomal Abnormality and Deletion of MicroRNAs

The impact of specific genomic alterations on dysregulated microRNAs in uterine fibroids was previously investigated using comparative genomic hybridization [42]. Chromosomal regions of 1p36.33-p36.23 and 13q26.1-q27.1 are regions of deletion overlapping among uterine fibroids [25, 61]. Members of the cancer-inhibitory miRNA family mir-200a/b are located in the region of loss at 1p36, while the 13q26-27 region harbors miR-15b and miR-16-2. Loss of the miR-15 and miR-16 cluster is associated with aggressive tumor growth [62]. These findings indicated that alteration of these genomic regions harboring cancer-related miRNAs may be related to the tumorigenesis of uterine fibroids.

5.4.4 Racial Differences in MicroRNA Expression in Uterine Fibroids

Although several previous reports using genome-wide approaches revealed a fraction of microRNAs were commonly dysregulated in uterine fibroids [42, 43, 45–47], the levels of the expression varied among races [45, 47, 50]. Wang et al. reported that uterine fibroids from African-Americans more strongly expressed let-7 than those from Caucasian [45]. The expression profiles from other racial groups (Asian and Hispanic) appear to be in between those of black and white women [45]. Chuang et al. reported that the levels of miR-200c were lower in uterine fibroids from African-Americans than those from Caucasians [50]. Pan et al. indicated several microRNAs including miR-21 were more strongly expressed in Caucasians than in African-Americans [47]. Uterine fibroids tend to develop more frequently, grow more rapidly, and are more symptomatic in African-Americans than in other racial groups. These facts suggest that regulatory roles of microRNAs are responsible for the difference of incidence, size, and growth rate between races.

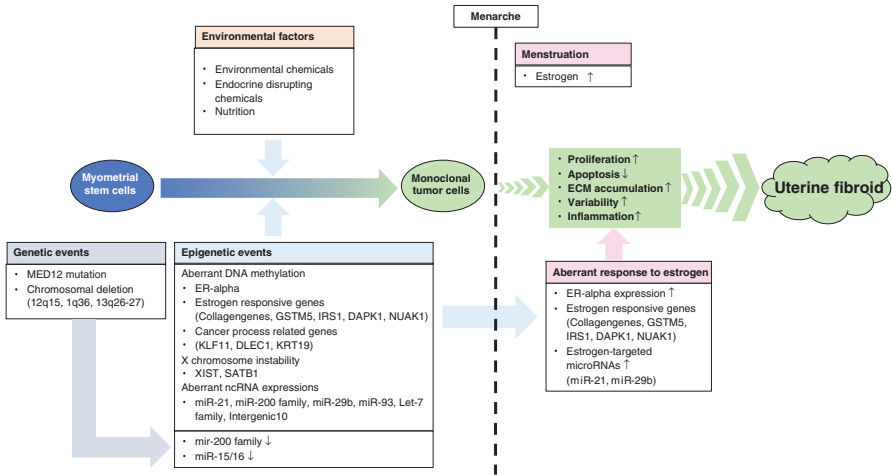


Fig. 5.2 Hypothesis of the pathogenesis and development of uterine fibroids

5.4.5 Long Noncoding RNAs and Uterine Fibroids

Long noncoding RNAs regulate gene expression in a variety of biological processes [63]. In a study of the global expression of lncRNAs in uterine fibroids, 252 lncRNAs were dysregulated in small fibroids and 816 were dysregulated in large fibroids compared to the myometrium [44]. This suggests that the degree of dysregulation is positively correlated with tumor size. In addition, the expressions of many lncRNAs were correlated with the mRNA expressions of the neighboring genes. For example, in vitro knockdown of the lncRNA intergenic10, which is often upregulated in uterine fibroids, decreased expression of the neighboring gene (ADAM12) and inhibited the proliferation of fibroid cells. These results suggest that not only microRNAs but also lncRNAs such as Intergenic10 contribute to the growth of uterine fibroids via their cis mRNAs.

Conclusion

We propose the following hypothesis for the pathogenesis of uterine fibroids (Fig. 5.2). Tissue stem cells in the myometrium transform to tumor cells by aberrant DNA methylation, histone modification, and microRNA expressions induced by factors such as environmental exposures. Aberrant DNA methylation and its related transcriptional aberration in cancer-related genes such as IRS1 may represent a critical initial mechanism that triggers transformation of a single tissue stem cell to a tumor cell, which will eventually develop into a monoclonal fibroid tumor. Downregulation of genes associated with tumor repression such as GSTM5, KLF11, DLEC1, and KRT19 by dysregulated DNA methylation may also contribute to the transformation. Aberrant DNA methylation in uterine fibroids has been implicated in the increase of ER-alpha expression. Dysregulated

ncRNAs, e.g., miR-21 and genes of the miR-200 and let-7 families, also may have important roles in tumorigenesis of uterine fibroids. Genomic alteration such as chromosomal deletion is also involved in the aberrant expression of microRNAs including miR-200 family genes and miR-15/16. After menarche, the tumor cells aberrantly respond to estrogen and gradually proliferate and differentiate to form uterine fibroid nodules. Aberrant DNA methylation of the promoters of ER-alpha-target genes, e.g., COL4A1, COL6A3, DAPK1, and NUA1, is responsible for the aberrant response to estrogen. In addition, the responses to estrogen and progesterone could be modified by dysregulated microRNAs such as miR-21 and miR-29b. Some epigenetic factors such as microRNAs and lncRNAs may affect the size of uterine fibroids. Moreover, there are differences in epigenetic statuses including DNA methylation and microRNAs between races. These findings suggest that epigenetic status can explain not only the pathogenesis but also diverse characteristics of uterine fibroids. Taken together, these facts suggest that aberrant epigenetic modifications contribute to the pathogenesis of uterine fibroids and may lead to the development of new strategies for treatment.

References

1. Wise LA, Laughlin-Tommaso SK. Epidemiology of uterine fibroids: from menarche to menopause. *Clin Obstet Gynecol.* 2016;59(1):2–24. <https://doi.org/10.1097/GRF.0000000000000164>.
2. Tamura H, Kishi H, Kitade M, Asai-Sato M, Tanaka A, Murakami T, et al. Clinical outcomes of infertility treatment for women with adenomyosis in Japan. *Reprod Med Biol.* 2017;16(3):276–82. <https://doi.org/10.1002/rmb2.12036>.
3. Laughlin SK, Schroeder JC, Baird DD. New directions in the epidemiology of uterine fibroids. *Semin Reprod Med.* 2010;28(3):204–17. <https://doi.org/10.1055/s-0030-1251477>.
4. Reis FM, Bloise E, Ortiga-Carvalho TM. Hormones and pathogenesis of uterine fibroids. *Best Pract Res Clin Obstet Gynaecol.* 2016;34:13–24. <https://doi.org/10.1016/j.bpobgyn.2015.11.015>.
5. Kim M, Costello J. DNA methylation: an epigenetic mark of cellular memory. *Exp Mol Med.* 2017;49(4):e322. <https://doi.org/10.1038/emm.2017.10>.
6. Alinovi R, Goldoni M, Pinelli S, Ravanetti F, Galetti M, Pelosi G, et al. Titanium dioxide aggregating nanoparticles induce autophagy and under-expression of microRNA 21 and 30a in A549 cell line: a comparative study with cobalt(II, III) oxide nanoparticles. *Toxicol In Vitro.* 2017;42:76–85. <https://doi.org/10.1016/j.tiv.2017.04.007>.
7. Priya ES, Kumar TS, Singh PR, Balakrishnan S, Arunakaran J. Impact of lactational exposure to polychlorinated biphenyl causes epigenetic modification and impairs Sertoli cells functional regulators in F1 progeny. *Reprod Sci.* 2017;1933719117699707. <https://doi.org/10.1177/1933719117699707>.
8. Sadakierska-Chudy A, Frankowska M, Jastrzebska J, Wydra K, Miszkiel J, Sanak M, et al. Cocaine administration and its withdrawal enhance the expression of genes encoding histone-modifying enzymes and histone acetylation in the rat prefrontal cortex. *Neurotox Res.* 2017;32(1):141–50. <https://doi.org/10.1007/s12640-017-9728-7>.
9. Walker CL. Epigenomic reprogramming of the developing reproductive tract and disease susceptibility in adulthood. *Birth Defects Res A Clin Mol Teratol.* 2011;91(8):666–71. <https://doi.org/10.1002/bdra.20827>.

10. Auclair G, Weber M. Mechanisms of DNA methylation and demethylation in mammals. *Biochimie*. 2012;94(11):2202–11. <https://doi.org/10.1016/j.biochi.2012.05.016>.
11. Dogan N, Wu W, Morrissey CS, Chen KB, Stonestrom A, Long M, et al. Occupancy by key transcription factors is a more accurate predictor of enhancer activity than histone modifications or chromatin accessibility. *Epigenetics Chromatin*. 2015;8:16. <https://doi.org/10.1186/s13072-015-0009-5>.
12. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res*. 2011;21(3):381–95. <https://doi.org/10.1038/cr.2011.22>.
13. Mai A, Cheng D, Bedford MT, Valente S, Nebbioso A, Perrone A, et al. Epigenetic multiple ligands: mixed histone/protein methyltransferase, acetyltransferase, and class III deacetylase (sirtuin) inhibitors. *J Med Chem*. 2008;51(7):2279–90. <https://doi.org/10.1021/jm701595q>.
14. Maekawa R, Yagi S, Ohgane J, Yamagata Y, Asada H, Tamura I, et al. Disease-dependent differentially methylated regions (D-DMRs) of DNA are enriched on the X chromosome in uterine leiomyoma. *J Reprod Dev*. 2011;57(5):604–12. <https://doi.org/10.1262/jrd.11-035A>.
15. Wei LH, Torng PL, Hsiao SM, Jeng YM, Chen MW, Chen CA. Histone deacetylase 6 regulates estrogen receptor alpha in uterine leiomyoma. *Reprod Sci*. 2011;18(8):755–62. <https://doi.org/10.1177/1933719111398147>.
16. Liu B, Li J, Cairns MJ. Identifying miRNAs, targets and functions. *Brief Bioinform*. 2014;15(1):1–19. <https://doi.org/10.1093/bib/bbs075>.
17. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. *Cell*. 2013;152(6):1298–307. <https://doi.org/10.1016/j.cell.2013.02.012>.
18. Deng Q, Becker L, Ma X, Zhong X, Young K, Ramos K, et al. The dichotomy of p53 regulation by noncoding RNAs. *J Mol Cell Biol*. 2014;6(3):198–205. <https://doi.org/10.1093/jmcb/mju017>.
19. Zhang J, Zhang P, Wang L, Piao HL, Ma L. Long non-coding RNA HOTAIR in carcinogenesis and metastasis. *Acta Biochim Biophys Sin Shanghai*. 2014;46(1):1–5. <https://doi.org/10.1093/abbs/gmt117>.
20. Li S, Chiang TC, Richard-Davis G, Barrett JC, McLachlan JA. DNA hypomethylation and imbalanced expression of DNA methyltransferases (DNMT1, 3A, and 3B) in human uterine leiomyoma. *Gynecol Oncol*. 2003;90(1):123–30.
21. Yamagata Y, Maekawa R, Asada H, Taketani T, Tamura I, Tamura H, et al. Aberrant DNA methylation status in human uterine leiomyoma. *Mol Hum Reprod*. 2009;15(4):259–67. <https://doi.org/10.1093/molehr/gap010>.
22. Maekawa R, Sato S, Yamagata Y, Asada H, Tamura I, Lee L, et al. Genome-wide DNA methylation analysis reveals a potential mechanism for the pathogenesis and development of uterine leiomyomas. *PLoS One*. 2013;8(6):e66632. <https://doi.org/10.1371/journal.pone.0066632>.
23. Ono M, Maruyama T, Masuda H, Kajitani T, Nagashima T, Arase T, et al. Side population in human uterine myometrium displays phenotypic and functional characteristics of myometrial stem cells. *Proc Natl Acad Sci U S A*. 2007;104(47):18700–5. <https://doi.org/10.1073/pnas.0704472104>.
24. Ono M, Qiang W, Serna VA, Yin P, Coon JS V, Navarro A, et al. Role of stem cells in human uterine leiomyoma growth. *PLoS One*. 2012;7(5):e36935. <https://doi.org/10.1371/journal.pone.0036935>.
25. Commandeur AE, Styer AK, Teixeira JM. Epidemiological and genetic clues for molecular mechanisms involved in uterine leiomyoma development and growth. *Hum Reprod Update*. 2015;21(5):593–615. <https://doi.org/10.1093/humupd/dmv030>.
26. Sato S, Maekawa R, Yamagata Y, Tamura I, Lee L, Okada M, et al. Identification of uterine leiomyoma-specific marker genes based on DNA methylation and their clinical application. *Sci Rep*. 2016;6:30652. <https://doi.org/10.1038/srep30652>.
27. Navarro A, Yin P, Monsivais D, Lin SM, Du P, Wei JJ, et al. Genome-wide DNA methylation indicates silencing of tumor suppressor genes in uterine leiomyoma. *PLoS One*. 2012;7(3):e33284. <https://doi.org/10.1371/journal.pone.0033284>.
28. Vanharanta S, Wortham NC, Laiho P, Sjoberg J, Aittomaki K, Arola J, et al. 7q deletion mapping and expression profiling in uterine fibroids. *Oncogene*. 2005;24(43):6545–54. <https://doi.org/10.1038/sj.onc.1208784>.

29. Gilden M, Malik M, Britten J, Delgado T, Levy G, Catherino WH. Leiomyoma fibrosis inhibited by liarozole, a retinoic acid metabolic blocking agent. *Fertil Steril*. 2012;98(6):1557–62. <https://doi.org/10.1016/j.fertnstert.2012.07.1132>.
30. Morelli C, Garofalo C, Sisci D, del Rincon S, Cascio S, Tu X, et al. Nuclear insulin receptor substrate 1 interacts with estrogen receptor alpha at ERE promoters. *Oncogene*. 2004;23(45):7517–26. <https://doi.org/10.1038/sj.onc.12080141208014>.
31. Esposito DL, Aru F, Lattanzio R, Morgano A, Abbondanza M, Malekzadeh R, et al. The insulin receptor substrate 1 (IRS1) in intestinal epithelial differentiation and in colorectal cancer. *PLoS One*. 2012;7(4):e36190. <https://doi.org/10.1371/journal.pone.0036190PONE-D-10-04875>.
32. Peng DF, Razvi M, Chen H, Washington K, Roessner A, Schneider-Stock R, et al. DNA hypermethylation regulates the expression of members of the Mu-class glutathione S-transferases and glutathione peroxidases in Barrett's adenocarcinoma. *Gut*. 2009;58(1):5–15. <https://doi.org/10.1136/gut.2007.146290>.
33. Weakley SM, Wang H, Yao Q, Chen C. Expression and function of a large non-coding RNA gene XIST in human cancer. *World J Surg*. 2011;35(8):1751–6. <https://doi.org/10.1007/s00268-010-0951-0>.
34. Sato S, Maekawa R, Yamagata Y, Asada H, Tamura I, Lee L, et al. Potential mechanisms of aberrant DNA hypomethylation on the x chromosome in uterine leiomyomas. *J Reprod Dev*. 2014;60(1):47–54.
35. Asada H, Yamagata Y, Taketani T, Matsuoka A, Tamura H, Hattori N, et al. Potential link between estrogen receptor-alpha gene hypomethylation and uterine fibroid formation. *Mol Hum Reprod*. 2008;14(9):539–45. <https://doi.org/10.1093/molehr/gan045>.
36. Maekawa R, Sato S, Okada M, Lee L, Tamura I, Jozaki K, et al. Tissue-specific expression of estrogen receptor 1 is regulated by DNA methylation in a T-DMR. *Mol Endocrinol*. 2016;30(3):335–47. <https://doi.org/10.1210/me.2015-1058>.
37. Claus R, Hackanson B, Poetsch AR, Zucknick M, Sonnet M, Blagitko-Dorfs N, et al. Quantitative analyses of DAPK1 methylation in AML and MDS. *Int J Cancer*. 2012;131(2):E138–42. <https://doi.org/10.1002/ijc.26429>.
38. Missaoui N, Hmissa S, Trabelsi A, Traore C, Mokni M, Dante R, et al. Promoter hypermethylation of CDH13, DAPK1 and TWIST1 genes in precancerous and cancerous lesions of the uterine cervix. *Pathol Res Pract*. 2011;207(1):37–42. <https://doi.org/10.1016/j.prp.2010.11.001>.
39. Gade P, Kimball AS, DiNardo AC, Gangwal P, Ross DD, Boswell HS, et al. Death-associated protein kinase-1 expression and autophagy in chronic lymphocytic leukemia are dependent on activating transcription factor-6 and CCAAT/enhancer-binding protein-beta. *J Biol Chem*. 2016;291(42):22030–42. <https://doi.org/10.1074/jbc.M116.725796>.
40. Bernard D, Augert A. NUAk1 links genomic instability and senescence. *Aging (Albany NY)*. 2010;2(6):317–9. <https://doi.org/10.18632/aging.100153>.
41. Hou X, Liu JE, Liu W, Liu CY, Liu ZY, Sun ZY. A new role of NUAk1: directly phosphorylating p53 and regulating cell proliferation. *Oncogene*. 2011;30(26):2933–42. <https://doi.org/10.1038/onc.2011.19onc201119>.
42. Zavadil J, Ye H, Liu Z, Wu J, Lee P, Hernando E, et al. Profiling and functional analyses of microRNAs and their target gene products in human uterine leiomyomas. *PLoS One*. 2010;5(8):e12362. <https://doi.org/10.1371/journal.pone.0012362>.
43. Georgieva B, Milev I, Minkov I, Dimitrova I, Bradford AP, Baev V. Characterization of the uterine leiomyoma microRNAome by deep sequencing. *Genomics*. 2012;99(5):275–81. <https://doi.org/10.1016/j.ygeno.2012.03.003>.
44. Guo H, Zhang X, Dong R, Liu X, Li Y, Lu S, et al. Integrated analysis of long noncoding RNAs and mRNAs reveals their potential roles in the pathogenesis of uterine leiomyomas. *Oncotarget*. 2014;5(18):8625–36. <https://doi.org/10.18632/oncotarget.2349>.
45. Wang T, Zhang X, Obijuru L, Laser J, Aris V, Lee P, et al. A micro-RNA signature associated with race, tumor size, and target gene activity in human uterine leiomyomas. *Genes Chromosomes Cancer*. 2007;46(4):336–47. <https://doi.org/10.1002/gcc.20415>.
46. Marsh EE, Lin Z, Yin P, Milad M, Chakravarti D, Bulun SE. Differential expression of microRNA species in human uterine leiomyoma versus normal myometrium. *Fertil Steril*. 2008;89(6):1771–6. <https://doi.org/10.1016/j.fertnstert.2007.05.074>.

47. Pan Q, Luo X, Chegini N. Differential expression of microRNAs in myometrium and leiomyomas and regulation by ovarian steroids. *J Cell Mol Med.* 2008;12(1):227–40. <https://doi.org/10.1111/j.1582-4934.2007.00207.x>.
48. Chuang TD, Khorram O. miR-200c regulates IL8 expression by targeting IKBKB: a potential mediator of inflammation in leiomyoma pathogenesis. *PLoS One.* 2014;9(4):e95370. <https://doi.org/10.1371/journal.pone.0095370>.
49. Chuang TD, Luo X, Panda H, Chegini N. miR-93/106b and their host gene, MCM7, are differentially expressed in leiomyomas and functionally target F3 and IL-8. *Mol Endocrinol.* 2012;26(6):1028–42. <https://doi.org/10.1210/me.2012-1075>.
50. Chuang TD, Panda H, Luo X, Chegini N. miR-200c is aberrantly expressed in leiomyomas in an ethnic-dependent manner and targets ZEBs, VEGFA, TIMP2, and FBLN5. *Endocr Relat Cancer.* 2012;19(4):541–56. <https://doi.org/10.1530/ERC-12-0007>.
51. Qiang W, Liu Z, Serna VA, Druschitz SA, Liu Y, Espona-Fiedler M, et al. Down-regulation of miR-29b is essential for pathogenesis of uterine leiomyoma. *Endocrinology.* 2014;155(3):663–9. <https://doi.org/10.1210/en.2013-1763>.
52. Fitzgerald JB, Chennathukuzhi V, Koohestani F, Nowak RA, Christenson LK. Role of microRNA-21 and programmed cell death 4 in the pathogenesis of human uterine leiomyomas. *Fertil Steril.* 2012;98(3):726–34.e2. <https://doi.org/10.1016/j.fertnstert.2012.05.040>.
53. Harmalkar M, Upraity S, Kazi S, Shirsat NV. Tamoxifen-induced cell death of malignant glioma cells is brought about by oxidative-stress-mediated alterations in the expression of BCL2 family members and is enhanced on miR-21 inhibition. *J Mol Neurosci.* 2015;57(2):197–202. <https://doi.org/10.1007/s12031-015-0602-x>.
54. Luu HN, Lin HY, Sorensen KD, Ogunwobi OO, Kumar N, Chornokur G, et al. miRNAs associated with prostate cancer risk and progression. *BMC Urol.* 2017;17(1):18. <https://doi.org/10.1186/s12894-017-0206-6>.
55. Mei LL, Qiu YT, Zhang B, Shi ZZ. MicroRNAs in esophageal squamous cell carcinoma: potential biomarkers and therapeutic targets. *Cancer Biomark.* 2017;19(1):1–9. <https://doi.org/10.3233/CBM-160240>.
56. Peng Q, Zhang X, Min M, Zou L, Shen P, Zhu Y. The clinical role of microRNA-21 as a promising biomarker in the diagnosis and prognosis of colorectal cancer: a systematic review and meta-analysis. *Oncotarget.* 2017;8(27):44893–909. <https://doi.org/10.18632/oncotarget.16488>.
57. Sims EK, Lakhter AJ, Anderson-Baucum E, Kono T, Tong X, Evans-Molina C. MicroRNA 21 targets BCL2 mRNA to increase apoptosis in rat and human beta cells. *Diabetologia.* 2017;60(6):1057–65. <https://doi.org/10.1007/s00125-017-4237-z>.
58. Bertsch E, Qiang W, Zhang Q, Espona-Fiedler M, Druschitz S, Liu Y, et al. MED12 and HMGA2 mutations: two independent genetic events in uterine leiomyoma and leiomyosarcoma. *Mod Pathol.* 2014;27(8):1144–53. <https://doi.org/10.1038/modpathol.2013.243>.
59. Peng Y, Laser J, Shi G, Mittal K, Melamed J, Lee P, et al. Antiproliferative effects by Let-7 repression of high-mobility group A2 in uterine leiomyoma. *Mol Cancer Res.* 2008;6(4):663–73. <https://doi.org/10.1158/1541-7786.MCR-07-0370>.
60. Bendoraitė A, Knouf EC, Garg KS, Parkin RK, Kroh EM, O'Briant KC, et al. Regulation of miR-200 family microRNAs and ZEB transcription factors in ovarian cancer: evidence supporting a mesothelial-to-epithelial transition. *Gynecol Oncol.* 2010;116(1):117–25. <https://doi.org/10.1016/j.ygyno.2009.08.009>.
61. Sandberg A. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: leiomyoma. *Cancer Genet Cytogenet.* 2005;158:1–26.
62. Aqeilan RI, Calin GA, Croce CM. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. *Cell Death Differ.* 2010;17(2):215–20. <https://doi.org/10.1038/cdd.2009.69>.
63. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem.* 2012;81:145–66. <https://doi.org/10.1146/annurev-biochem-051410-092902>.