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The Mechanism and Function of Epigenetics in Uterine Leiomyoma Development

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Abstract

Uterine leiomyomas, also known as uterine fibroids, are the most common pelvic tumors, occurring in nearly 70% of all reproductive-aged women and are the leading indication for hysterectomy worldwide. The development of uterine leiomyomas involve a complex and heterogeneous constellation of hormones, growth factors, stem cells, genetic, and epigenetic abnormalities. An increasing body of evidence emphasizes the important contribution of epigenetics in the pathogenesis of leiomyomas. Genome-wide methylation analysis demonstrates that a subset of estrogen receptor (ER) response genes exhibit abnormal hypermethylation levels that are inversely correlated with their RNA expression. Several tumor suppressor genes, including Kruppel-like factor 11 (*KLF11*), deleted in lung and esophageal cancer 1 (*DLEC1*), keratin 19 (*KRT19*), and death-associated protein kinase 1 (*DAPK1*) also display higher hypermethylation levels in leiomyomas when compared to adjacent normal tissues. The important role of active DNA demethylation was recently identified with regard to the ten-eleven translocation protein 1 and ten-eleven translocation protein 3-mediated elevated levels of 5-hydroxymethylcytosine in leiomyoma. In addition, both histone deacetylase and histone methyltransferase are reported to be involved in the biology of leiomyomas. A number of deregulated microRNAs have been identified in leiomyomas, leading to an altered expression of their targets. More recently, the existence of *side population* (SP) cells with characteristics of tumor-initiating cells have been characterized in leiomyomas. These SP cells exhibit a tumorigenic capacity in immunodeficient mice when exposed to 17β-estradiol and progesterone, giving rise to fibroid-like tissue in vivo. These new findings will likely enhance our understanding of the crucial role epigenetics plays in the pathogenesis of uterine leiomyomas as well as point the way to novel therapeutic options.

Keywords

DNA methylation, oxidative DNA demethylation, TET proteins, histone modification, miRNA, leiomyoma, stem cells, epigenetics

Introduction

Uterine leiomyomas, or fibroids, are the most common benign tumors of the reproductive tract and serve as the single most common indication for hysterectomies.[1,2] Uterine leiomyomas typically cause severe menstrual bleeding, pelvic pain, preterm labor, recurrent abortion, and infertility. Hysterectomy is currently the main treatment used in women who no longer desire childbearing. This surgery is associated with morbidity and mortality as along with a huge economic impact on the health care delivery system. In the United States, this translates into a cost in the range of US$5.9 to US$34.4 billion/year for uterine leiomyoma management annually.[3] Despite its high prevalence, knowledge about the exact pathogenesis of these tumors is still largely unknown.[4,5]

Epigenetics refers to changes in phenotype mediated by altered gene expression—these changes do not occur as a result of the alteration in DNA sequencing. There are 3 major mechanisms of epigenetic regulation: (a) DNA methylation mediated by DNA methyltransferases as well as active and passive DNA demethylation, (b) modification of histone proteins, and (c) microRNAs. The DNA methylation, histone modification, and miRNA as the mechanisms of epigenetic regulation are involved in a complex network to allow aberrant gene expression for cellular transformation and development of many diseases.[6,7] Thus, the understanding of these mechanisms may provide great opportunities for the optimization of diagnostic and prognostic systems as well as generation of novel therapeutic approaches.

DNA Methylation and Demethylation

Methylation of cytosine residues in CpG dinucleotides within the context of a CpG island is one of the common epigenetic regulation mechanisms in eukaryotes. Hypermethylation of

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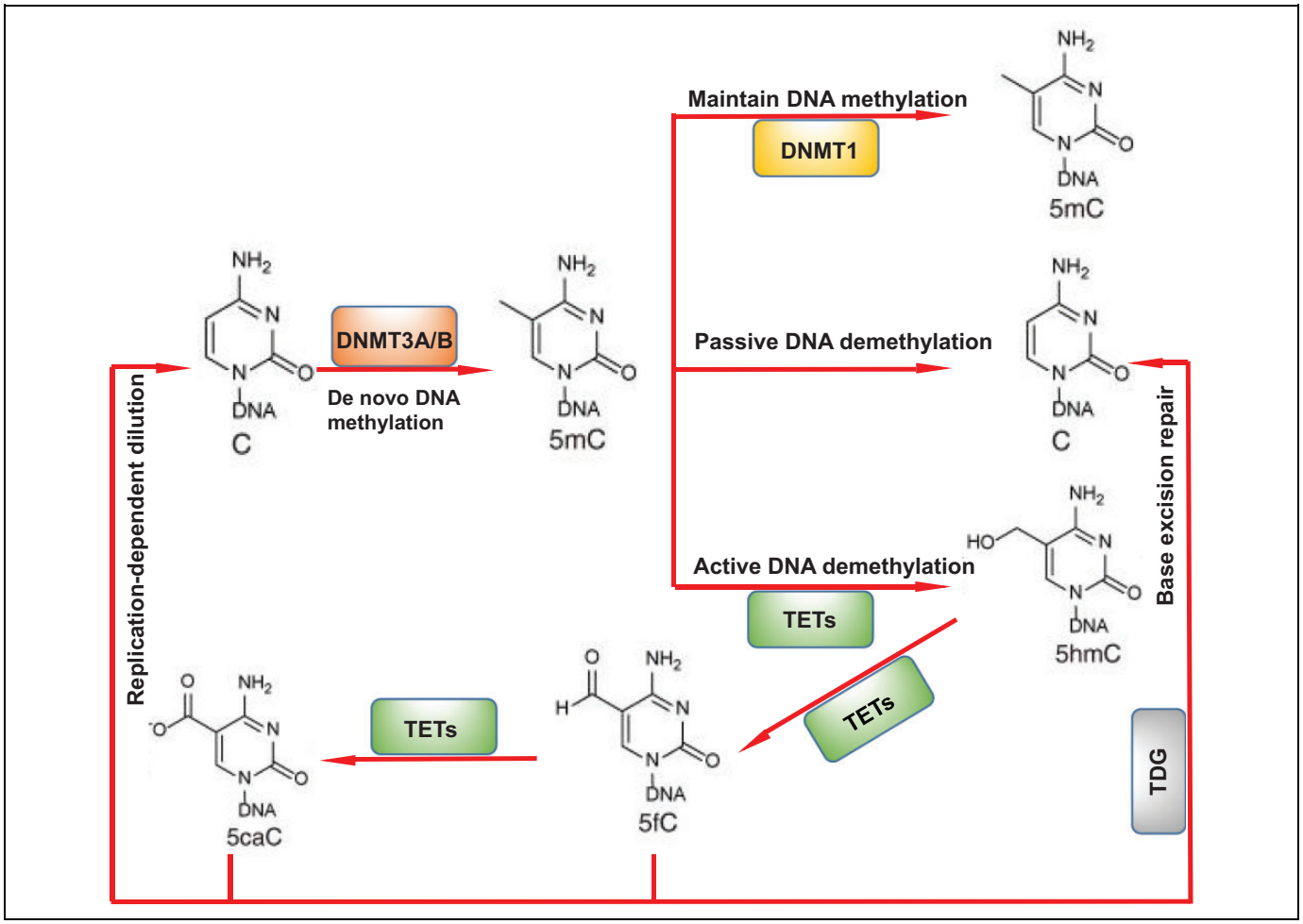

Figure 1. Schematic representation of DNA demethylation pathways. TET proteins can oxidize 5mC to generate 5hmC. 5hmC can also be further oxidized by TET proteins to produce 5fC and 5caC. The above 3 modified C, served as intermediates for DNA demethylation, can be diluted during DNA replication. On the other hand, 5fC and 5caC can be excised from DNA by TDG generating an abasic site as part of the base excision repair process that regenerates unmodified C. DNMT indicates DNA methyltransferase; TET, ten-eleven translocation protein; TDG, thymine DNA glycosylase; C, cytosine; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; 5fC, formylcytosine; 5caC, 5-carboxylcytosine.

CpG island in the promoter region generally results in repression of gene expression, while hypomethylation leads to active transcription.[8] Aberrant DNA methylation occurs in many diseases including cancers,[9-14] diabetes mellitus,[15,16] vascular diseases,[17] immune system-related disorders,[18] and skin diseases.[19] The methylation of cytosine is catalyzed by specific DNA methyltransferases that transfer a methyl group from the donor S-adenosyl methionine to the 5'-position of the pyrimidinic ring. Genomic methylations in mammals appear to be established by a complex interplay of at least 3 DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B).[20,21] DNMT1 is considered to maintain DNA methylation patterns during DNA replication. In contrast, DNMT3A and DNMT3B act primarily as de novo methyltransferases in establishing methylation patterns.[21-26] Furthermore, a number of alternatively spliced DNA methyltransferases or isoforms have been identified in a variety of cancer types. These isoforms either lack DNA binding or catalytic domains that alter gene expression and methylation patterns, which account for the complex epigenome network in cancers.[27-32]

DNA demethylation can occur passively during successive rounds of replication in the absence of functional DNA methylation maintenance machinery. However, this passive model does not adequately explain the loss of DNA methylation in nonreplicating cells, which has been reliably established.[33] In contrast, active DNA demethylation refers to an enzymatic process that removes or modifies 5-methylcytosine (5-mC) with regeneration of unmodified cytosine.[33-35] Ten-eleven-translocation (TET) enzymes convert 5-mC to 5-hydroxymethylcytosine (5-hmC), hence, TET-mediated DNA demethylation processes are considered as active DNA demethylation (Figure 1).

Modifications of Histone Proteins

Yet another key component in the epigenetic regulation of gene expression is posttranslational modifications of N-terminal

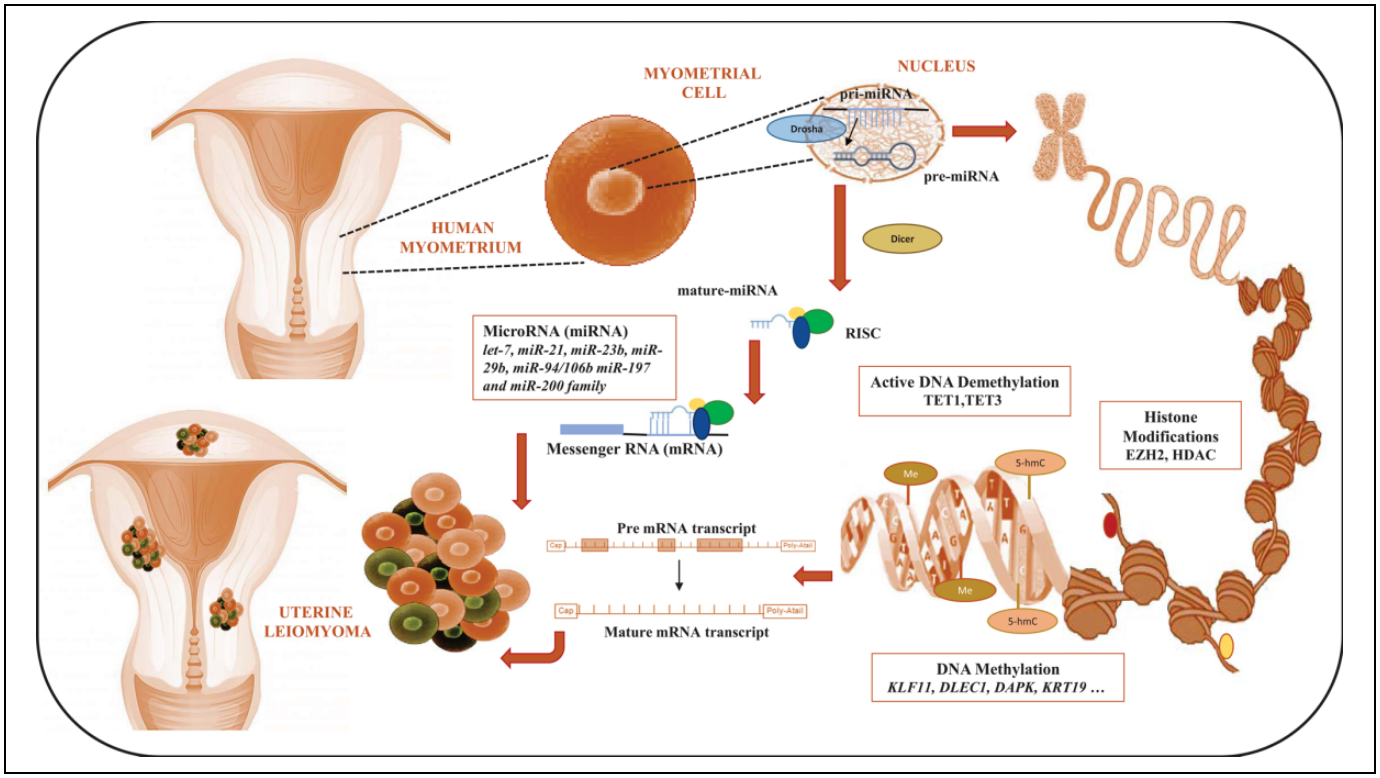

Figure 2. Schematic representation of epigenetic mechanisms involved in human leiomyoma formation. DNA methylation, histone modifications, and micro RNA (miRNA) play a crucial role in modulating the gene expression pattern in the pathogenesis of leiomyoma. *TET1* indicates ten-eleven translocation protein 1; *TET3*, ten-eleven translocation protein 3; *EZH2*, enhancer of zeste 2; *HDAC*, histone deacetylase; *Dicer*, an enzyme that cleaves double-stranded RNA (dsRNA) and pre-microRNA (pre-miRNA) into short double-stranded RNA fragments called small-interfering RNA and microRNA, respectively; RISC, RNA-induced silencing complex; *KLF11*, Kruppel-like factor 11; *DLEC1*, deleted in lung and esophageal cancer 1; *DAPK1*, death-associated protein kinase 1; *KRT19*, keratin 19.

tails or the globular domains of core histones. Histone modifications are involved in many biological processes[36-40] and alter DNA accessibility, thereby modulating the expression of genetic information. Epigenetic modifications of histone tails include acetylation, methylation, phosphorylation, ubiquination, and SUMOylation. In contrast to DNA methylation, histone methylation can result in either activation or repression of gene transcription, while histone acetylation is correlated with gene activation.[41,42] Varying combinations of histone modifications are believed to comprise a histone code that directs biological processes by the recruitment of specific chromatin-associated proteins leading to distinct gene expression patterns.[43]

Posttranslational modifications of histones is one of the major mechanisms for the assembly and compaction of chromatin.[44] Many enzymes, which are involved in their addition and removal, have been identified. These include histone acetyltransferases and deacetylases,[45] lysine methyltransferases and demethylases,[46,47] arginine methyltransferases and demethylases,[48] serine/threonine/tyrosine kinases and phosphatases,[49] and lysine ubiquitinases and deubiquitinases.[50,51] These enzymes exist in multi-subunit complexes and act on distinct amino acid residues of specific histones and within chromatin at certain genomic regions that allow a vast range of flexibility in regulating chromatin dynamics and signaling transmission.[52]

MicroRNAs

MicroRNAs (miRNAs) are evolutionarily conserved, small noncoding RNA molecules (21–2 3nt) that play an important role in transcriptional and posttranscriptional regulation of gene expression.[53] The miRNAs function by binding to complementary sequences within messenger RNA (mRNA) molecules, usually, but not exclusively, resulting in gene silencing via translational repression or target degradation.[54,55] At the same time, a single mRNA can be modulated by multiple different miRNAs, resulting in a sophisticated gene regulatory network. Since the first miRNAs were characterized in the early 1990s,[56] miRNAs have been found to be involved in multiple biological events including numerous diseases.[57-66]

Moreover, increasing evidence in human and experimental animal models has further substantiated the role epigenetic alterations play in reprogramming key sensing and signaling pathways leading to leiomyoma formation (Figure 2). The focus of this review is to provide a comprehensive summary of our current scientific knowledge of epigenetic factors related to human myometrium and their putative implications in leiomyoma formation.

DNA Methylation in Uterine Leiomyoma

The mechanism of deregulation of ER response genes in leiomyoma was recently described by Maekawa et al using genome-wide DNA methylation and mRNA profiling in paired specimens of leiomyoma and adjacent normal myometrium from Japanese women.[67] Although a similar methylation pattern between the myometrium with leiomyoma and the normal myometrium was observed, the methylation pattern in leiomyoma is different from the one in myometrium with leiomyoma or the normal myometrium. Importantly, among 120 genes whose DNA methylation and mRNA expression patterns differed between leiomyoma and the adjacent myometrium, 22 genes including Collagen Alpha-1(IV) Chain (*COL4A1*), Collagen Alpha-3(VI) Chain (*COL6A3*), glutathione S-transferase mu 5 (*GSTM5*), NUAK Family SNF1-Like Kinase 1 (*NUAK1*), the death-associated protein kinase I (*DAPK1*), have the consensus sequence of estrogen receptor (ER) response elements.[67] The latter one, a proapoptotic Ca(2+) calmodulin-regulated serine/threonine kinase, participates in a wide array of apoptotic systems initiated by interferon-γ, TNF-α, activated Fas, and detachment from extracellular matrix.[68] It was found that the mRNA and protein expression of *DAPK1* is frequently lost in various human cancer cells due to DNA methylation in the *DAPK1* promoter region.[69-73] Interestingly, *DAPK1* was also identified as a target gene for DNA hypermethylation in leiomyoma.[74] The DNA methylation analysis demonstrated that fully methylated methylation-specific PCR products in sera of patients with leiomyoma. In contrast, sera from otherwise healthy women showed only partially methylated DAPK sequences.

It is well known that hypermethylation of tumor suppressor genes and hypomethylation of oncogenes contributes to the development of tumorigenesis.[75,76] Another genome-wide DNA methylation study using the high-throughput Illumina Infinium Methylation Readchip (Illumina Inc., San Diego, California) and parallel mRNA expression profiles was performed by Navarro et al.[77] This study was conducted among African American women in which they examined uterine leiomyoma and matched adjacent myometrial tissue. The authors concluded that 55 genes showed differential promoter methylation with concomitant differences in mRNA expression in uterine leiomyoma, when compared to normal myometrium. *DLEC1*, Keratin 19 (*KRT19*), and *KLF11* were among the 3 top tumor suppressor genes found with the latter tumor suppressor gene, *KLF11* considered a target of progesterone or antiprogestins in uterine leiomyoma tissue. The methylation status of these genes was further investigated by bisulfite sequencing. The methylation levels of CpG islands located at the promoter region of *KLF11*, *DLEC1*, and *KRT19* were higher in leiomyoma when compared to adjacent myometrial tissue. These studies suggest the critical role of DNA hypermethylation in the pathogenesis of leiomyoma. Both of the previously mentioned independent studies were performed using genome-wide DNA methylation analysis and mRNA profiling techniques, however, the differential methylated genes and deregulated expressed genes identified by the 2 groups differed substantially. Possible explanations in this discrepancy may lie in (a) the difference in methylation and mRNA platforms used and (b) racial differences and attributes relative to the different gene expression and DNA methylation pattern in leiomyoma.

Additionally, aberrant DNA methylation status of X chromosome-related genes has been observed in leiomyoma. Sato et al[78] demonstrated a higher incidence of aberrant DNA hypomethylation on the X chromosome when compared to the whole genome in uterine leiomyoma. The aberrant hypomethylation gene includes Testis-Specific Protein Y Encoded-Like 2 (*TSPYL2*), which was hypomethylated in 68% of multiple leiomyoma samples. However, the expression of *TSPYL2* was not upregulated in leiomyoma samples.[78]

Leiomyomas are estrogen-dependent tumors. A growing body of evidence within the literature has investigated a link between the ER signaling and epigenetic regulation. Estrogen receptor α (*ER*α) is highly expressed in uterine leiomyomas when compared to normal myometrium, suggesting its role in the pathogenesis of uterine leiomyomas.[79] Recent studies demonstrate that *ER*α expression is regulated by DNA methylation in uterine leiomyoma formation. Asada et al[80] reported a potential link between *ER*α gene expression and hypomethylation. They compared the DNA methylation status around the *ER*α promoter region (−1188 to +299) in myometrium and leiomyoma using the bisulfite sequencing approach and demonstrated that 49 CpG sites in the proximal promoter region of *ER*α gene exhibit no cytosine methylation in either normal myometrium or leiomyomas. However, at 7 CpG sites in the distal promoter region of ERα gene, variation appeared in DNA methylation status between normal myometrium and leiomyoma. The hypomethylation status of ERα distal promoter region is correlated with high *ER*α mRNA expression—such a correlation does not generally seem to be conserved in other uterine diseases. For example, Hori et al[81] reported that *ER*α transcription from the distal promoter, rather than from the proximal promoter, predominates in the proliferative phase of the normal menstrual cycle because hypermethylation of the proximal promoter region was not observed during that phase. However, methylation of the promoter regions of *ER*α gene did not correlate with lack of ERα protein in human patient samples with endometrial disease, including stromal sarcoma, hyperplasia, and adenocarcinoma. In their study, they examined the cytosine methylation status in the proximal promoter region in exon 1 and the distal promoter region in exon 1' of the *ER*α gene in various human endometrial diseases by semiquantitative competitive polymerase chain reaction assay using restriction enzymes (*Hpa*II, *Not*I, and *Sac*II). One possible explanation for this discrepancy may lie in the location of CpG sites they examined. Additionally, the use of different methods in determining the methylation status of the *ER*α promoter region in uterine disease may further explain this incongruence. Yet another possible explanation may lie in the translational failure from RNA to protein within the samples examined. Finally, the methylation pattern in the promoter

Table 1. Validated Epigenetic Targets in Uterine Leiomyoma.

Validated Target Genes	Epigenetic Mechanism	Species	References
ERα	DNA methylation	Human	Hori M et al[81]
ERα	DNA hypomethylation	Human	Asada et al[80]
DAPK1	DNA methylation	Human	Hafner et al[74]
KLF11	DNA methylation	Human	Navarro et al[77]
DLEC1	DNA methylation	Human	Navarro et al[77]
KRT19	DNA methylation	Human	Navarro et al[77]
IRS1	DNA methylation	Human	Maekawa et al[67]
COL4A1	DNA methylation	Human	Maekawa et al[67]
GSTM5	DNA methylation	Human	Maekawa et al[67]
TSPYL2	DNA hypomethylation	Human	Sato et al[78]
OCRL	DNA hypomethylation	Human	Sato et al[78]
HMGA2	let-7 miRNA	Human	Wang et al[28]
TUBB,CYPIB1, CTBP2	miR-200a	Human	Zavadil et al[82]
F3, IL8	miR-94/106b	Human	Chuang et al[83].
ZEB1/ZEB2,TIMP2, FBLN5,VEGFA	miR-200c	Human	Chuang et al[84]
IKBKB	miR-200c	Human	Chuang et al[85]

Abbreviations: *DAPK1*, death-associated protein kinase 1; *DLEC1*, deleted in lung and esophageal cancer 1; ERα, estrogen receptor α; *HMGA2*, high-mobility group A2 protein; *KLF11*, Kruppel-like factor 11; *KRT19*, keratin 19; *IRS1*, insulin receptor substrate 1; *COL4A1*, collagen Alpha-1(IV) Chain; *GSTM5*, glutathione S-Transferase M5; *TSPYL2*, TSPY-Like 2; *OCRL*, oculocerebrorenal syndrome of Lowe; *HMGA2*, high mobility group AT-hook 2; *TUBB*, tubulin, beta class I; *CYP1B1*, cytochrome P450 1B1; *CTBP2*, c-terminal binding protein 2; *F3*, coagulation factor III ; *IL8*, interleukin 8 ; *ZEB1*, zinc finger E-box binding homeobox 1; *ZEB2*, zinc finger E-box binding homeobox 2 ; *TIMP2*, TIMP metallopeptidase inhibitor 2; *FBLN5*, fibulin 5 ; *VEGFA*, vascular endothelial growth factor A; *IKBKB*, inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta.

region of *ERα* between myometrium and endometrial disease may differ in response to environmental factors. The validated methylation-regulated targets in leiomyoma are summarized in Table 1.

Active DNA Demethylation in Uterine Leiomyoma

Reduced levels of 5-hmC have been found in various solid tumors, indicating that TET enzymes may contribute to cellular transformation via regulation of DNA demethylation.[86-88] In addition, TET proteins oxidize 5-mC not only to 5-hmC but also to 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) in a stepwise manner.[89,90] As a new epigenetic modification, 5-hmC may be a useful biomarker for the diagnosis of cancers. These studies challenge the traditional view of DNA methylation as permanent and suggest that DNA methylation in the promoter region may be transient under certain conditions. First evidence of the dysregulation of 5-hmC in uterine leiomyoma was provided by Dr Bulun's group.[91] They demonstrated that an epigenetic imbalance in the 5-hmC content of leiomyoma tissue, caused by upregulation of the TET protein 1 (TET1) and TET protein 3 (TET3) enzymes, contributes to promoting proliferation of human uterine leiomyoma. In contrast to general findings which suggest that 5-hmC levels from malignant cancers including human skin, breast, liver, lung, prostate, pancreatic, and melanoma, are lower, studies from Bulun group showed that 5-hmC levels from leiomyoma were significantly higher when compared to normal myometrial tissue. The increase in 5-hmC levels was associated with the upregulation of TET1 or TET3 mRNA and protein expression in leiomyoma tissue. TET1 or TET3 knockdown significantly reduced 5-hmC levels in leiomyoma cells and decreased cell proliferation. Similarly, treatment with 2-HG, a competitive TET enzyme inhibitor, significantly decreased both 5-hmC content and cell proliferation of leiomyoma cells. These studies suggest that an unusual DNA methylation/demethylation dynamic may be attributable to the alteration of the epigenome and phenotype in leiomyoma. Interestingly, TET1 upregulation leading to a global increase in 5-hmC levels was also found in mixed lineage leukemia (MLL)-rearranged leukemia,[92] indicating that TET proteins may play an oncogenic role in certain tumors.

Histone Modifications in Uterine Leiomyoma

Although epigenetic analysis for the pathogenesis of leiomyoma hasbeen focused on DNA methylation, a couple of studies have demonstrated that histone modification is directly involved in leiomyoma development.[93,94]

The plasticity of gene expression patterns in the developing fetus allows for adaptations of growing tissues/organs to various environmental stimuli in optimizing fetal survival. However, when the in utero environment is suboptimal, permanent developmental reprogramming of the epigenome could take place. Environmental exposure during development can alter susceptibility later in life to adult diseases, including uterine leiomyoma.[95] Currently, the best experimental animal model for studying uterine leiomyoma is the Eker rat model. Eker rats carry a germ line mutation in the tuberous sclerosis complex-2 tumor suppressor gene.[96] Greathouse et al[95] demonstrated that when exposed to diethylstilbestrol (DES) as newborns, adult Eker rats manifested permanent changes in gene expression of the myometrium throughout their adult lifetime. They demonstrated that 171 genes were differentially expressed in leiomyoma, relative to normal myometrium using microarray-based analysis. Among them, several genes (S100 Calcium Binding Protein G [*Calbindin D9K*]; Deiodinase, Iodothyronine, Type II [*Dio2*]; growth differentiation factor 10 [*Gdf10*]; carbonic anhydrase 8 [*CA8*]; glutamate receptor, ionotropic, AMPA 2 [*Gria2*]; and matrix metallopeptidase 3 [*mmp3*]) with putative estrogen responsive elements and confirmed estrogen responsive in the myometrium of 5-month-old rats were reprogrammed by neonatal DES exposure. In animals carrying a germ line defect in Tsc-2, early life exposure to DES during development of the uterus increased tumor-suppressor gene penetrance from 65% to >90% and tumor multiplicity and size.

Tumors that developed in exposed animals displayed an enhanced proliferative response to steroid hormones relative to tumors that developed in unexposed animals, suggesting that gene–environment interactions through epigenetic regulation are important determinants of tumor risk including leiomyoma.[96,97]

Histone methyltransferase enhance of zeste homolog 2 (EZH2) is a member of the polycomb repressive complex 2 (PRC2), which catalyzes trimethylation of lysine 27 of histone H3. Abnormal EZH2 expression has been associated with various cancers.[98,99] Dr Walker's group reported on xenoestrogen-induced regulation of EZH2 and histone methylation via ER signaling to phosphatidylinositol 3-kinase (PI3K)/serine-threonine protein kinase (AKT).[93] They found that in response to both 17β-estradiol (E2) and xenoestrogen diethylstilbestrol, ER signaling via PI3K/protein kinase B phosphorylated EZH2 at S21 and reduced levels of trimethylation of lysine 27 on histone H3 in hormone-responsive cells.[93] A further study indicated that another environmental estrogen, genistein, also induced PI3K/AKT nongenomic ER signaling to the histone EZH2.[94] These studies provide a direct link between xenoestrogen-induced nuclear hormone receptor signaling and modulating of epigenetic machinery during developmental reprogramming in response to environmental estrogen in leiomyoma.

More recently, long noncoding RNAs (lncRNAs) have emerged as important players in the regulation of gene expression in a variety of biological processes.[100-108] The LncRNAs may provide potential guides to complex with chromatin-modifying proteins and recruit their catalytic activity to specific sites in the genome, thereby modifying chromatin states and modulating gene expression.[109,110] For instance, lncRNA has functional links with PRC2,[110] and the direct interactions between PRC2 and RepA/Xist RNAs target PRC2 in cis to the mammalian X chromosome.[111] The involvement of lnc RNAs would provide targeting specificity and introduce new regulatory capabilities.[112] So far the role of lncRNAs in pathogenesis of uterine fibroids has not been identified, therefore, it is encouraged to determine how the lncRNAs alter the chromatin state, especically how lncRNAs interact with polycom group proteins to influence the gene expression pattern through epigenetic machinery.

Histone deacetylases (HDACs), a class of enzymes that remove acetyl groups from an e-N-acetyl-lysine amino acid, also participate in the regulation of gene expression. The HDAC has been shown to be involved in the regulation of tumor suppressor gene, *KLF11*'s function relevant to uterine disease.[113] *KLF11* belongs to the family of Sp1/Krüppel-like zinc finger transcription factors that play important roles in a variety of cell types and tissues.[114,115] KLF11 was found to be diminished in uterine leiomyomas.[116] Zheng et al[113] recently suggested that KLF11 repressed most endometrial cytochrome (CYP) enzymes in Ishikawa cells. They further demonstrated that KLF11 bound to the estrogen-metabolizing enzyme CYP3A4 promoter GC elements and thereby repressed its promoter and enzymatic function. This repression was epigenetically mediated as KLF11 recruited the coreceptor SIN3A/HDAC resulting in selective deacetylation of the CYP3A4 promoter. Moreover, this repression was pharmacologically reversible with an HDAC inhibitor. KLF11 is highly expressed in reproductive tissues, suggesting that cofactor (HDAC) binding likely plays a critical role in the regulation of transcription factors related to the development of uterine diseases including leiomyoma.

Besides DNA methylation-mediated regulation of ERα expression, Wei et al reported that HDAC6 regulated ERα in uterine leiomyoma.[117] HDAC6 belongs to class I HDAC family. It is a unique member of the HDAC family that primarily localizes to the cytoplasm.[118] Wei et al found that HDAC6 expression is mainly detected in the cytoplasm of smooth muscle cells associated with *ERα* expression. In uterine leiomyomas, a strong staining for the HDAC6 in the cytoplasm was frequently observed, where weak staining of HDAC6 was seen in matched normal myometrium. Silencing of HDAC6 expression using the small-interfering (siRNA) approach led to a significant reduction in ERα protein levels but not *ERα* mRNA levels. Furthermore, treatment with lysosome inhibitor CQ, but not with a proteasome inhibitor (MG132), blocked the depletion of ERα protein levels by HDAC6 siRNA, suggesting that HDAC6 siRNA decreased the ERα protein levels by promoting the degradation of ERα protein in the lysosome. Although the role of histone modification on *ERα* signaling regulation in leiomyoma has not been characterized, a previous study demonstrated that H3K27 methylation imposed ligand-dependent of the *ERα*-dependent apoptotic response via Bcl-2 in breast cancer cells.[119]

Interplay Between Epigenetics and Genomics

An increasing body of evidence shows that a link between genetics and epigenetics occurs in many biological events including tumorigenesis. For instance, silencing of DNA Mismatch Repair Protein (*MLH1*) gene expression due to its promoter methylation prevents the normal activation of the DNA repair gene, leading to genomic instability in colorectal cancer formation.[120] Another DNA repair gene, O^6-methylguanine-DNA methyltransferase (*MGMT1*), is hypermethylated in cancers and inactivation of *MGMT1* is associated with *TP53* and *K-Ras* mutations.[121-123] Although little is known regarding the relationship between genetic and epigenetics in uterine leiomyoma, Moore et al[124] previously reported that the chromosomal aberration, t (10:17), in uterine leiomyoma disrupted histone acetyltransferase, monocytic leukemia zinc finger protein-related factor (MORF).[125] Since MORF is a member of the MYST family (the name of this family is derived from its 4 founding members: MOZ, YBF2/SAS3 and, SAS2, and TIP60) of histone acetyltransferases,[126] it is conceivable that dysfunction of MORF by genetic disruption alters chromatin regulation and confers a distinct gene expression pattern in leiomyoma pathogenesis.

Dysregulation of miRNA in Uterine Leiomyoma

Micro-RNAs are deregulated in many biological pathways that may lead to the pathogenesis of leiomyoma. A number of

studies have been conducted to perform profiling and function analyses of miRNAs in human uterine leiomyoma using microarray and deep sequencing.[82,127-129] These studies demonstrate that many miRNAs regulating cellular processes including cell proliferation, apoptosis, cell adhesion, WNT signaling, mitogen-activated protein kinase (MAPK) signaling, nuclear factor κB (NF-κB) activation, and insulin signaling are deregulated in leiomyoma when compared to normal tissues. Importantly, the predicted targets of these deregulated miRNAs including let-7, miR-21, miR-23b, miR-29b, and miR-197 in leiomyoma play an important role in the pathogenesis of leiomyoma. For example, let-7, whose expression is upregulated in leiomyoma, targets high-mobility group A2 protein (HMGA2), which has been implicated in the pathogenesis of mesenchymal tumors such as leiomyoma, lipoma, and hamartoma.[130] Furthermore, subsets of miRNAs are strongly associated with race and tumor size in human uterine leiomyomas.[128] In addition to HMGA2, Fitzgerald et al[131] reported that increased miR-21 levels are predicted to decrease programmed cell death (PDCD-4) expression. In many malignant tumors, PDCD-4 is downregulated and acts as a tumor suppressor, however, PDCD-4 exhibits a unique expression profile, with almost complete absence of PDCD-4 in normal myometrium and high overexpression of PDCD-4 proteins in leiomyoma. Knockdown of miRNA-21 increases PDCD-4 levels in immortalized leiomyoma and myometrium cells indicating that miRNA-21 can regulate PDCD-4 expression. Furthermore, several other miRNA direct targets have been characterized in leiomyoma.[82,83,84] More recently, Chuang et al[85] reported that miR-200c regulates *IL8* expression by direct targeting *IKBKB* and alteration of NF-κB activity in leiomyoma.

A functional and essential role of miR-29b in the uterine leiomyoma formation was recently determined by Qiang et al.[132] In uterine leiomyoma xenografts, restoring miR-29b inhibited the accumulation of extracellular matrix (ECM) and the development of solid tumors, indicating that the downregulation of miR-29b is essential for uterine leiomyoma tumorigenesis. In addition, 17β-estradiol and progesterone downregulated miR-29b and upregulated mRNAs for multiple collagens in uterine leiomyoma xenografts. This study suggests that excessive ECM production in uterine leiomyoma is regulated by steroid hormones via downregulation of miR-29b.[132] The validated miRNA-regulated targets in leiomyoma are summarized in Table 1.

Epigenetic Regulation of Stem Cells in Leiomyoma, Future Directions

Somatic stem cells (SSCs) are undifferentiated cells, presented throughout the body, that multiply by cell division to replenish dying cells and regenerate damaged tissues and thereafter differentiate into tissue-specific types. The SSCs create the dynamic system required for cellular/tissue homeostasis. Accordingly, tumor stem cells have the capacity for self-renewal and tumor maintenance and growth.

Many chromatin regulators are required for development, stem cell maintenance, and differentiation. Epigenetic mechanisms allow genetically identical cells to stably adopt different phenotypes by controlling the transcriptional availability of different regions of genome packing or opening different regions of the chromatin. The combination of transcriptional factors and chromatin remodeling factors might be essential for different aspects of stem cell phenotype.[133] Emerging evidence indicates that PRC1 and PRC2 and HDAC1- and HDAC2-containing complexes (NuRD, Sin3, and CoREST) play a crucial role in stem cell function and cancer pathogenesis.[134-136] Moreover, an increasing body of evidence suggests that specific CpG methylation regions are altered during differentiation of multipotent stem cells.[137-140] Recently, a new epigenetic landscape has been described by genome-wide mapping of 5-mC and 5-hmC and active/repressive histone code marks associated with DNMT3A expression in hematopoietic stem cells.[7]

Uterine leiomyomas are monoclonal tumors that arise from the uterine smooth muscle tissue.[2,141] A limited number of genetic defects transmitted by germ cells have been associated with familial uterine leiomyoma syndromes. So far, there are no published data showing the involvement of epigenetic changes in these patients, for example, hereditary leiomyomatosis and renal cell carcinoma which are linked with germ line mutation of fumarate hydratase.[142-144] Although the cellular origin of uterine leiomyoma remains largely unknown, several lines of evidence suggests that each leiomyoma originates from the transformation of a single somatic stem cells.[145-153] Moreover, stem cells derived from leiomyoma carry Med12 mutations, which suggests that at least 1 genetic hit is initially required to transform myometrial stem cells.[2]

The existence of putative SSCs was first identified in 2007 in mouse myometrium and nonpregnant human myometrium using 5-bromo-2'-deoxyuridine (which permits the identification of label-retaining cells), and side population technique, respectively.[145,154] A subset of myometrial cells isolated from human myometrium (side population) exhibit characteristics similar to stem cells. In contrast to the main population of myometrial cells, the side population of myometrial cells are capable of generating functional human myometrial tissues efficiently when transplanted into the uteri of severely immunodeficient mice.[145] Subsequently, the leiomyoma-derived side population, which have stem cell characteristics, were characterized and demonstrated to be responsible for cell proliferation and tumor growth[146,151] (Figure 3). Although leiomyomas have a lower percentage of side population cells when compared to normal myometrium,[150] the wingless-type MMTV integration site family (WNT)/β-catenin pathway in leiomyoma stem cells is activated in a paracrine manner leading to the promotion of tumor growth. WNT/β-catenin signaling is a key regulator of multiple aspects of tumorigenesis,[155] embryonic development, and tissue homeostasis. In a cell coculture system, where both leiomyoma stem cells and mature myomentrial cells exist, estrogen–progesterone selectively induced nuclear translocation of β-catenin, leading to the proliferation of leiomyoma stem cells.[147] Accordingly, aberrant regulation of

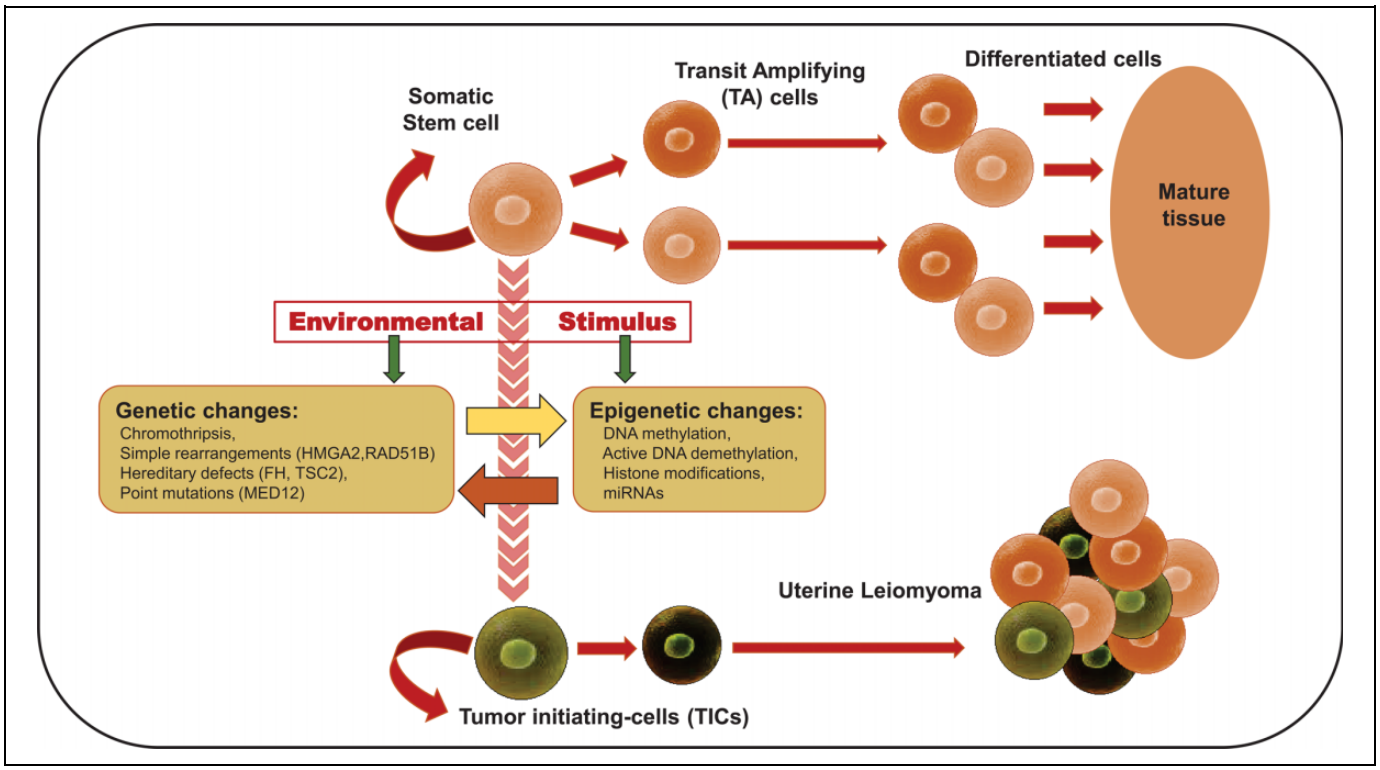

Figure 3. Hierarchy of differentiation of stem cells and specific genetic/epigenetic abnormalities relevant to human leiomyoma formation. Environmental factor-induced genetic/epigenetic abnormalities alter the gene expression pattern and function of undifferentiated stem/transit amplifying (TA) cells leading to the formation of a population of proliferating cells, called tumor-initiating cells (TICs), which differ from the rest and could develop into a uterine leiomyoma. *HMGA2* indicates high mobility group AT-Hook 2; *RAD51B*, RAD51 Paralog B; *FH*, fumarate hydratase; *TSC2*, tuberous sclerosis 2; *MED12*, mediator complex subunit 12.

the canonical signaling (WNT/β-catenin/glycogen synthase kinase 3 (GSK-3)-3 axis) has been reported to be involved in the formation and maintenance of cancer stem cells.[156]

Improved approaches in the characterization and isolation of myometrium and leiomyoma derived SSCs will shed new light on how deregulated epigenetic factors promote the pathogenesis of leiomyoma and provide a potential therapeutic approach for targeting leiomyoma stem cells.

Concluding Remarks

Aberrant epigenetic changes including DNA methylation, histone modifications, and miRNAs are common molecular lesions in tumor cells. Although great progress has been made in understanding the epigenetic mechanisms related to tumor development, little is known about the mechanisms and function of epigenetics in uterine leiomyoma formation. Recently, several approaches including mapping of the genome-wide distribution of 5mC and oxidized 5mc derivatives have led to a better understanding as to the epigenome in tumor biology and stem cell somatic cell reprogramming.[157-159] The dynamic of active DNA demethylation and DNA methylation in leiomyoma provides a network for regulating DNA methylation status across the genome.

In contrast to intensive genome-wide studies on DNA methylation and miRNAs, little is known about the regulation of histone modifications globally as well as the interplay between DNA methylation and histone modifications in the development and pathogenesis of leiomyoma. These studies will lead to a better understanding as to the mechanisms and pathology of leiomyoma formation. Comprehensive studies on histone modifications are needed to fully explore several available and underdeveloped histone-based therapeutic agents for the treatment of uterine leiomyoma.

Similarly, recent studies demonstrating the existence of side populations with stem cell characteristics in myometrium and leiomyoma will be helpful in determining how leiomyoma stem cells are derived and what role epigenetic events play during these processes. Understanding the abnormal signaling and epigenetic regulation within leiomyoma stem cells will provide new opportunities to develop an efficient therapeutic approach, capable of effectively reducing the severity and size of uterine leiomyoma while avoiding side effects.

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