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Latest Evidence in the Pathogenesis of Uterine Fibroids

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Abstract:

Uterine fibroids (UFs) are extremely common neoplasm affecting up to 8 out of 10 women during middle or late reproductive years leading to significant morbidity. Some of the serious complications associated with UFs include abortion, abnormal foetal formation, obstructed labour, postpartum haemorrhage in pregnancy and premature deliveries in pregnant women whereas heavy and prolonged menstrual bleeding, pelvic pressure, anaemia, urinary incontinence, constipation and infertility were discovered in non-pregnant women. Hormonal irregulation particularly estrogen hormone followed by genetics, epigenetics, environmental factors, and lately gut microbiota have been associated in the pathogenesis of UFs. The most common indication for UFs is hysterectomy, whereas medications available were less effective in most of the cases. There are various review articles that have been published on UFs in the past decade. However, up to date review that comprehends epidemiology, pathogenesis, physiopathology and classifications of UFs is not available Thus, our goal is to explore and scrutinize every aspects within the topic to help provide absolute understanding on UFs.

Keywords: Uterine Fibroid, neoplasm, estrogen, pathogenesis, hysterectomy

1.0 Introduction:

Uterine fibroids (UFs), otherwise known as leiomyomas or myomas are a common pelvic neoplasm occurring in women of a reproductive age. These fibroids are benign tumours originating from the myometrial smooth muscle cells. Besides smooth muscle, UFs also comprised of extracellular matrix, for instance, collagen, proteoglycan and fibronectin. Depending on the study population and diagnostic method, the prevalence of UFs were reported to be around 4.5% to 68.6%, with black women having two to threefold increased risk as compared to white women(1). However, the prevalence tends to be underestimated as only approximately 25 to 50% of women becomes symptomatic, experiencing symptoms such as heavy bleeding, bleeding between periods, pressure on the bladder and painful sexual intercourse which greatly influenced their quality of life(2). In this context, a cross-sectional study revealed that UFs had a moderate to significant impact in the quality of life among 64% of women with UFs(3).Among the treatment options available, hysterectomy is the only definitive treatment which involves the surgical removal of the entire uterus.UFs have also been the main indication for hysterectomy in the United States, which comprised about 50% of all hysterectomy cases(4,5). In addition, UFs significantly burdened the health care

system, with a total direct cost of 2 billion per year attributed to UFs alone. Among this, majority of the cost are due to inpatient care, particularly hysterectomy (6). The cause of UFs is multifactorial, with multiple pathway being identified to contribute to the formation of UFs. Among this, hormones such as oestrogens and progesterone, excessive extracellular matrix and epigenetics have been linked to the aetiology of UFs(7–9). Thus, this review aims to discuss and present comprehensive knowledge on the prevalence, physiopathology and pathogenesis, of uterine fibroids.

2.0 Prevalence of Uterine Fibroids

Generally, UFsoccurs in 50-60% of women rising to 70-80% by the age of 50(10). As UFs is an asymptomatic condition, the prevalence tends to be underestimated as most of the epidemiologic studies focused on symptomatic womenrather than the asymptomatic women and those who attended to gynaecological clinics. However, study conducted in US on randomly selected women of age between 35- 49 years showed that the incidence of UFs at the age 35 was 60%, increasing to more than 80% by age of 50 inAfrican American women. The exact prevalence of UFs isbelievedto beremained

Table 1: The Prevalence of Uterine Fibroids at different country

Country	Year conducted	Diagnostic test	Prevalence	Reference
United Kingdom **France** **Canada** **USA** **Brazil** **Germany** **Korea** **Italy**	2009	Self-report	4.5% (n=1,503) 4.6% (n=1,465) 5.5% (n=851) 6.9% (n=7,685) 7.0% (n=5,543) 8.0% (n=1,951) 9.0% (n=1,353) 9.8% (n=1,396)	Zimmermann et al(2)
France **Germany** **Italy** **Spain** **United Kingdom**	2010	Self-report	26.4% (n=358) 34.3% (n=345) 42.4% (n=351) 37.9% (n=352) 36.8% (n=350)	Downes et al(11)
Korea	2013	Data obtained from Korean National Health Insurance Service (NHIS) cohort data	2.43% (n=302,760)	Lee et al(12)
Nigeria	2014	Sonography	6.83% (n=176)	Ukwenya et al(13)
South India	2014-2016	Sonography, Histology	37.65% (n=136)	Munusamy et al(14)
India	2015-2017	Ultrasound	11.6% (n=522)	Srilatha et al(15)
US	2016	Self-report	9%	Marsh et al(16)
China	2016	Data extracted from Global Burden of Disease Study 2016	4.10% (n=27, 169,312)	Ji et al(17)

in the shadow. However, studies reported on the prevalence of UFs by various regions and countries were summarized in Table 1.

3.0 Physiopathology of Uterine Fibroids

Uterine Fibroids occurs from the excessive growth of smooth muscles and connective tissuesof the endometrium. According to WHO, UFs is defined as “a benign, smooth-muscle tumour that has several variant morphological features”(18). Two stages have been identified in the development of UFs, which is the conversion of normal myocytes to abnormal myocytes with a decreased contractile organelles and a higher rate of cell division and the subsequent development into fibroid(19). The development of fibroids is classified into 4 phases according to the collagen content and the rate of proliferation of the myocytes. Going across the different phases, the collagen content seemed to increase with a decrease in proliferation and a decrease in density of micro-vessel. Most of the fibroids identified in phase 3contains an estimation of 10-50% collagen with early senescence at the end of phase 3(20). The arrangement of myocytes of UFswas observed to be disorganized as compared to the normal myometrium. As the extracellular matrix accumulates across the phases, this causes the distance between myocytes and the distance of myocytes to the capillaries to increase that leads to ischemia. Eventually, ischemia will lead to atrophy of the myocytes(20).

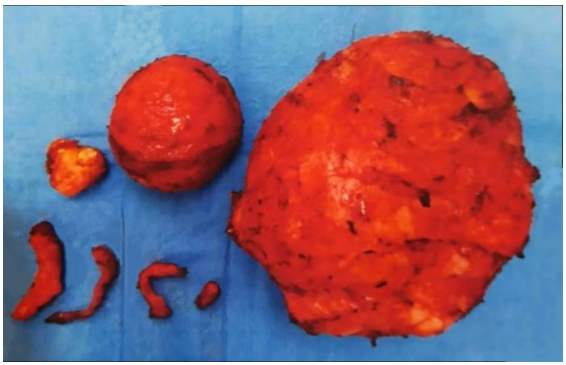

Figure 1.Different sizes of uterine fibroids

The size of fibroids depends on various factors, mainly with elevated oestrogen hormones, which relatively leads to an increase in the size of UFs. Based on findings, the size of an UFs can be as small as apple seed to as big as honey dew, with diameter of more than 10cm. Figure 1 shows the different sizes of fibroids removed from a women.

Classifications of Uterine Fibroids

Different classifications for UFs have been addressed in studies, however UFs are commonly classified according to their anatomical location into subserosal, intramural and submucosal fibroids(18,21). The most common UFs is the subserosalfibroid which is asymptomatic unless the size is very big. It arises from the myometrium and grows out towards the serosal surface. It may be sessile or pedunculated. The intramural UFs are present within the uterine wall myometrium and may affect the shape of the uterine cavity. They may appear with symptoms such as infertility or menorrhagia while submucosal UFs are symptomatic at large. Same as all types of UFs, submucosal UF sarises from the myometrium and it grows towards the endometrial cavity, protruding it.Submucous UFs can be further classified into type 0 (fibroid polyp), type I (less than 50% is contained within the myometrium) and type II (more than 50% intramural extension) (22). According to Nguefack et al., 89.4% of the UFs is classified as submucous, 74.5% were intramural UFs while 10.6% were subserous(23). The fibroid with the most significant effect towards pregnancy and implantation rate issubserosal fibroid followed by intramural and submucosal. Studies have also reported the detrimental effect of submucosal and intramural UFs towards pregnancy rate, with fertility being improved following myomectomy(24–26). A consistent report on subserosal fibroids showed no negative impact towards pregnancy rate and there was also no improvement observed following myomectomy(26,27).

The International Federation of Gynecology and Obstetrics (FIGO) classifications for Uterine fibroids are summarized Table 2(28).

Table 2. FIGO classification of Uterine Fibroids

Stages	Description
0	Pedunculated intracavitary submucosal
1	<50% intramural submucosal
2	≥50% intramural submucosal
3	Intramural fibroid in contact with endometrium or 100% intramural
4	Completely located intramurally
5	Subserosal that is ≥50% located intra-murally
6	Subserosal that is <50% located intra-murally
7	Subserosal pedunculated
8	Others (such as cervical, parasitic)

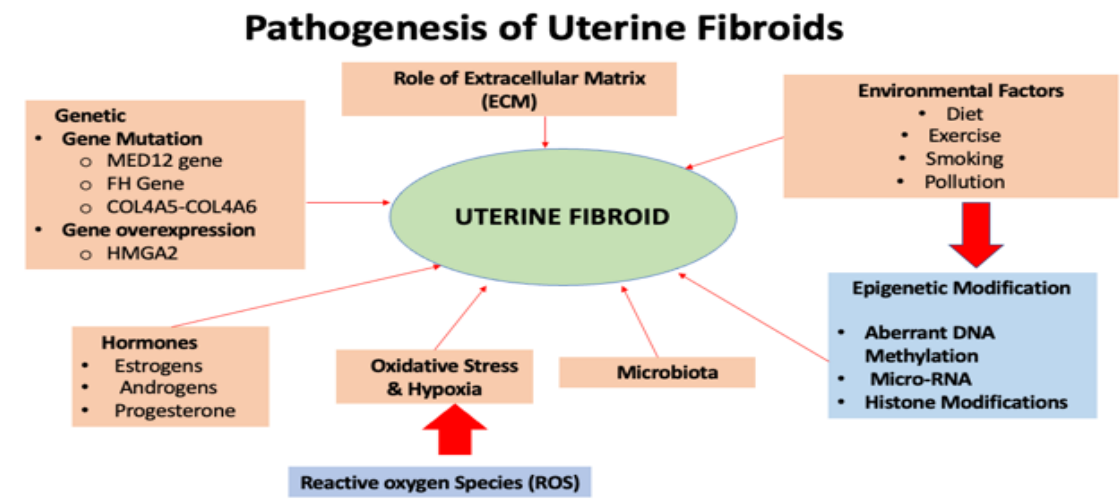

Figure 2. Pathogenesis of uterine fibroids summarized

Hormones role in Uterine Fibroids

Uterine fibroids have been linked to various pathogenesis as shown in figure 2, with hormonal role being one of the major contributors to UFs. UFs are commonly observed during the reproductive years of woman with a peak incidence at around 40 years old, with no cases being reported before puberty. In addition to that, association between the use of oral contraceptives with UFs has also been found with a higher risk of UFs occurring in women who used oral contraceptive at a young age, suggesting the role of estrogen in the pathogenesis of UFs(29). Following that, studies have identified the role of progesterone in the development of UFs by involving in the regulation of genes involved in proliferation and apoptosis. (30)Knowing the fact thatUFsis hormone dependent, the role of each hormone specifically estrogen, progesterone and androgen on UFs are illustrated in detail as below.

Estrogenin UFs

High level of estradiolwas shown to increase the risk of UFs in midlife women without any

fibroids previously(31).There were studies reported on increase in estrogen receptor mRNA andtranscription factor in patients with UFs as compared to individual without UFs (32–34). Supportingthis, another study mentioned that UFshas about 40% higher concentration of estrogen receptor as compared to normal myometrium(35). The greater the prevalence of UFsduring the first few years before reaching menopause, and when missed ovulation occurs more frequently with a decrease in progesterone level suggest that estrogen have a more significant role in UFs as compared to progesterone(36).

Furthermore, studies have also reported an increased aromatase expression in women with UFs as compared to normal myometrium(34,37–39). The ability of aromatase to convert androstenedione to estrogen, thereby allowing the leiomyoma cells to synthesize sufficient estrogen resulting in cell proliferation and hence, fibrosis(39). These results are in line with the findings that the use of aromatase inhibitor is able to reduce leiomyoma cell growth and may also shrink the tumour(40–42).

Estrogen has been found to promote leiomyoma via various mechanisms. Among them, it has been hypothesised that estrogen stimulates leiomyoma via cytokines, growth factors or apoptosis-inducing factors. (43) The ability of 17-beta estradiol in down-regulating p53, a tumor suppressor protein, is also proposed as a possible mechanism as to how estrogen contributes to UFs(44). Protein p53 is involved in the regulation of cell cycle by affecting the expression of other factors involved in cell proliferation and cell death, such as p21 or Bax(45–47). It arrests cell cycle particularly at the G1 phase, and permits damaged DNA to be repaired(45). The reduction in the level of p53 by estrogen thereby reduces apoptosis and allows the tumour to proliferate.

The other important functions of estrogen in the development of UF sis the increase of progesterone receptor, causing the tissues to be more sensitive towards progesterone(48).

Androgen in UFs

In previous studies, androgen was not identified as a factor for UFs development. However, one study by Women's Health Across the Nation (SWAN) has changed the perspective on androgen when they identified the role of androgen in UFs which increases the odd of women by 1.33 times in developing UFs. The mechanism proposed is due to the ability of androgen, in particular testosterone to be converted by aromatase to estradiol. Prior to this, another study have also demonstrated an increased level of androgen receptor protein by 100% in leiomyoma tissue, which highlighted androgen receptor involvement in the cell division of leiomyoma cells(49). This study also suggest , androgen receptor promotes the cell growth in leiomyoma via the insulin-like growth factor-1 (IGF-1)-mediated myometrial cell proliferation and the myeloid cell leukemia 1 (MCL1)-mediated regulation of myometrial cell apoptosis(49). This is attributed to the need of androgen receptor for IGF-1 receptor protein stability and myometrial cell proliferation via ligand-independent mechanisms(50) while MCL1 (an anti-apoptotic protein)(51) is involved in the androgen receptor-activated pathway that mediates the anti-apoptotic function in myometrial cell(49).

Progesterone in UFs

Studies have proved, the level of progesterone receptor is significantly higher in patient with UFs, suggesting that activation of progesterone receptor may be favourable for leiomyoma growth(52,53). Progesterone produces its action by acting on progesterone receptor which consists of two types, PR-A and PR-B.

One of the mechanisms proposed is the ability of progesterone to increase the expression of Bcl-2 protein. Bcl-2 is a proto-oncogene which have the ability to block apoptosis and lead to reduced cell death and increase the proliferation when there is an increased expression of Bcl-

2, resulting in tumor(52). Retinoid acid (RA), a natural metabolite of vitamin A, is also involved in signalling, which plays an important role in specifying cell identities and control gene expression via the interaction with specific nuclear receptors(54), hence the reduction in RA may leads to uncontrolled gene expression, hence fibroids. Omar et al found that RA synthesis genes are greatly repressed in the presence of progesterone while RA catabolic enzyme is increased by progesterone, thereby reducing the concentration of RA available. All this thus creates a favourable environment that supports cell division, and increase the risk of UFs (52).

Environmental Factors and Epigenetics in Uterine Fibroids

Diet and UFs

Limited literatures reported on the effect of diet on pathogenesis of UFs. Based on a cohort study conducted by Gao et al., women who consumed milk or soybean frequently are relatively at a higher risk of developing UFs(55). This has been postulated to be due to the animal or plant estrogen-like substance that is well-known to be rich in milk or soybean, which may exert estrogen-like functions, stimulating the growth of UFs(56–58).

On the other hand,the effect of alcohol consumption on UFs is controversial. However, based on independent studies reported(59,60),there is a positive association between alcohol consumption and the development of leiomyoma by increasing the activity of aromatase in the liver resulting in conversion of androgens to estrogens, which increases the proliferation activity of estrogen(61). Alcohol also interrupts with the metabolism of estrogen, causing an accumulation of endogenous estrogen(62–64). Alcohol may also cause an increase in luteinizing hormone release, causing an increase in estradiol release from the ovaries(65). On the contrary, a recent systematic review and meta-analysis reported that myoma risk is not associated with alcohol intake(66). However, the particular study is limited by the number of studies included in the analysis.

Apart from that, the risk of UFs was shown to have an inverse relationship with fruit intake, specifically with citrus fruit and dietary vitamin A. It has been proposed that vitamin A may reduce UFs risk through the retinoic acid pathway, which has been reported in several studies showing the altered expression of retinoic acid in patients with UFs as compared tonormal myometrium (67,68). When ingested, vitamin A is converted to more active compound, including RA, in which the RA is important to control gene expression to reduce the risk to UFs. In a study conducted by Catherino et al.(69), it has been reported that there is a reduction in genes involved in the formation of RA in leiomyoma, with an increase in genes involved in the metabolism of RA, thereby contributing to the reduction of active RA. The author also reported a higher rate of RA metabolism in leiomyoma in comparison to myometrium tissue. Complement to this is another study by Malik et al.(68) who concluded that a down-regulation of cell proliferation, RA metabolism and TGF-beta regulation was also observed when UFs was exposed to all-trans retinoic acid (ATRA).

Exercise and UFs

Study conducted by Wyshak et al. reported that former non-athletes were at 1.4times greater risk of developing UFs as compared to athletes(70). Supporting this, another study reported that the development of UFs was decreased in both African-American and White women who have exercised for an estimated duration of at least 4 hours of vigorous activity per week(71).

Several mechanisms have been proposed to explain the protective effect of exercise on UFs.A systemic review and meta-analysis on the effect of physical activity on sex hormones also concluded that physical activity decreases the level of circulating sex hormones(72). Exercise has been suggested to affect estrogen metabolism, such that an

increased estrogen metabolite is formed, reducing the concentration of estrogen in the body(73). However, there were also studies showing that estrogen level is not being affected by exercise(74,75). Another mechanism proposed is the ability of exercise to increase the level of sex hormone-binding globulin, thereby reducing the bioavailability of circulating estrogen(76,77).

Smoking and UFs

Few studies have reported an inverse relationship between smoking and uterine fibroid(78–81).Parazzini et al., reported that the incidence of UFs was 10% lesser in current smokers than the control group(79).Study by Ross et al.(81), also identified a dose-dependent relationship between smoking and UFs, where the chain smokers showed one third lesser risk than the non-smokers which might be due to the anti-estrogenic effect of smoking(82,83). It was reported that the components of tobacco smoke causes the upregulation of a number of genes, including CYP1A1 that catalyzed the 2-hydroxylation of estradiol(84). This reduces the bioavailability of the estrogenically potent 16α-hydroxylation. Nicotine in cigarette smoke also inhibits granulose cell aromatase in a dose-dependent manner, reduces the conversion of androgens to estrone(85).

On the contrary, the study conducted by Wong et al. found that midlife women who never or was a former smoker who were exposed to environmental tobacco smoke(≥ 1 person-hour/week) has a higher risk of developing fibroids as compared to women who were not exposed. The study also reported the difference in the risk of UF sin women who is a former smoker and those who never smoked, with a two times risk in women who is a former smoker(86). Another prospective cohort study also reported that smoking were unrelated to risk of UFs(60). A recent systemic review and meta-analysis on cigarette smoking and risk of uterine myoma concluded that smoking does not exert a significant effect on uterine myoma. However, this study is limited by the number of studies in each sub-analysis(87).

Pollution and UFs

Chronic exposure to fine particulate matter (PM2.5) have been associated with a higher incidence of UFs. In the same study, living closer to the roadway was found to have a small elevation in the risk of UFs, though not statistically significant (88). This may be due to the presence of environmental endocrine disrupting chemicals (EDC), including diethylstilbestrol(89,90), phenols (91) and polychlorinated biphenyls (92), that have all shown to be associated with UFs. Most of the EDCs are synthetic compounds that produce estrogenic effect and are able to interact with hormone transport proteins (93). Air pollution being an important source of polycylic aromatic hydrocarbons (PAH)(94) and the other components present in the air demonstrated involvement in hormonal activity and was shown to be bound to aryl-hydrocarbon repector (AhR) in in vitro studies (95–97). AhR is a receptor that mediates the toxicity of EDC with xeno-estrogenic activies(98). AhR participates cellular differentiation and proliferation by interacting with the genes that are involved with it, hence an overexpression of AhR may lead to increased cell proliferation and resulting in fibroid formation (99,100). A study conducted by Bidgoli et al. showed that those living at a closer distance to companies producing PAH were shown to have a higher risk of UFs with AhR over-expression (93).

Aberrant DNA-methylation in UFs

Early studies have reported on the DNA global hypomethylation in leiomyoma tissues as compared to normal myometrium, while 95% of DNMT1, a DNA methyltransferase responsible for methylation was either equally or overexpressed in leiomyomas, that is proposed to reflect the increased cell proliferation(9). This result is reasonable as hypermethylation of gene will lead to gene silencing, thus hypomethylation in UFs may be responsible for the increased expression of gene that is associated with

estrogen as well as cell proliferation. DNMT3A and 3B however have a decreased expression in UFs, suggested to be associated with the global hypomethylation(9). This study thus suggests the possibility of epigenetic being a part of pathogenesis for UFs. Another genome-wide analysis conducted on 18 African American women observed that the promoter of 55 genes were differentially methylated with concomitant differences in the expression of mRNA in women with UFs as compared to normal myometrium (101). The promoter region of three known tumour suppressor genes, KLF11, DLEC1 and KRT19 were studied using bisulfide genomic sequencing. CpG islands present at the promoter region were identified to be hypermethylated in women with UFs as compared to adjacent myometrium (101). The researchers further reported on an inverse relationship between methylation of DNA with gene silencing. These studies further support the role of epigenetic in the development of UFs(101).

It has been found that the mRNA of estrogen and its transcription is elevated in UFs, which might be contributed by the epigenetic mechanisms. This is supported by Asada et al., who have identified that the promoter region of ER-α is hypomethylated in 9 out of 11 patients in leiomyoma as compared to normal myometrium. The ER-α mRNA level was also higher in those patients, which showed that the aberrant DNA methylation is associated with the expression of ER-α mRNA (102.)

In addition, Maekawa et al. identified 120 genes that have a varied DNA methylation and mRNA expression between leiomyomas and the normal myometrial cells (103). Coherent with previous studies that showed an increased expression of COL4A1 and COL4A2 in UFs, this study also showed that COL4A1 and COL4A2 gene are hypomethylated with an increased transcription in leiomyoma (103). Collagen is the most predominant part of the extracellular matrix in UFs. An exacerbate production of collagen and hydroxylation of collagen was associated with an increased size and fibroid formation (103). IRS1 was also shown to be hypomethylated with increased expression. COL4A1 and COL4A2 and COLRA1 gene was shown to have a decreased expression in IRS1 knockout mice in a separate study, which suggests that IRS1 is involved in UFs by increasing the gene expression of these collagen genes (104).

Micro-ribonucleic acids (mi-RNA) in UFs

The study conducted by Wang et al. (2007) (105) was one of the earliest studies conducted on the transcriptome analysis of the uterine tissue that involved miRNA. The study revealed that 45 miRNA among the 206 miRNA analysed were significantly up or down regulated in patient with UFs as compared to normal myometrium. Let-7 family, miR-21, miR-23b, miR-29b and miR-197 are among the five most common dysregulated miRNA. This study also reported that some of the miRNAs varied with race and tumour size. Following this, various other studies that have been conducted to investigate the role of miRNA in UFs have also reported an aberrant expression of miRNA in UFs as compared to normal myometrium, which is proposed to contribute to their aberrant mRNA levels and transcription (106,107).

As UFs is estrogen-dependent in its development, the level of miRNAs that are associated with sex-steroid receptor such as miR-21, miR-34a, miR-125b and miR-150 were found to be higher in leiomyoma (107). Coherent to the study mentioned earlier conducted by Wong et al., Georgieva et al.(106) also found that miR-21 is being overexpressed in UFs. Overexpression of miR-21 was found to elevate the gene and expression of TGF-β3 and an increased proliferation of leiomyoma cells were observed (108). When TGF-β inhibitor was used in Eker rats induced with UFs, the incidence and multiplicity of UFs was greatly reduced, thus supporting the hypothesis that the increased expression of TGF-β caused by miR-21 is associated with fibroid development(109). A recent study investigated on the expression

and effect of miR-129 on UFs also reported a decreased expression of miR-129 in UFs(110). A high expression of miR-129 plays a role in cell cycle by inducing apoptosis and was found to reduce proliferation of cells by reducing the expression of TET1 (110). Hence, the low level of miR-129 in UFs allows the fibroid cells to proliferate, and plays a role in the pathogenesis of UFs. The data obtained from all these studies therefore highlights the role of microRNA in the development of UFs and therefore it can be considered as a potential target for treatment.

Histone Modification in UFs

Histone modification has also been associated with UFs. A study conducted on primary cell cultures from UFs and normal myometrium found that histone deacetylase (HDAC) level was higher in leiomyoma cells that was treated with estradiol, while the activity of histone acetyltransferase (HAT) was barely detected. An independent study by Wei et al. also showed that the level HDAC6 is significantly increased in UFs sample as compared to myometrium. In addition, it was found that the level of HDAC6 is positively associated with ER-α, while when HDAC6 was silenced, ER-α expression diminished which inhibited the growth of leiomyoma cells (111).

In 2014, there was another study conducted to investigate the epigenetic mechanism of KLF11 in regulating CYP450 enzyme (112). It was found that KLF11 regulate CYP450 by first binding to the GC element promoter of CYP3A4. Following this, KLF11 will then attract and bind to SIN3/HDAC. Subsequently, HDAC deacetylates histone on the CYP3A4 promoter, which causes the chromatin to compact and thus silencing the CYP3A4 gene (112). Knowing that KLF11 mRNA level is lower in UFs than normal myometrium (101), the decreased KLF11 and increased expression of CYP3A4 may be responsible for metabolizing estrogen to its active metabolite which contributes to the development of UFs. These therefore supports the involvement of histone modification as part of the pathogenesis for UFs.

Genetics in Uterine Fibroids

Table 3. Frequency of MED12 mutations in Uterine Fibroids on different populations

Country	Year	Ethnicity	Frequency	Reference
Finland	2011	-	70% (159/225)	Mäkinen et al(122)
Korea	2012	Asian	52.2% (35/67)	Je et al(121)
USA	2012	Black American	78% (18/23)	McGuire et al(116)
		White American	66% (79/120)	
Japan	2013	Asian	80% (36/45)	Matsubara et al(118)
Finland	2014	-	85.5% (65/76)	Heinonen et al(115)
Southern United States	2015	African American, Caucasian and Hispanic	63.63% (92/143)	Halder et al(123)
China	2015	Asian	54.39% (93/171)	Ye et al.(124)
Iran	2016	Asian women	31.07% (32/103)	Sadeghi et al(119)
Russia	2016	Russian	51.5% (63/122)	Osinovskaya et al(117)
China	2017	Asian	43.6% (158/362)	Wu et al(125)
South Korea	2018	Asian women	66.67% (40/60)	Lee et al(120)

Mutation of MED12 gene in UFs

MED12 is one of the important genes involved in the development of UFs, by bridging the interaction between MED13 and cyclin C-CDK8 that activates the kinase activity of CDK8, thereby regulate gene transcription(113). In a study conducted by Mittal et al, it was shown that mice that expressed MED12c.131G>A variant showed chromosomal rearrangements with a greater sized leiomyoma lesions, suggesting the importance of this mutation in the pathogenesis of UFs(114).

Various studies have reported on the association of UFs with the mutation of MED12, with frequency varying from 31.07% to 85.5% in different populations (Table 3). Among the mutations, missense is the most prominent mutation reported in most of the studies(115–121).

Tommaso et al. reported on the effect of MED12 gene missense mutations on leiomyoma, uterine myometrium and psudo capsule samples leading to an overexpression of IGF-2 suggest its mechanism in causing UFs(126). In addition, studies conducted by Kämpjärvi and co-authors reported the mutations on exon 1 and exon 2 of MED12 interferes with the interaction between MED12 and cyclin C-CKD8/19, and thereby terminating the mediator-associated CDK kinase activity by interfering with cell cycle regulation(127). Several studies have reported on the association of MED12 with the β-catenin/ Wnt pathway(128–133).Kim et al reported on the activation of the Wnt signalling pathway causing β-catenin to translocate into the nucleus, and bound to MED12 and intact mediator, activating the gene expression (131).However, Perot et al. reported, 71.4% of the MED12-expressed mutated tumours displayed only membranous β-catenin while the others does not express β-catenin(134). The author further concluded that there was no association between MED12 mutations and β-catenin localization(134). Further supporting this, another study combining mRNA and miRNA differential expression between UFs and myometrium reported on the downregulation of Wnt pathway causing an upregulation of the focal adhesion pathway in UFs, suggesting MED12 mutation in leiomyoma may not act through the β-catenin/ Wnt pathway(135).

Overexpression of HMGA2 gene in UFs

About 50% of uterine leiomyoma showed abnormal karyotype. Among this, 20% were attributed to the 12q14-q15 mutation, affecting HMGA2(136). High-mobility group (HMG) proteins are heterogeneous, non-histone chromosol proteins that are grouped according to their electrophoretic properties into one of the three distinct families(137). HMGA2(previously was known as HMGIC) genes encodes non-histone proteins that bind to DNA, which participates in the pathway that involves protein complexes assembly, leading to conformational changes in the structure of chromatin. It is also involved in regulation of gene transcription, cell differentiation, apoptosis and proliferation (138).

Decreased expression of HMGA2 causes a

Table 4. Frequency of *HMGA2* mutations in leiomyoma

Country	Year	Frequency	Reference
Chicago	2015	10.1% (18/178)	Bertsch et al(145)
Brazil	2018	69.0% (29/42)	Mello et al(147)
Spain	2018	65% (13/20)	Galindo et al(146)
Finland	2016	28.7% (27/94)	Mehine et al(143)
Finland	2017	Conventional UFs: 24.6% (16/65) Histopathological UFs variant: 13.8% (13/94)	Makinen et al(148)

decrease in VEGF-A, VEGF-C and FGF-2 in oral squamous cell carcinoma, which are the markers for angiogenesis(139), suggesting the role of HMGA2 in promoting angiogenesis in cancer.This is further supported by the study conducted by Helmke et al. that the expression of fibroblast growth factor 2 (FGF-2) are significantly higher in those with a mutated HMGA2 as compared to those with a normal karyotype in women with UFs. The author also mentioned that the FGF-2 expression and the level of HMGA2 overexpression have a linear relationship with the tumour size(140).A recent study reported that the angiogenic expression, specifically, VEGFA, EGF, bFGF, TGFα, VEGFR1 and VEGFR2 is significantly higher in leiomyomas with HMGA2as compared with leiomyomas with MED12 and myometrium (141). It was also proposed that the effect of HMGA2 on angiogenesis might act through IGF2BP2 and pAKT as the overexpression of HMGA2 upregulated IGF2BP2 and pAKT(141). According to Liu et al., it was found that the overexpression of HMGA2 causes a significant increase in the level of PCNA (a measure of cell growth) and a reduction in the level of p21 (cyclin-dependent kinase inhibitor 1)(142). This suggests the importance of HMGA2 in the proliferation of leiomyoma. Further in this study, HMGA2 was shown to bind to the promoter of p62 thus suppressing its transcription.The reduced level of p62 increases the expression of estrogen receptor-α which enhances the proliferation of UFs(142). The author concluded that there is a causal role of the HMGA2-p62-ERα axis in the increased ERα expression in HMGA2-UFs, and is thus a possible target in the treatment of HMGA2-UFs(142). In addition, HMGA2-LM was found to significantly up-regulate the proto-oncogene pleomorphic adenoma gene 1 (PLAG1), suggesting the possibility of it inducing tumorigenesis through the activation of PLAG1(143). Previous studies proposed that MED12 and HMGA2 mutations are mutually exclusive events in leiomyomas(133,143,144). This is supported by Bertsch et al. that found that HMGA2 overexpression occur exclusively in leiomyomas without MED12 mutation(145).

However, in contrary to the previous studies, recent studies observed that HMGA2 over-expression were present significantly in leiomyoma that also showed mutations in MED12, indicating the possibility of co-occurrence in patients with leiomyoma(146,147). Table 4 summarizes the frequency of HMGA2 mutations in different studies.

Fumarate hydratase (FH) gene mutation in UFs

Fumarate hydratase (FH) is a tumour suppressor gene since most of the tumours that have resulted from its mutation were found to exhibit a biallelic inactivation of this gene, leading to a condition known as hereditary leiomyomatosis and renal cell cancer (HLRCC) (149,150). It was found that 75-100% of women with FH mutation developed UFs(151,152). However, FH gene mutation as a leading cause for UFs is relatively rare as compared to MED12 and HMGA2, which accounts for about 0.4-1% of leiomyoma cases(153,154).

The mechanism as to how the mutation in FH gene causes tumorigenesis in HLRCC remains unclear, however the most common mechanism proposed is the activation of hypoxia pathway caused by the effect of FH deficiency on HIF1 transcription factor. The bi-allelic deactivation of FH in the tumour cells altered the production of ATP from oxidative phosphorylation, resulting in a shift to aerobic glycolysis (Warburg effect). The increased fumarate level stabilize the hypoxia-inducible factor (HIF)-1α and an increased in VEGF and GLUT1(155–157).However, there were studies reported on the accumulation of fumarate modified the cysteine residue of KEAP1 and causes the succination of KEAP1, affecting the action of KEAP1 in repressing NRF2 mediated antioxidant response leading to accumulation of NRF2 (158,159). In addition to that, the accumulation and activation of NRF2 have been reported to be associated with many cancers (160).Supporting this is a study conducted by Mehineet al. which reported NRF2 pathway

was the most significantly dysregulated pathway in UFs associated to FH mutations, while the H1F1α pathway was not significantly affected(143).

COL4A5-COL4A6 gene mutation in UFs

COL4A5-COL4A6 gene found on chromosome X, have been associated with UFs. Alport Syndrome, a genetic disorder has a specific COL4A5-COL4A6 deletion presented together with diffuse leiomyomatosis(161). Whole-genome sequencing revealed a small portion of patients with diffuse leiomyomatosis showed deletion of COL4A5-COL4A6 gene (162). IRS4 gene which is close to COL4A5, was found to be highly expressed in UFs with COL4A5-COL4A6 deletion(143,162). IRS4 gene codes for insulin receptor substrate 4, which was identified to play a significant role in the PI3K pathway(163). The activation of the PI3K-AKT-mTOR pathway leads to an increased cell proliferation, resulting in fibroid(164,165). In addition, IRS4 also enhanced the effect of IGF-I in overcoming cell cycle arrest, which also contributes to the increased cell proliferation. (166)

Microbiota in Uterine Fibroids

Recent studies have reported on the association of gut microbiota to the development of UFs. It has been identified that intestinal microbiota is able to affect systemic estrogen level, supporting the estrobolome concept, thus affecting diseases and cancers that are associated with estrogen(167). These are attributed to the β-glucuronidase that is secreted by the gut flora, which is an enzyme that deconjugates estrogen(167). Thus, this affects the endogenous metabolism of estrogen by altering the enterohepatic circulation of estrogen, and increases the free estrogen that is able to bind to estrogen receptors to exert its physiological effect. This is in line with a previous study that showed that the exposure to antibiotics reduce intestinal flora, which results in large amount of conjugated estriol being excreted unchanged in the faeces and a reduction in urinary estrogen(168), which supports the function of gut microbiota in affecting estrogen level.

Lignans are one of the classes of phytoestrogens that are derived from plants. In humans, lignans are metabolized into enterolignans by the gut microbiome. Several studies have established the relationship between lignan or enterolignan exposure and the risk of breast cancer, with high lignan or enterolignan exposure reducing the breast cancer risk(169–171). After ingesting lignan, it has to be metabolized by the gut microbiome into the lignan metabolites enterolignans, enterolactone and enterodiol before it is able to be absorbed into the systemic circulation to produce any physiological effect(172). One of the mechanisms proposed is that enterolactone being structurally similar to estrogen, is able to inhibit enzymes responsible for estrogen synthesis as well as metabolism, thereby reducing the concentration of estrogen(173). Enterolactone has also been shown to reduce the activation of NF-κB and prevent the degradation of I-κB, resulting in a decrease in TNFα production(174). NF-κB is a transcription factor that is involved in regulating angiogenesis, and thus is often activated in cancer cells. In contrast to this, another study observed no significant association between enterolignan with NF-κB(174,175). However, it was proposed that enterolignan reduces the proliferation of cancer cells via VEGF-associated pathways(175). Therefore, dysbiosis in the gut flora can affect the metabolism of lignan and thus will affect the development of cancer. This is also highly applicable in UFs as it is highly estrogen-dependent. This hypothesis is supported by Atkinson et al. that have concluded that there is a modest inverse association between lignan excretion and uterine fibroid risk***(176).***

Role of extracellular matrix in Uterine Fibroids

Several studies showed an altered extracellular matrix in patients with UFs as compared to normal. Collagen consists of a large component in the ECM of fibroid, which

is at least 37%(177). The collagen content was found to be 3.7-fold higher in UF sas compared to the normal myometrium(178). Fibroids have been reported to be stiffer than the normal myometrium, with collagen being one of the contributors(179). A recent study by Jayes et al. have shown that the stiffness of and size of fibroid are not associated with the collagen content(177). However, cross-linking of collagen was reported to be present more frequently in UFs and it was thought to attribute to the stiffness in fibroids(178,180). It has been known that mechanotransduction affects cell activity by converting physical forces into biochemical signals, which affects cellular activity such as proliferation and apoptosis. With that being said, a change in stiffness as observed in fibroids will thus affect the regulation of cell proliferation and apoptosis.

In analysing the GAG in leiomyoma, the composition seemed to be altered with dermatan sulphate and chondroitin sulphate levels being increased as compared to normal tissues, with a decreased heparin sulphate(181). Dermatan sulphate consists of L-glucuronic acid and D-glucuronic acid. A positive correlation has been reported between D-glucuronic acid content in dermatan sulphate with the size of tumor.Berto et al. reported that a higher proportion of D-glucuronic acid was found in dermatan sulphate, which may have contributed to the increased tumour size.(181)

Another proteoglycan involved in the pathogenesis of UFs is versican, which was found to be overexpressed in leiomyoma tissues(182,183). Versican binds to certain GAG affecting cell growth and differentiation. It was reported that the isoform V0 and V1 of versican were upregulated in symptomatic UFs women as compared to asymptomatic women(182). This elevation could affect the stiffness and mechanical stress of the cells. Versican knockdown in UFs cells was found to have decreased estrogen receptor-1 and progesterone-A mRNA expression, making a potential target for treatment (182).

When there is a change in the ECM component, it will affect the stiffness and thus, affecting the development of fibroid. In the normal myometrium, the homeostasis is achieved by the assistance of matrix metalloproteinases (MMP) which is involved in remodelling and degrading of certain constituents of the ECM such as collagen, which contributes to the stiffness of ECM(184). Studies throughout the years have proved that the activity of MMP-2 is significantly higher in UFs as compared to control(185–187). MMP-2 mediates the degradation of collagen type IV as well as other components of ECM consequently, interfering with differentiation and proliferation(188,189).

Oxidative stress and hypoxia in Uterine Fibroids

Studies have reported on the association of oxidative stress and hypoxia to UFs. The subset of myometrial cells, known as side population of myometrium cells (myoSP) proliferated into larger leiomyoma tumour as compared to the myometrial main population (myoMP) when it is transplanted into immune-deficient mice, indicating that these myometrial stem cells play a vital role in the development of UFs(190). Interestingly, it was observed that these myoSP proliferates in vitro in hypoxic condition while it is not able to proliferate efficiently in vitro under normal condition.The greater cell proliferation achieved by both myoSP and myoMP in hypoxic condition suggests that low oxygen condition may be a driving force in the differentiation and proliferation into UFs(191).

Ishikawa et al. have reported that hypoxia increased hypoxia inducible factor 1α (HIF-1α) protein expression in the leiomyoma smooth muscle cells, with an upregulation of mRNA of several HIF-responsive genes (ALDOA, ENO1, LDHA, VEGFA, PFKFB3, and SLC2A1)(192). The increased expression of HIF-1α protein was suggested to reduce apoptosis and increase proliferation by repressing p53, a tumour suppressor protein and by increasing the expression of BCL-2, an

anti-apoptosis protein(193).

In addition to that, the oxidative stress markers and antioxidants have been shown to vary significantly in leiomyoma cells as compared to the normal myometrium. Several studies have reported that the level and activity of several antioxidants such as thiol, superoxide dismutase and catalase were decreased in UFs(194–196). On the contrary, the antioxidant enzyme activities such as catalase and glutathione peroxidase was shown to increase in other independent studies(197,198). However, these studies have the same conclusion, which is the increased in activity of these antioxidant enzymes is due to the compensatory mechanism to the mild oxidative stress in UFs. One of the mechanisms identified as to how ROS affects cell proliferation is through the PDGF-induced MAPK1/MAPK3 pathway. ROS is a critical intermediate in this pathway and the use of NADPH oxidase inhibitor DPI reduces ROS and was shown to inhibit the platelet derived growth factor (PDGF)-induced MAPK1/MAPK3 activation, suggesting a vital role of ROS in UFs proliferation.(199)

Conclusion

Uterine fibroids are a very common benign condition that affects women throughout the world. The pathogenesis is multifactorial and unclear, however various studies were continuously being carried out to identify the possibility of different factors in contributing to the pathogenesis of UFs. Early studies have identified the dependency of fibroid on sex hormones for its growth and development. However, genetic seems to play an important role as well, with different frequency of genetic mutation occurring in different ethnic, race and demography. Similarly, environmental factor, epigenetic, microbiota and extracellular matrix have also been implicated in the development of UFs.

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