

Chapter

1

Pathophysiology of Uterine Fibroids

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1.1 Introduction

Uterine fibroids are a major cause of morbidity in women [1]. Fibroids have variable clinical presentations, depending on size and location. These include pelvic pain (20–40% of patients), bleeding (30% of patients), and anaemia. Fibroids are the leading cause of hysterectomy in the United States [2]. Bleeding from fibroids generally presents as abnormal uterine bleeding (AUB) and often prolonged or heavy menstrual bleeding (HMB) [3]. Fibroids that bleed are more frequently submucosal or extend into the endometrial cavity. Other problems related to fibroids include problems with implantation [1], preterm labour, recurrent loss of pregnancy, obstruction of labour, and urinary incontinence [4]. Medical costs for patients with fibroids in the United States were estimated as 4.1–9.4 billion US dollars in 2010 [2].

The self-reported prevalence of uterine fibroids in women from France, Germany, Italy, the United Kingdom, the United States, Canada, Brazil, and South Korea has ranged from 4.5% in the United Kingdom to 9.8% in Italy [5]. The highest prevalence was seen in women aged 40–49 years in the United Kingdom (9.4%) and Italy (17.8%). The worldwide mortality from fibroids in developed countries has remained about 0.01/100,000 women over the last 25 years [6].

There is a variable risk of developing fibroids in women, depending on race, hormone exposure, age, diet, and other factors. Some factors may be modified and, if altered, have the potential to reduce the impact of fibroid-related morbidity. Herein, we review these factors and the associated genetic changes that have been described to date.

1.2 Aetiology

1.2.1 Definition

Uterine leiomyomas, or fibroids, are common, benign, smooth muscle tumours of the uterus.

Fibroid growth involves clonal uterine smooth muscle cell expansion along with production of a large amount of extracellular matrix. Uterine fibroids are commonly found presenting after puberty and diminishing after menarche [7]. Uterine fibroids usually become symptomatic in women during their fourth and fifth decades, and 20–40% of women develop symptoms related to their fibroids [8].

1.2.2 Epidemiology

A number of factors have been linked to the development of fibroids and the different patterns in which they become clinically evident. Some factors are modifiable and may be useful in promoting preventive health care. Numerous risk factors for fibroid development have been described, several of which may be affected by changes in diet or lifestyle (Table 1.1).

1.2.2.1 Fibroid Growth Rates

The Fibroid Growth Study followed 262 fibroids in 38 black and 34 white premenopausal women over a 12-month period [9]. Black women 35 years of age or older had a 2.8-fold increased risk of rapid fibroid growth (>20% increase in volume over 6 months) when compared to similar white women [10]. Fibroids from women more than 45 years of age grew more rapidly in black women (increase in volume of 15% over 6 months) than in white women (increase in volume of 2% over 6 months) [9]. White women, but not black women, had a decrease in growth rate with age. Rapid growth was seen more frequently in young white women than in older white women. The frequency of fibroids with rapid growth did not vary by age in black women. Fibroid volume changes in a 6-month period ranged from –89% to +138%. Both black and white women had a median increase in volume of 9% over 6 months. About 34% of fibroids increased in volume by 20%, and 7% experienced a 20% decrease in volume over 6 months. Fibroid shrinkage was generally

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Table 1.1 Risk factors for fibroid development have been described, several of which may be influenced by changes in diet or lifestyle

	Effect	May be modified with
Race		
Black women have		
• ↓ urinary 2-HE/16α-HE metabolite excretion	↑ oestrogen exposure	Diet: high-fibre, low-fat diet
• ↓ vitamin D blood levels	↑ fibroid risk	↑ sun exposure, oral vitamin D
• ↑ aromatase blood levels	↑ oestrogen exposure	Diet: high-fibre, low-fat diet
Oestrogen		
Early age of menarche		
	↑ oestrogen exposure associated with ↑ cellular proliferation ↑ risk of genetic mutation ↑ expression of progesterone receptors	Diet: high-fibre, low-fat diet
Progesterone		
	Associated with suppressed apoptosis (↑ bcl-2 expression) ↑ TGF-β expression ↑ cellular proliferation ↑ extracellular matrix formation	

associated with loss of arterial blood supply as indicated by contrast enhancement. Women often had fibroids with varying growth and shrinkage [7].

Fibroid growth rates were faster in women with a single fibroid than in women with multiple fibroids [9]. Fibroid growth rates did not differ by patient BMI, parity, size, or location. About 62% of women undergoing surgical resections of fibroids without hysterectomy have been reported to have a recurrence of a fibroid at least 2 cm in diameter within 5 years, and 9% required surgery for this recurrence [11].

1.2.2.2 Age

Fibroids are often diagnosed in older premenopausal women. Most fibroids occur in women in their thirties or forties, associated with chronic exposure to oestrogen and progesterone throughout the reproductive life course [12]. The Seveso Women’s Health Study found fibroids in 21.4% of Italian women aged 30–60 who were screened by ultrasound [11]. Most fibroids diagnosed by fibroid screening are symptomatic. Ultrasound identified fibroids in 3.3% of Swedish women aged 25–32 years and 7.8% of women aged 33–40 years. A woman’s age at birth of the last child was inversely related to risk of fibroids [13]. Fibroids have not been described in prepubertal girls [12].

1.2.2.3 Race

Most studies evaluating race-related risk of developing fibroids were performed in the United States. The incidence of fibroids by age 50 was estimated as 70% for white women and 80% for African-American or black women [7]. Black women had a lower urinary 2-hydroxyoestrone/16α-hydroxyoestrone metabolite ratio than white women [14]. 2-hydroxyoestrone metabolites have less oestrogenic activity than 16α-hydroxyoestrone metabolites, possibly leading to greater oestrogen exposure in black women. Black women had a three times greater risk of clinically symptomatic fibroids, and were younger at first diagnosis, had more severe disease, and underwent hysterectomy more frequently than white women [12]. Fibroids were detected about 10 years earlier in black women than in white women [15, 16].

A random selection of adult women in an American health plan found that 35% of premenopausal women had a previous diagnosis of fibroids and that 51% of the premenopausal women with no clinical diagnosis had ultrasound-detectable fibroids [7]. Black women had fibroids more frequently than white women (cumulative incidence by age 50, >80% vs. 70%, OR = 2.9). There was little change in risk after

adjustment for parity or body mass index (BMI). White women appeared to have an increasing risk with increasing age, while black women had a constant higher risk. Parity was not a risk factor in black women, and about two-thirds of white women were nulliparous. Black women were diagnosed at a younger age than white women (33 vs. 36 years). Black women were more likely to have multiple fibroid tumours than white women (73% vs. 45%). This difference was greatest in younger women (35–39 years, 74% vs. 31%). Clinically relevant premenopausal fibroids were present in about half of black women and 25% of white women [7]. A much lower incidence was reported in 334 Swedish women screened with ultrasound [17]. Only about 5% of all Swedish women were affected and only 8% of women 33–40 years of age. Pathologic study of 2-mm sectioned hysterectomy specimens obtained from adult American women demonstrated fibroids in 77% [18]; 84% of these women had multiple fibroids.

The Study of Environment, Lifestyle and Fibroids evaluated 1,696 black American women with ultrasound and found 22% had at least one fibroid 0.5 cm in diameter [15]. Prevalence increased with age: 10% for women 23–25 years of age and 32% for women 32–35 years of age.

The incidence of newly diagnosed fibroids has been reported to be about 3% per year in reproductive-age black women [11]. It has been reported that 73% of black women and 45% of white women have multiple fibroids [7]. Hispanic women have been reported to have a similar incidence of fibroids as white women [4].

1.2.2.4 Obesity

BMI is inversely associated with sex hormone-binding globulin level and these levels are higher in obese patients [19]. Thus women with increased BMI may have more bioavailable circulating oestrogens and androgens. Obesity has been associated with a modest increased risk of fibroids in several, but not all, international studies [13, 19]. Each 10 kg increase in weight in adults was associated with an 18% increase in the development of fibroids [11]. BMI during adolescence has not to date been associated with an increased risk of fibroids.

1.2.2.5 Diet and Exercise

Several studies have suggested an association between diet and the development of fibroids. Increased

consumption of red meat (RR = 1.7) and decreased consumption of green vegetables (RR = 2.0) have been associated with an increased risk of fibroids [11, 12]. A double-blinded randomized study showed that intake of green tea extract was associated with a reduction in uterine fibroid size and clinical severity, and an improvement in quality of life, compared to placebo control [20].

The dietary habits of 22,583 premenopausal black women were evaluated for their association with the development of fibroids [21]. Fruit and vegetable intake were each inversely associated with the risk of fibroids (2 servings/day vs. 2 servings/week). Citrus fruit had the highest inverse association (3 servings/week vs. 1 serving/month). Increasing fruit and vegetable consumption was observed with increasing patient age and multivitamin use. Risk was not associated with intake of vitamins C or E, folate, or carotenoids.

The National Institute of Environmental Health Sciences Uterine Fibroid Study correlated plasma vitamin D levels with the ultrasound diagnosis of fibroids in randomly selected 35- to 49-year-old women [22]. Moreover, 50% of white women and 10% of black women had normal plasma levels of 25-hydroxy vitamin D (>20 ng/mL). Women with normal levels of 25-hydroxy vitamin D had a 32% lower risk of having fibroids. Sun exposure for more than 1 hour per day was also associated with reduction in risk of having fibroids (adjusted OR = 0.6). Vitamin D deficiency is more common among black women, as their pigment inhibits sunlight-related stimulation of vitamin D production in the skin [23].

Premenopausal vegetarian women excreted three times more faecal oestrogen, had less urinary oestrogen excretion, and had 15–20% lower plasma oestrogen levels than non-vegetarian women [14]. Increased faecal excretion of oestrogen was linked to decreased deconjugation of faecal oestrogen that is needed for its reabsorption. Postmenopausal women placed on a low-fat diet experienced a 17% decrease in plasma oestradiol concentrations.

The effect of exercise on fibroid pathophysiology is not clear. While former college athletes were less likely than non-athletes (RR = 0.72) to develop fibroids, other differences such as diet and weight were not accounted for [14].

1.2.2.6 Caffeine

The Black Women's Health Study found an increased risk of fibroids in the subgroup of women less than

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35 years of age who drank over three cups of coffee a day or consumed more than 500 mg caffeine a day [19]. This association was not found when women of all ages were included in the evaluation.

1.2.2.7 Alcohol

Alcohol intake has been associated with the development of fibroids in most studies [19]. A report on Japanese women found fibroids, diagnosed by ultrasound, more frequently in those in the highest third of alcohol intake, compared to those in the lowest third (OR = 2.8, about 1 drink/day vs. non-drinkers). Current drinkers in the Black Women's Health Study also had a higher risk than non-drinkers. The highest risk, a 60% increase, was with women drinking beer (7+ drinks/week vs. non-drinkers).

1.2.2.8 Smoking

Smoking is historically associated with a decreased risk of fibroid formation [12]. Smoking two packs of cigarettes a day was associated with an 18% increase in the development of fibroids [11]. More recent studies, including the National Institute of Environmental Health Sciences Uterine Fibroid Study, showed no association between risk of fibroids and smoking [19]. Protective effects found in earlier studies have been attributed to bias introduced by improper selection of the control groups.

1.2.2.9 Menarche

Early age of menarche increases a woman's overall lifetime exposure to oestrogen and is associated with a higher risk for fibroids [13]. Women who are ≤ 10 years at menarche have a higher risk of fibroids than women who were 12 years of age or more at menarche (RR = 1.24) [14].

1.2.2.10 Menopause

Uteri evaluated pathologically using 2 mm sections were found to have similar numbers of fibroids whether they were obtained from pre- or postmenopausal women. However, the fibroids were smaller in postmenopausal women. Postmenopausal women have a decrease in circulating oestrogen and progesterone levels, and this has been attributed to fibroid shrinkage [14]. These findings could however be affected by selection bias, as postmenopausal women were often treated more conservatively and did not undergo hysterectomy.

1.2.2.11 Parity

High concentrations of oestrogen and progesterone are found in pregnancy and with oral contraceptive (OC) use, two conditions associated with a decreased risk of fibroids [12]. Pregnancy longer than 20 weeks in duration has been associated with decreased risk of fibroid formation. Women undergoing childbirth had a lower risk of fibroids than women who never had children (RR = 0.5) [14]. A progressive decrease in risk was seen with increasing number of births. Pregnancy has been associated with a five-fold reduction in the incidence of fibroids [11]. This number may be biased, as women with fibroids are less likely to become pregnant or deliver at term.

1.2.2.12 Oral Contraceptives (OCs)

The use of OCs in adult women is associated with a decrease in clinically evident fibroids [12, 24]. Women taking oral contraceptives for more than 12 years had 50% fewer fibroids than similarly aged women who did not take oral contraceptives [11]. This study may have been biased because women with fibroids tend to have more trouble with conception and take oral contraceptives for shorter periods than women without fibroids. Nevertheless, the relative risk of developing fibroids was shown to decrease in a dose-dependent fashion with the duration of oral contraceptive use. The Nurses' Health Study II, a study of 95,061 American women, found that oral contraceptive use between the ages of 13 and 16 years was associated with an increased risk of clinically symptomatic fibroids [24].

1.2.2.13 Hormone Replacement Therapy

Symptoms related to fibroid growth often decrease after menopause, when circulating oestrogen and progesterone levels decline. It has been reported in several studies that women who take hormones after menopause may experience renewal of these symptoms [12].

Postmenopausal women with small asymptomatic uterine fibroids were randomly treated with either transdermal oestradiol (E2) plus medroxyprogesterone acetate (MPA) or conjugated equine oestrogen (CEE) plus MPA [25]. Women treated with transdermal E2 plus MPA had larger fibroids after 1 year, while women treated with conjugated equine oestrogen plus MPA had no change in fibroid size. In a separate study, 70 postmenopausal women with

fibroids were treated with either transdermal 17 β -oestradiol patches plus MPA or calcium carbonate for about 11 months [26]. No change in fibroid size was observed in either treatment group. Post-menopausal women with solitary uterine fibroids that were taking CEE and MPA for 3 years were evaluated for fibroid growth [27]. This hormone replacement combination increased fibroid size, although the main effect was observed during the first 2 years.

1.2.2.14 Tamoxifen

Animal studies have shown that tamoxifen inhibits the growth of fibroids [28]. These findings have not been reproduced in women, where results have been mixed. A randomized trial evaluating the effect of tamoxifen on fibroid growth is not available.

1.2.2.15 Xeno-oestrogens

Xeno-oestrogens have been hypothesized to antagonize fibroid growth. Xeno-oestrogens are synthetic or natural chemicals with oestrogenic activity. Synthetic xeno-oestrogens include polychlorinated biphenyls, bisphenol A, the pesticide DDT, and phthalates. Natural xeno-oestrogens are generally plant-derived chemicals called phyto-oestrogens. Phyto-oestrogens are converted into oestrogenic substances by bacterial degradation during digestion and absorbed [14]. Soy and flax contain the greatest quantities of these compounds. The anti-oestrogenic effects sometimes reported with phyto-oestrogens may be due to their ability to compete with natural oestrogens such as oestradiol at the oestrogen receptor (ER).

1.2.3 Theories of Fibroid Growth

Historically, the development of cancer has been modelled into four stages, these being initiation, promotion, progression, and malignant transformation. Initiation introduces changes in the normal cellular machinery and is thought to be caused by mutagens in the local tissue environment. Promoters introduce additional changes in the cellular machinery that selectively enhance the growth of initiated cells. Promotion is considered reversible. Removal of the promoter results in reversion of cells into the previous phenotype. Tumour progression is a permanent change in cellular function and can lead to malignant transformation [14, 29, 30]. Complete carcinogens are able to promote cellular change through all four stages, while incomplete carcinogens cannot.

Promotion and progression are not well defined in fibroids and are considered together here.

More recently, malignant transformation has been explained by loss of function or gain of function mutations in tumour suppressor genes and oncogenes [29, 30]. The formation and growth of some benign tumours appears to have some characteristics of all these models, while not progressing to an invasive or metastatic phenotype. Factors related to the formation and growth of uterine leiomyomas are illustrated in Figure 1.1.

1.2.3.1 Initiation

While many genetic events have been described in fibroids, the initiating events are not clear [14]. Hypothesized initiating events include chronic exposure to high levels of oestrogen and progesterone, leading to increased cellular proliferation and an associated introduction of genetic mutations through errors during gene copying. Increased levels of ERs are found in fibroids and may be a contributing factor. Genetic findings in fibroids similar to those found in scars have led to the 'injury' hypothesis [31]. Here, vasoconstrictive factors secreted during menses are thought to lead to ischaemia and local uterine muscle injury [14]. Another potential initiating factor may be activation of genes associated with the familial or inherited occurrence of fibroids [14, 32, 33].

1.2.3.2 Promotion and Progression

Oestrogen is more generally accepted as the principal promoter of fibroid growth [14]. Several observations suggest oestrogens contribute to fibroid development and growth. Oestradiol binds to oestrogen receptor α (ER α), activating transcription of multiple genes, including the progesterone receptor (PR) [28]. Fibroids have been observed to increase in size after exposure to exogenous oestrogen, the increased levels of oestrogen found in pregnancy, or hormone replacement therapy. They have been observed to decrease in size after menopause or gonadotrophin-releasing hormone (GnRH) agonist treatment [8]. Leiomyoma (fibroid) cells transfected with an inactive ER α mutant suppressed both ER α - and PR-related gene expression [28]. Expression of IGF-I, a growth factor related to cellular proliferation, is not found in leiomyoma samples obtained from women with decreased oestrogen levels [34].

Fibroids have been observed to have a greater number of PRs (both A and B forms) than normal

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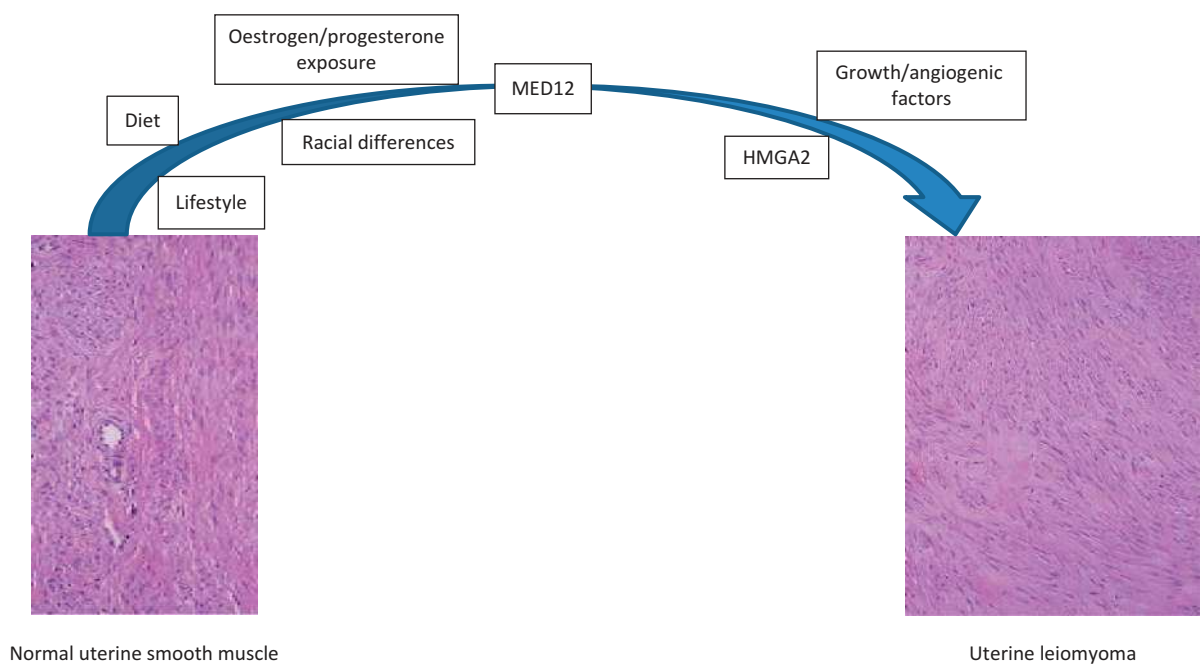


Figure 1.1 Factors related to the formation and growth of uterine leiomyomas. Normal uterine smooth muscle tissue is thought to transform into a uterine leiomyoma under the influence of multiple forces (see Tables 1.1–1.3). Altered expression of MED12 appears to play a major role in the development of uterine leiomyomas.

uterine muscle [8]. Animal studies have shown that PR-A is linked to ovulation and the anti-proliferative effects of progesterone in the uterus, and that PR-B is associated with mammary gland development [28].

Progesterone is also thought to contribute to tumour promotion [14]. Progesterone binds to the PR, activating genes related to suppression of apoptosis, cellular proliferation, and extracellular matrix formation [28]. Anti-progestins will block these effects. The mitotic index of fibroids increases during the progesterone-rich luteal phase of the menstrual cycle. Leiomyoma proliferative activity in post-menopausal women has been shown to increase after administration of oestrogen plus progestin, but not with oestrogen alone [28]. The administration of progestins with GnRH antagonists is associated with an inhibition of the GnRH antagonist effect on uterine shrinkage [8].

Progesterone has been shown to increase the expression of the growth factor, epidermal growth factor (EGF), and the anti-apoptotic protein bcl-2 by binding to progesterone response elements found on these genes. EGF activates c-Myc, a transcription factor that increases cellular proliferation and decreases apoptosis by down-regulating Bcl-2 expression [28].

IGF-I, IGF-II, and IGF-II receptor, but not IGF-I receptor type, are more highly expressed in fibroid tissues than in normal uterine muscle cells [34]. In vitro studies have shown that IGF-I stimulates the proliferation of leiomyoma cells. In vitro treatment with the selective progesterone receptor modulator (SPRM) asoprisnil decreases the expression of IGF-I in fibroids, but not in normal uterine smooth muscle cells [34].

The SPRMs mifepristone, asoprisnil, ulipristal acetate, and telapristone acetate have been reported in clinical trials to suppress uterine bleeding and decrease leiomyoma size [28, 34]. Ulipristal acetate (UPA) is an SPRM that has been shown to initiate apoptosis in uterine fibroids and to decrease fibroid size [35]. Treatment with UPA did not alter GnRH blood levels and maintained oestradiol levels above 1.05×10^{-3} pm/L [35]. Use of Depo-MPA, an injectable progestin contraceptive, has been associated with a reduced risk of fibroid development [19]. Both progestins and anti-progestins may diminish ER signalling and leiomyoma cell growth, suggesting cross-talk occurs between ER and PR signalling [13]. Risk factors for fibroid formation that frequently affect the expression of cellular factors are listed in Table 1.2.

Table 1.2 Risk factors for fibroid formation that frequently affect the expression of cellular factors that control cell growth

Associated Findings	
Increased expression in fibroids	
• Oestrogen receptors	↑ leiomyoma cell proliferation
• Progesterone A and progesterone B receptors	↑ leiomyoma cell proliferation
• IGF-I, IGF-II, and IGF-II receptors	↑ leiomyoma cell proliferation
• EGF	↑ cellular transcription ↓ apoptosis (↓ bcl-2)
• TGF-β3	↑ leiomyoma cell proliferation
• bFGF	↑ leiomyoma cell proliferation
Increased expression in fibroid extracellular matrix:	
• Greater volume of matrix than that found in normal uterus	
• bFGF	↑ angiogenesis
• Decorin	Organizes type I and II collagen
• TGF-β3	↑ ECM formation

1.2.3.3 Effectors

Oestrogen and progesterone mediate their effects through transcriptional activation or suppression of growth factors and their receptors, and by direct activation or suppression of growth factor pathways.

Aromatase

Most endogenous oestrogen is thought to originate from the ovary [28]. Oestrogen is also produced by ovarian aromatization of androgens released from the adrenal glands and ovaries [28]. Aromatase P450 in the ovary converts androstenedione, testosterone, and 16α-hydroxyandrostenedione to oestrone (E1), oestradiol-17β (E2), and oestriol (E3), respectively [36]. Aromatase P450 is expressed 1.5–25 fold more in leiomyomas than in normal uterine smooth muscle cells. The high level of aromatase activity results in higher local oestrogen levels, contributing to fibroid growth. Blocking this activity with aromatase inhibitors results in fibroid shrinkage similar to that reported with GnRH antagonists, even if peripheral oestrogen levels are elevated.

Black women have higher levels of aromatase activity in their fibroids than white women [37]. Increased fibroid aromatase activity has been associated with an increased prevalence of uterine fibroids and clinical presentation at a younger age.

Growth Factors

Basic fibroblast growth factor (bFGF) stimulates uterine fibroid cell growth. Fibroids have increased cellular expression of basic fibroblast growth factor (bFGF), and a large pool of bFGF is found in the abundant extracellular matrix (ECM) that is present in fibroids [12]. Growth factors secreted by uterine muscle cells act to promote angiogenesis [13]. These factors include bFGF (angiogenesis), parathyroid-hormone-related protein (PTH-RP) (a potent vasorelaxant), prolactin (angiogenesis), and prolactin cleaved by cathepsin D (inhibits angiogenesis). Matrix components that promote angiogenesis include bFGF, heparin-binding EGF, and PDGF.

Expressions of TGF-β, IGF 1 and 2, PDGF, and EGF also contribute to fibroid cell growth and the formation of ECM, and increased expression is demonstrated in fibroids [13, 38]. TGF-β3 appears most consistently elevated in fibroids, compared to normal uterine smooth muscle cells [34]. The highest expression of TGF-β3 was observed during the progesterone-rich secretory phase. Asoprisnil, an SPRM, decreases TGF-β3 expression [39].

Dermatopontin (DPT) is an extracellular matrix protein that has been hypothesized to regulate TGF-β activity [10]. The expression of DPT is lower in fibroids than in normal uterine muscle and also lower in fibroids from older black women than in those from older white women. Expression of fibulin 1, the calcium-related migratory and endocytotic molecules netrin 1 and stoning 1, and pyruvate dehydrogenase kinase isoenzyme IV was similarly down-regulated [10]. Fibroids share cellular changes found in fibrotic pathologies including expression of type I and III collagen and TGF-β [12].

DNA samples from fibroid and normal uterine muscle obtained from women participating in the Fibroid Growth Study have been analysed using Affymetrix Gene Chip expression arrays [10]. A key finding in fibroid samples was down-regulation of receptor tyrosine-protein kinase erbB-2 (ERBB2) expression. Down-regulation of ERBB2 appeared linked to down-regulation of dermatopontin. SNAIL2, a mediator of epithelial–mesenchymal transition

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(EMT), and cyclin B2 were over-expressed in fibroid tissue. The von Hippel-Lindau (VHL) gene, a normally expressed hypoxia-induced gene and a tumour suppressor gene, and PARP1 were frequently over-expressed in fibroids, while expression of EGFR was frequently down-regulated [10]. PARP1 expression was frequently associated with over-expression of PRKDC, catenin- β 1, and MED12 [10].

Bcl-2 inhibits apoptosis and is over-expressed in fibroids, especially in the secretory phase [28]. Oestrogen and progesterone may have an impact on expression of these growth factors. The cell proliferation marker Ki67, proliferating cell nuclear antigen (PCNA) expression, and mitotic counts in human fibroids are highest during the luteal and secretory phases [28].

Hormone Receptors

Oestrogen and progesterone receptors (ER and PR) appear to contribute to fibroid growth. Increased expression of ER and PR-A and PR-B are reported in fibroids, compared to normal uterine muscle [13, 28]. Leiomyoma cells transfected with an inactive ER α mutant suppresses both ER α - and PR-related gene expression [28]. The selective ER modulator (SERM) raloxifene has been shown to decrease leiomyoma cell proliferation in vitro and cause uterine fibroid regression in animal models [28]. A randomized study of raloxifene in postmenopausal women also demonstrated a significant decrease in fibroid size. These findings were not reproduced in premenopausal women where the higher levels of circulating oestrogen were thought to counteract the inhibitory effects of raloxifene. In another study, premenopausal women randomized to raloxifene plus GnRH had a greater decrease in fibroid size than women treated with GnRH alone, supporting the above hypothesis.

Fibroids have increased cellular expression of the bFGF receptor [40], and fibroids that bleed have higher expression of the type I bFGF receptor. Expression of TGF receptors is increased in fibroids, a finding similar to fibrotic disease states [12].

1.2.3.4 Genetic Findings

Inheritance

Several types of familial expression of fibroids have been observed [12]. Increased expression of fibroids has been found in women with Reed's syndrome (with subcutaneous myomas), Bannayan-Zonana syndrome (with other benign tumours including lipomas

and haemangiomas), Alport's syndrome, cutaneous leiomyomatosis, intravenous leiomyomatosis, and gastrointestinal stromal tumours [12, 16, 33]. Hereditary defects in hereditary leiomyomatosis and renal cell cancer (HLRCC – fumarate hydratase, FH), tuberous sclerosis (TSC2), Birt-Hogg-Dubé (folliculin – FLCN), and the high-mobility group protein 2a (HMG2a) have also been associated with the development of fibroids [13, 33].

Cytogenetics

About 60% of fibroids have normal cytogenetic studies [16]. Patients with cytogenetic abnormalities most frequently have a 12:14 translocation, trisomy 12, a 6:10 translocation, or deletions of chromosome 3 and 7. The most common finding is the 12:14 translocation, occurring in about 20% of women with cytogenetic abnormalities [16]. Fibroids with a chromosome 12:14 translocation have increased expression of high-mobility group DNA-binding protein (HMGA2) compared to normal uterine smooth muscle. In vitro studies blocking fibroid HMGA2 activity led to cell senescence. Fibroids with abnormal cytogenetic findings are generally larger than fibroids with no cytogenetic abnormalities [32].

Microarray Studies

Microarray studies of fibroids and matched normal uterine smooth muscle demonstrate increased expression of cellular retinoic acid-binding protein II, the inotropic glutamate receptor GluR2, insulin-like growth factor II, TGF- β , and CD24 [16]. TGF- β 3 expression in fibroids is three times greater than that of normal adjacent uterine smooth muscle. Decreased expression of alcohol dehydrogenase, prostaglandin E receptor, EP3 subtype, ATP-binding cassette subfamily A, activating transcription factor 3, and dermatopontin is observed. These genes are associated with uterine muscle growth, development, and differentiation, and the development of extracellular matrix. About a quarter of all differentially expressed genes were related to extracellular matrix formation [16].

The genetic features found in fibroids are similar to those found in keloid scar formation [31]. Pirfenidone blocks TGF- β expression and has been administered to block collagen formation in disease states characterized by fibrosis. A pilot study of eight women demonstrated a 32–56% reduction in fibroid size after 3 months of pirfenidone treatment. It is

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interesting that microarray studies did not detect alterations in ER and PR isoforms or any nuclear receptor cofactor expression that augments transcription of steroid hormones [16].

Genomic Screening

In a study, 457,044 single nucleotide polymorphisms (SNPs) were evaluated in a series of over 9,600 fibroids and normal uterine muscle controls [41]. Three loci were associated with fibroid development, chromosome 10q24.33, 22q13.1, and 11p15.5. The associated SNPs were rs7913069 (OR = 1.47), rs12484776 (OR = 1.23), and rs2280543 (OR = 1.39), respectively. Genes of interest on chromosome 10q24.33 included SLK (STE20-like kinase) and AKAP13 (A-kinase anchor protein-13). SLK is found in proliferating myoblasts and has a role in cellular differentiation and motility. AKAP13 is found to be associated with cytoskeletal filaments in leiomyoma cells and facilitates cellular responses to mechanical stress, such as that found with abnormal excessive extracellular matrix deposition.

Genetic Findings

In one study, 7.8% of fibroids had rearrangement of chromosome 12q14–15, 69.9% had a mutation in the transcription factor MED12, and 22.3% had a different or no genetic abnormality identified [42]. MED12 mutations are thus the most common mutation found in fibroids [28, 32]. MED12 regulates transcription and binds with ERα and ERβ. Loss of function of MED12 through physiologic changes or mutation has been associated with increased expression of the TGF-β receptor, increasing the influence of TGF-β on fibroid metabolism [37]. Specific genetic mutations have been associated with the development or growth of leiomyoma as listed in Table 1.3.

Leiomyoma stem/progenitor cells have been identified as the leiomyoma-derived side population (SP) cells [43, 44]. These cells can be divided into three groups based on CD34 and CD49b expression. It has been estimated that about 6% of fibroids are leiomyoma stem cells. These cells are CD34⁺/CD49b⁺ and have low expression of ERα and PR. Fibroid stem cells contain a MED12 mutation, while normal uterine stem cells do not [44, 45].

Mutations in MED12 may be an early change in uterine smooth muscle transformation. The pattern of tumour growth is demonstrated in animal models where CD34⁺/CD49b⁺ cells form leiomyoma-like

Table 1.3 Specific genetic mutations have been associated with the development or growth of leiomyoma

Mediator of RNA polymerase II transcription, subunit 12 homolog (MED12)

*MED12 mutations are frequently found in leiomyoma
MED12 mutations are found in fibroid stem cells, not normal uterine stem cells*

MED12:

- Binds to oestrogen receptors α and β
- ↑ cellular transcription
- ↑ progesterone-related responses
- Can activate Wnt/β-catenin signalling in normal uterine smooth muscle
 - MED12-Wnt/β-catenin signalling:
 - ↑ cellular proliferation
 - ↑ TGF-β3 expression

High-Mobility Group AT-Hook 2 (HMGA2)

HMGA2:

- ↑ expression is found in a smaller subset of leiomyoma
- Mutations in HMGA2 are associated with
 - Larger fibroids
 - Solitary fibroids
- Normally will ↓ expression of the cellular senescence factors p16INK4a and p16Arf
- A nearby HPV-binding site has been hypothesized to affect HMGA expression

tumours with progesterone treatment and CD34[−]/CD49b[−] cells form flat fibrotic lesions. CD34⁺/CD49b⁺ cells are characterized by the greatest expression of the stem cell markers KLF4, NANOG, SOX2, and OCT4. These cells can proliferate to maintain their lineage or to give rise to CD34⁺/CD49b[−] leiomyoma intermediate cells. These cells comprise about 7% of cells and have high expression of ERα and PR. Leiomyoma intermediate cells appear to differentiate into leiomyoma differentiated cells, which comprise about 87% of cells. These cells are CD34[−]/CD49b[−] and have the highest expression of ERα and PR.

While leiomyoma stem cells have the lowest expression of ERα and PR, they demonstrate marked proliferation in response to oestrogen and progesterone. This proliferation is dependent on the local presence of leiomyoma or normal uterine smooth muscle cells, suggesting the presence of an oestrogen- and progesterone-mediated paracrine loop. The wntless-type (Wnt)/β-catenin pathway may be involved in this paracrine

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signalling [28]. Human normal uterine smooth muscle cells treated with oestrogen and progesterone secrete Wnt ligands which stimulate nuclear translocation of β -catenin co-cultured leiomyoma stem cells, activating genes related to cell growth and proliferation [46]. β -catenin binds to MED12 in normal uterine smooth muscle cells, activating transcription. Mutations in MED12 have been shown to activate Wnt/ β -catenin signalling in normal human uterine smooth muscle cells [46]. Selective inhibition of Wnt/ β -catenin activity abrogated cell growth and proliferation in vitro and decreased tumour growth in animal models. The activated Wnt/ β -catenin pathway also stimulates TGF- β 3 expression, a stimulator of cellular proliferation and extracellular matrix formation [37]. TGF- β 3 is also thought to inhibit clotting locally, leading to increased uterine bleeding [47].

In a study, 58.8% of fibroids analysed had a mutation in MED12, all of which occurred in bp 130 or 131 [45]. Codon 44 mutations were observed in 95.8% of fibroids with mutations; 41.7% of these were bp 131 mutations G to A, 18.8% were bp 130 G to A mutations, 16.7% were bp 130 G to C mutations, and other mutations occurred less frequently. MED12 mutations were found in 80% of fibroids with a normal karyotype and in no fibroids with chromosome 12q14–15 rearrangements. Fibroids with a MED12 mutation and a normal karyotype were smaller tumours than those with HMGA2 rearrangements. Fibroids with bp 130 or 131 G to A mutations were larger than fibroids with other mutations in this area.

HMGA2 expression in patients with chromosome 12q14–15 rearrangements are known to be strongly up-regulated [48]. HMGA2 down-regulates the expression of p16INK4a and p14Arf, which both have cellular senescence promoting activity [48]. Fibroids with mutations in HMGA2 were larger than those without a mutation [37]. In 70.0% of fibroids, HMGA2 mutations presented clinically as solitary nodules, and 85.5% of fibroids with a MED12 mutation presented clinically with multiple, clonally individual, fibroids [42]. These findings suggest that (1) different genetic forces may drive fibroid formation and that (2) forces exist to form different MED12 mutations in an individual patient. It is noteworthy that the herpes papilloma virus (HPV) insertion site is 50–100 kbp from the HMGA2 gene [19]. The effect of HPV infection on fibroid growth is not known.

The abundant ECM found in fibroids contains enzymes to break down the matrix and allow

endothelial cell migration as well as collagens I and III, and bFGF, which stimulates angiogenesis [38]. Decorin, a proteoglycan that helps organize type I and II collagen, is present in ECM in greater amounts. Decorin modulates the activity of TGF- β 1 in ECM and has been shown to inhibit angiogenesis. A higher than normal molecular late form of decorin is found in an abnormal distribution pattern in fibroid ECM. ECM found in fibroids consists primarily of collagen, fibronectin and proteoglycans [20]. Collagen in ECM has an abnormal fibril structure and orientation compared to that found in normal uterine tissue [49]. Large amounts of the glycosaminoglycan dermatan sulfate are found in fibroid ECM.

1.2.4 Fibroid Classification Systems

Fibroid classification systems are meant to convey the risk of fibroid-associated morbidity. There is currently no internationally fully accepted staging system for the categorization of fibroids. Bleeding is a common feature of clinically relevant fibroids. Uterine fibroids may be classified according to the International Federation of Gynecology and Obstetrics (FIGO) PALM-COEIN system [50]. Fibroids in this system are classified and assigned a numerical description according to their submucosal (0 – pedunculated intracavitary, 1 – <50% intramural, 2 – $\geq$ 50% intramural) or other location (3 – contacts endometrium 100% intramural, 4 – intramural, 5 – subserosal $\geq$ 50% intramural, 6 – subserosal <50% intramural, 7 – subserosal pedunculated, 8 – other). Number and size of fibroids are not included in this system.

The FIGO classification system complements that of the European Society of Hysteroscopy that has classified fibroids according to luminal extension [51]. Type 0 fibroids are pedunculated, submucosal, tumours without intramural extension. Type I fibroids are submucosal, sessile tumours with less than 50% intramural extension. Type II fibroids are submucosal, sessile tumours with 50% or more intramural extension [51]. This classification indicates which tumours are more easily resected for the treatment of fibroid-associated uterine bleeding.

1.2.5 Uterine Sarcoma

The uterus is the most common anatomical subsite and leiomyosarcomas are the most common histological subtype. The published literature exposes a