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Norihiro Sugino
Editor

Uterine Fibroids and Adenomyosis

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ISSN 2364-1932 ISSN 2364-219X (electronic)
Comprehensive Gynecology and Obstetrics
ISBN 978-981-10-7166-9 ISBN 978-981-10-7167-6 (eBook)
https://doi.org/10.1007/978-981-10-7167-6

Library of Congress Control Number: 2018944594

Printed on acid-free paper

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd. part of Springer Nature
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Uterine fibroids are common uterine tumors in reproductive-age women. However, the pathogenesis of uterine fibroids is still poorly understood. Complex networks of multiple factors such as growth factors, cytokines, extracellular matrix, apoptosis, and estrogen/progesterone actions have been reported to be involved in the pathogenesis of uterine fibroids to date. Recently, accumulating evidence suggests that both genetic and epigenetic factors are likely to be involved in the generation and development of uterine fibroids. *MED12* mutations have also been found to play a role in the tumorigenesis of uterine fibroids. Comprehensive epigenomic analyses have demonstrated that DNA methylation is involved in the pathogenesis of the fibroids, a potential cause of which may be environmental factors. In addition, a new concept of the involvement of stem cells/progenitor cells in the generation of uterine fibroids has been developed. This recent research on the genomics and molecular biology of uterine fibroids has enabled us to better understand the pathogenesis and pathophysiology of this benign tumor.

Adenomyosis is also a benign uterine tumor in reproductive-age women. Recently, women with adenomyosis who wish to become pregnant are increasing in number because the average age of marriage is increasing due to changes in women's lifestyles. Consequently, the influence of adenomyosis on fertility is not negligible. Furthermore, accumulating reports have shown that obstetric complications are closely associated with adenomyosis in pregnant women. Therefore, we need to better understand the pathogenesis of adenomyosis, possible mechanisms of adenomyosis-induced infertility, recent treatments for adenomyosis, and obstetric complications in pregnant women with adenomyosis.

For these subjects, outstanding experts have come together to provide a detailed discussion of basic research and clinical aspects of uterine fibroids and adenomyosis. I believe that this book is beneficial not only for gynecologists and obstetricians but also for basic researchers who are interested in uterine tumors.

Yamaguchi, Japan — Norihiro Sugino

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Histopathology of Uterine Leiomyoma

1

Yoshiki Mikami

Abstract

Uterine leiomyoma shows a wide spectrum of morphologies and clinicopathological features. Thus, in both the clinical and pathological settings, it can be difficult to distinguish from leiomyosarcoma. In particular, patients scheduled for conservative laparoscopic procedures need careful preoperative evaluation, because in rare cases microscopic examination may instead reveal leiomyosarcoma. Clinicians should therefore be familiar with the variety of manifestations and imaging characteristics of both leiomyoma and leiomyosarcoma. In this chapter, the morphologic features and current diagnostic criteria for leiomyoma, as opposed to leiomyosarcoma, and the controversies regarding the diagnosis of borderline tumors are presented. The focus includes smooth muscle tumors of uncertain malignant potential.

Keywords

Uterus · Fibroid · Leiomyoma · Pathology · Variants

1.1 Introduction

Leiomyoma, also called "fibroid," is the most common benign mesenchymal tumor of the uterus. It shows a wide spectrum of morphologies and clinicopathological features such that both clinically and pathologically it can be difficult to distinguish leiomyoma from leiomyosarcoma. In the current standard of practice, patients favor conservative treatment of leiomyoma, especially laparoscopic surgery employing morcellation, rather than hysterectomy. However, in rare cases, on subsequent

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_1

microscopic examination, the resected tumor will be diagnosed as leiomyosarcoma. Therefore, the preoperative evaluation of patients with suspected leiomyoma is crucial and requires that clinicians are familiar with the various clinicopathological expressions and imaging characteristics of leiomyoma and leiomyosarcoma. In this chapter, the morphologic features and current diagnostic criteria for leiomyoma, as distinct from leiomyosarcoma, are presented. The controversies regarding the diagnosis of borderline tumors are discussed as well, focusing on smooth muscle tumors of uncertain malignant potential (STUMPs).

1.2 General Aspects and Clinical Features

Leiomyoma is defined as a benign smooth muscle tumor characterized by a variety of morphological features. As the most common uterine neoplasm, it is identified in up to 75% of hysterectomy specimens. The uterine corpus accounts for 98% of the anatomic sites, and among up to 80% of patients, multiple tumors are detected. Although the majority of leiomyomas are asymptomatic, one-third of the patients will show clinical manifestations within the reproductive ages of 30–50, and 10% will undergo surgical management of the tumor. The clinical manifestation of leiomyoma depends on the size, location, and secondary changes of the tumor. Common symptoms include pelvic pain, mass, and genital bleeding. In pregnant women, the tumor may rapidly enlarge, while in menopausal women or those who have undergone oophorectomy, the mass typically shrinks. Leukocytosis occurs in association with secondary infection; erythrocytosis is rare and is induced by erythropoietin production by the tumor.

The management of leiomyoma patients varies based on the clinical manifestations and patient preference. Treatment options range from hormonal therapy to the removal of the tumor(s), whether by enucleation or total hysterectomy. Minimally invasive surgery, and specifically morcellation, has recently gained wider acceptance because of the short recovery time and the resulting improvement in the quality of life following tumor removal. However, careful preoperative evaluation of patients with suspected leiomyoma is mandatory because the diagnosis may turn out to be leiomyosarcoma. The latter accounts for 1–2% of all uterine malignancies, and in these cases the minimal procedure is inappropriate. The incidence of these unsuspected leiomyosarcomas is estimated to be <1% [1, 2]. Parker et al. reported that only one of 371 cases of apparent leiomyoma proved to be leiomyosarcoma [3]. Seidman reported that among 1091 uterine mesenchymal tumors removed by morcellation, two (0.18%) were malignant, including one endometrial stromal sarcoma (ESS) and one leiomyosarcoma [4]. In that particular series, surveillance was followed by laparotomy in 14 patients, and in nine (64.3%) intra-abdominal dissemination was identified, which included one cellular leiomyoma, four STUMPs, and four leiomyosarcomas [4]. A constellation of findings, including rapid enlargement of the mass, tumor heterogeneity, and the possible presence of necrosis, as seen on imaging studies, suggests the diagnosis of leiomyosarcoma. Such cases are deemed "suspicious," which is an indication for hysterectomy. However, there is significant

overlap in the clinical features of leiomyoma and leiomyosarcoma, and rapid enlargement of the tumor per se is a non-specific finding. Chan et al. reported older age, black race, large tumor size, and extrauterine disease as factors suggestive of leiomyosarcoma, based on data retrieved from the Surveillance, Epidemiology, and End Results database [5].

1.3 Pathological Features

The current WHO classification, published in 2014, defines eight morphologic subtypes and four variants of leiomyoma. The latter, which account for approximately 10% of cases, are characterized by distinct growth patterns and clinicopathological features (Table 1.1) [6].

1.3.1 Gross Features

Uterine leiomyomas are typically multiple. Individual tumors range from 5 to 10 cm in their greatest dimension and can be microscopic or form a bulky mass exceeding 10 cm (Fig. 1.1a). On gross examination, these tumors are typically elastic hard, well-demarcated, and have smooth borders. Their cut surfaces are whitish to grayish, with a whorl or arabesque appearance due to interlacing fascicles of smooth muscle cells (Fig. 1.1b). Highly cellular variants and lipoleiomyoma may be yellowish in color (Fig. 1.1c); the former tend to be soft and fleshy on their cut surfaces. Secondary changes in the gross appearance of these tumors consist of hydropic changes, infarction-type necrosis (Fig. 1.1d), cystic degeneration, and dystrophic calcifications. Hydropic portions are translucent and in rare cases may result in the formation of a cystic cavity due to the accumulation of extracellular fluid. Hydropic change is also a feature of dissecting (cotyledonoid) leiomyoma. Infarction-type necrosis is recognized by the reddish to tan color of the tumor and is referred to as "beefy-red" or "red degeneration." The ill-defined, confluent intramyometrial growth of numerous nodules is a characteristic of diffuse leiomyomatosis, and intravenous growth is seen in intravenous leiomyomatosis.

Table 1.1 The WHO 2014 classification of leiomyoma [6]

Cellular leiomyoma
Leiomyoma with bizarre nuclei
Mitotically active leiomyoma
Hydropic leiomyoma
Apoplectic leiomyoma
Lipomatous leiomyoma (lipoleiomyoma)
Epithelioid leiomyoma
Myxoid leiomyoma
Dissecting (cotyledonoid) leiomyoma
Diffuse leiomyomatosis
Intravenous leiomyomatosis
Metastasizing leiomyoma

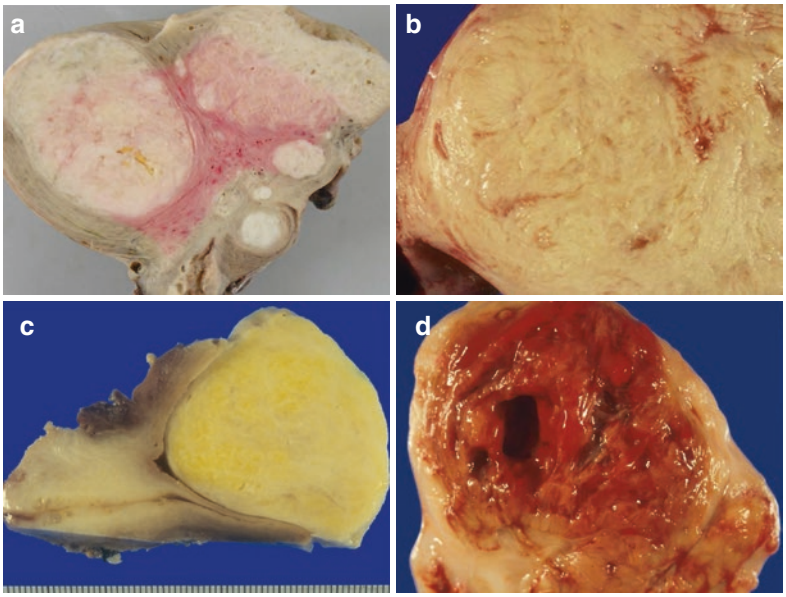

Fig. 1.1 Leiomyoma. (**a**) The gross features of the cut surface show well-demarcated, whitish to grayish nodules in the myometrium. (**b**) Whorl formation due to interlacing fascicles of neoplastic cells, seen on the tumor's cut surface. (**c**) Lipoleiomyoma is recognizable by its yellowish cut surface, due to the intermingling of mature adipocytes. (**d**) Tan and friable areas indicate infarct-type degeneration

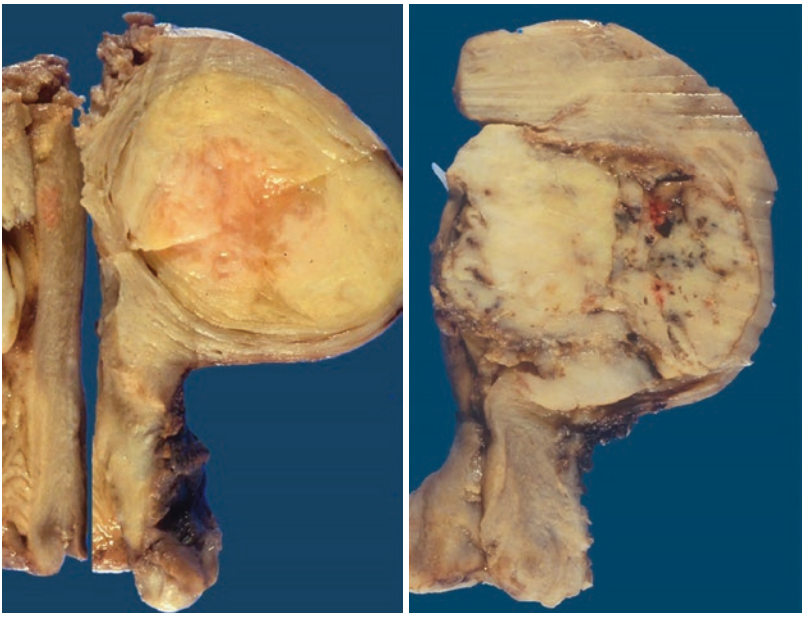

Fig. 1.2 Leiomyoma (right) and leiomyosarcoma (right). Leiomyoma is a well-demarcated mass with smooth borders and a whorl-like appearance, while leiomyosarcoma has irregular borders and a fleshy cut surface, with occasional areas of hemorrhagic necrosis

Table 1.2 Gross appearances of leiomyoma and leiomyosarcoma

	Leiomyosarcoma	Leiomyoma
Number	Single (60–80%)	Multiple
Size	Average: 6–9 cm	Average: 3–5 cm
Cut surface	Elastic, soft, fleshy, fascicular appearance obscured, yellowish-whitish, tan, poorly demarcated, regular border	Elastic hard, fascicular pattern, whitish, well-demarcated
Hemorrhage and necrosis	Common	Frequent

Depending on the location, uterine leiomyoma is divided into submucosal, intramural (interstitial), and subserosal leiomyomas. Subserosal and submucosal tumors can be pedunculated, with a narrow pedicle. Pedunculated submucosal leiomyoma may protrude through the cervical canal and external os. Occasionally, detachment of the tumor from the uterus results in a so-called parasitic leiomyoma. The gross features of typical leiomyoma vs. leiomyosarcoma are summarized in Fig. 1.2 and Table 1.2.

1.3.2 Microscopic Features

Leiomyoma is typically composed of interlacing fascicles of spindle cells with an eosinophilic fibrillary cytoplasm (Fig. 1.3). The cell borders are indistinct, and the nuclei are elongated and blunt-ended (cigar-shaped). Nucleoli are absent or small, and mitotic figures are minimal. Nuclear palisading, thus mimicking schwannoma, is seen in some leiomyomas (Fig. 1.4). In the ordinary leiomyoma of pregnant patients, the cells may show nuclear enlargement, an abundant cytoplasm, and increased mitotic figures (Fig. 1.5). As mentioned above, secondary changes occur to various degrees in these tumors.

1.3.2.1 Cellular Leiomyoma

Occasionally, leiomyoma will show hypercellularity when compared with the normal myometrium of the uterine corpus and is thus referred to as a cellular leiomyoma (Fig. 1.6). The term "hypercellular" describes tumors with features of blue-cell tumor and a resemblance to endometrial stromal nodule or low-grade ESS. The neoplastic cells are short and spindle-shaped, with scant cytoplasm and a homogeneous nuclear morphology. The fascicular pattern may be obscured, particularly in the central area.

1.3.2.2 Leiomyoma with Bizarre Nuclei

Previously called atypical leiomyoma, symplastic leiomyoma, or bizarre leiomyoma, this variant is characterized by an admixture of large cells with multilobated or multiple nuclei that have an irregular configuration and nuclear hyperchromatism. These cells occur against a background of ordinary leiomyoma (Fig. 1.7). The distribution of the bizarre cells ranges from only focal to extensive. Frequently, the nuclei are smudgy and may show intranuclear cytoplasmic pseudo-inclusions. Mitotic figures are minimal,

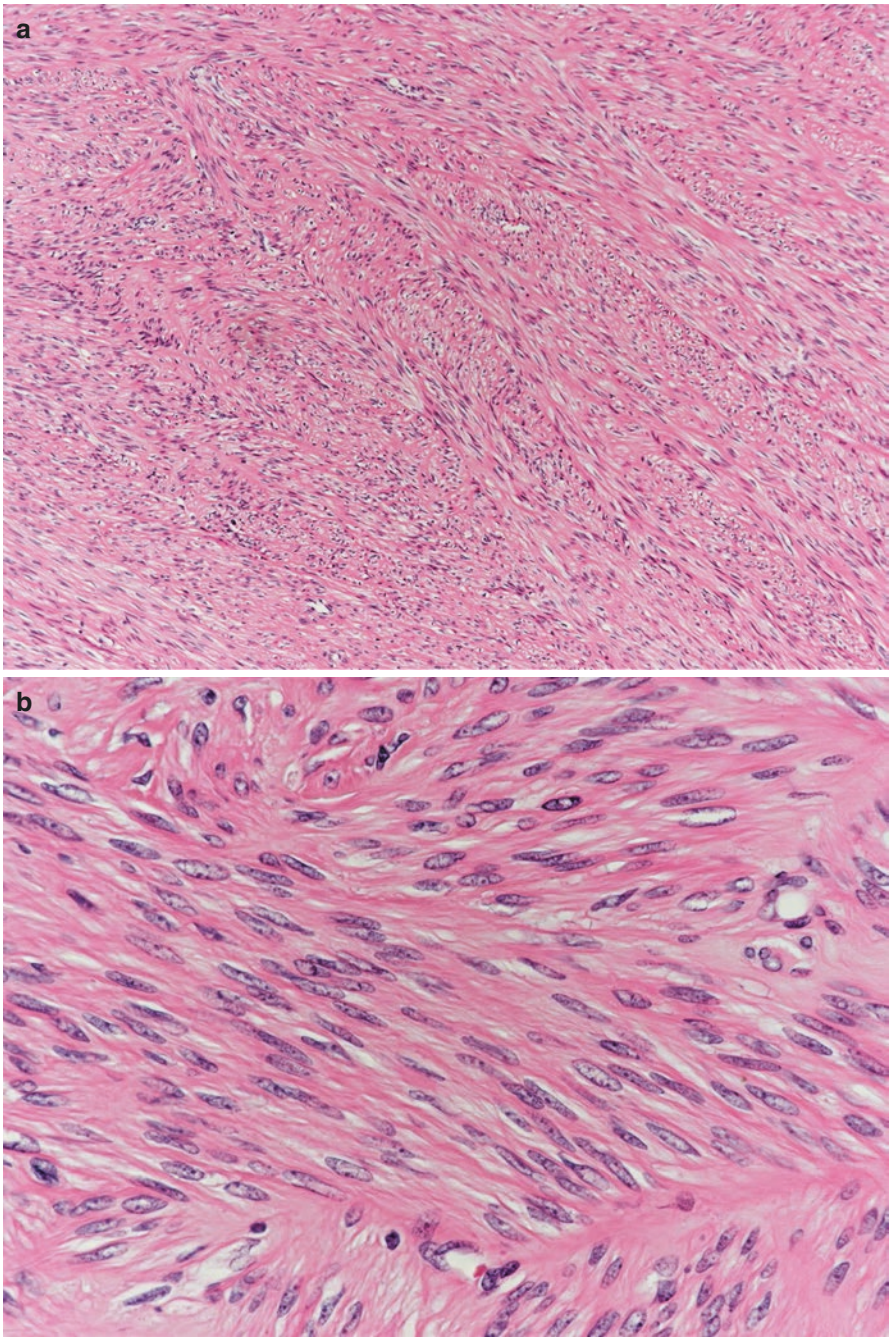

Fig. 1.3 Ordinary leiomyoma. (**a**) Interlacing fascicles of spindle cells. (**b**) The high-power view shows cells with elongated, blunt-ended nuclei and a fibrillary eosinophilic cytoplasm

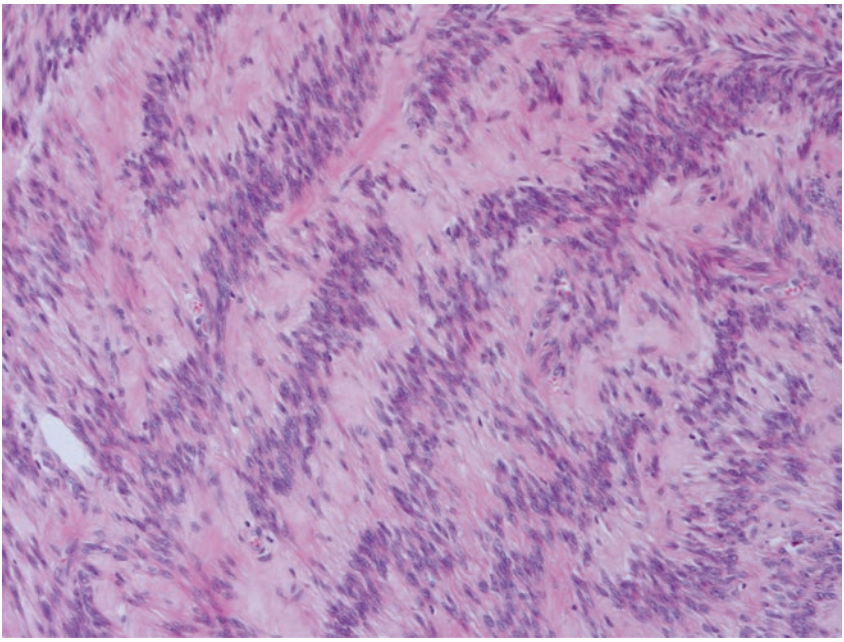

Fig. 1.4 Nuclear palisading in this leiomyoma results in its schwannoma-like appearance

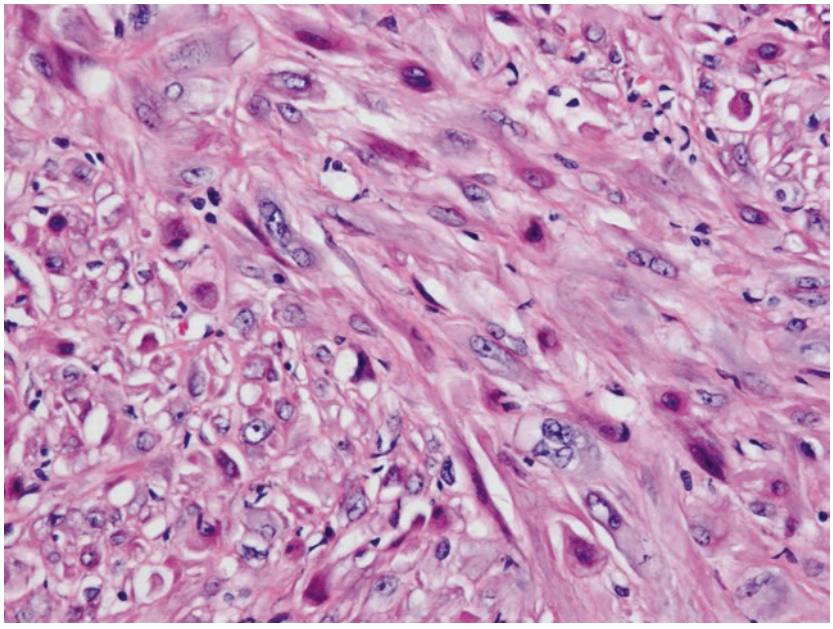

Fig. 1.5 Leiomyoma from a pregnant woman. The tumor cells contain abundant eosinophilic cytoplasm and enlarged nuclei

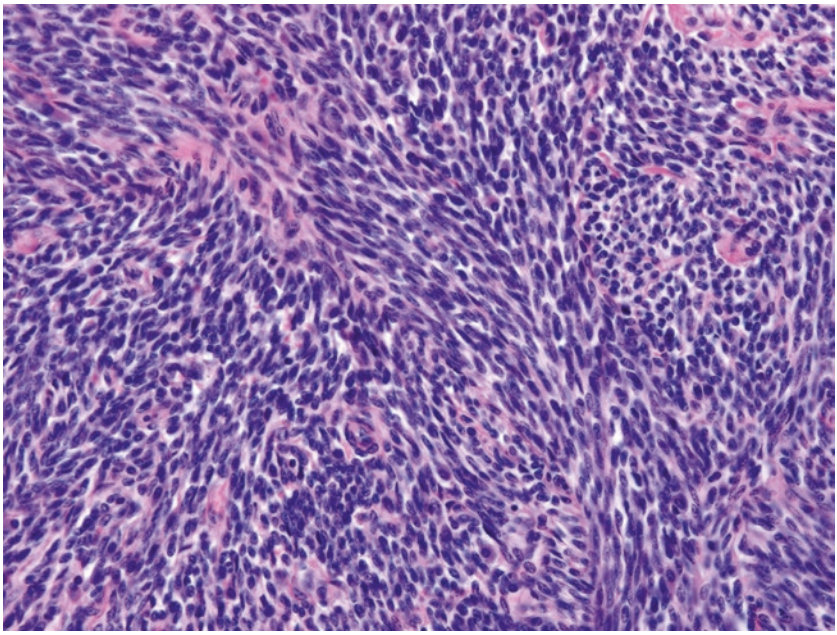

Fig. 1.6 Highly cellular leiomyoma. Features of a blue-cell tumor composed of spindle cells with elongated nuclei and scant cytoplasm impart a resemblance of the tumor to low-grade endometrial stromal sarcoma, although interlacing fascicular pattern of growth suggests the diagnosis of leiomyoma

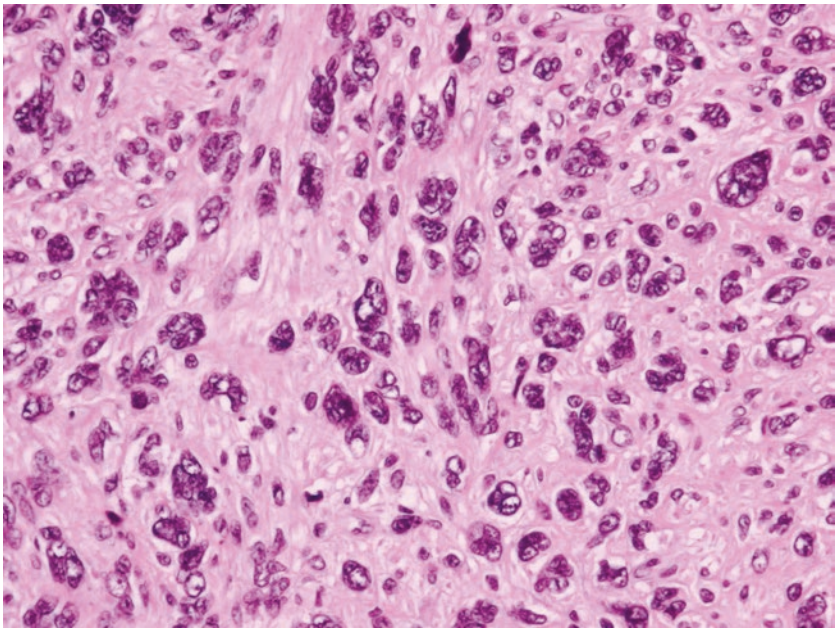

Fig. 1.7 Leiomyoma with bizarre nuclei. This tumor is composed of large and irregularly shaped nuclei with hyperchromasia. Both foci of coagulative tumor necrosis and an increased number of mitotic figures are absent

and coagulative tumor necrosis is absent, although infarct-type areas may be seen. Recent studies suggest that this variant is genetically heterogeneous. In a subset of cases, somatic mutation or deletion of the fumarate hydratase gene is detected [7, 8]. These tumors are characterized by staghorn vessels, prominent eosinophilic nucleoli with perinuclear halos, and eosinophilic cytoplasmic pseudo-inclusions, which are also features of hereditary leiomyomatosis and renal cell carcinoma, as discussed below [7]. The patients with this particular subtype show benign clinical course [9, 10].

1.3.2.3 Mitotically Active Leiomyoma

Tumor in which the number of mitotic figures is increased to 5 or more per 10 high-power fields (10HPF) and without coagulative tumor necrosis or diffuse moderate to severe nuclear atypia is diagnosed as mitotically active leiomyoma [6]. The mitotic counts may be more than 10 per 10HPF, but atypical mitotic figures are absent. This particular variant is frequently seen in women of reproductive age and may be associated with hormonal therapy. With their hypercellularity and/or bizarre nuclei, these tumors can be a diagnostic challenge.

1.3.2.4 Hydropic Leiomyoma

Liquefaction degeneration can be seen to varying degrees in leiomyoma (Fig. 1.8). Edematous change imparts a clear appearance to the tumor and when extensive results in cystic cavity formation, which should be distinguished from myxoid

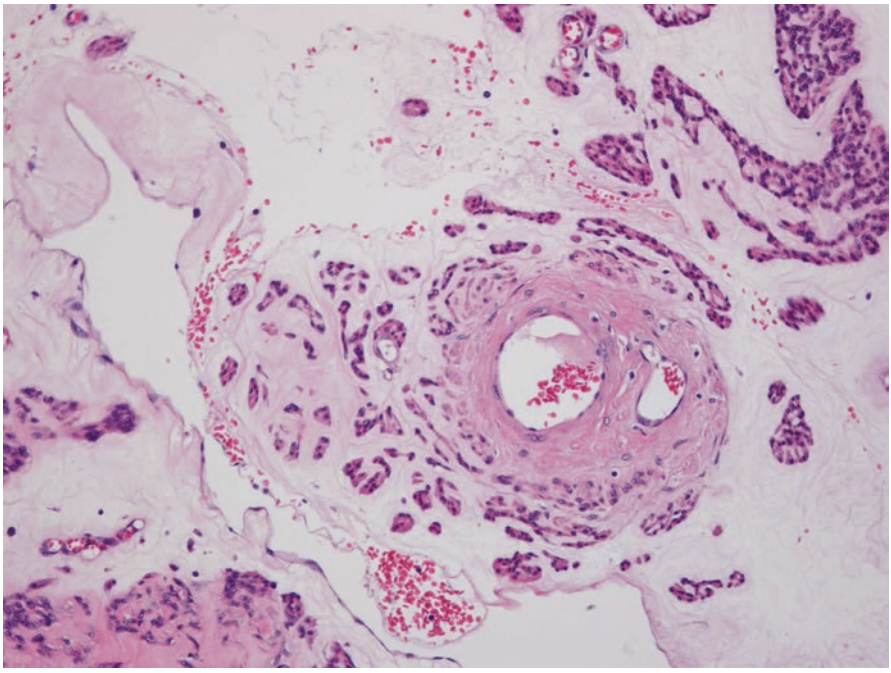

Fig. 1.8 Hydropic leiomyoma is characterized by liquefaction-type degeneration due to the accumulation of extracellular fluid in the widening of spaces between bundles of spindle cells and vessels. The neoplastic cells are arranged in cords

degeneration, described below. Tumors with extensive hydropic change may be confused with myxoid leiomyosarcoma and in those characterized by a perinodular distribution with intravenous leiomyomatosis [11].

1.3.2.5 Apoplectic Leiomyoma

This variant refers to a tumor with extensive infarct-type necrosis that develops in a patient on progesterone therapy. The necrotic area is generally extensive, and in the periphery there may be a granulation tissue-type reaction with or without hemorrhage (Fig. 1.9), surrounded by hypercellular areas with increased mitotic figures. In later stages, the necrotic foci are replaced by extensive hyalinization.

1.3.2.6 Lipomatous Leiomyoma (Lipoleiomyoma)

A mixture of ordinary leiomyoma and mature adipocytes defines lipomatous leiomyoma (Fig. 1.10). The adipocyte content varies, and tumors with abundant adipocytes appear yellowish on gross examination. While it is not uncommon to see a minimal number of adipocytes intermingled with leiomyoma, there are no quantitative criteria for the designation of this variant. Lipoleiomyoma may arise as a consequence of lipomatous metaplasia in a pre-existing leiomyoma. The subset of these tumors with anomalous arterial blood vessels is considered to be choristomatous in nature [12].

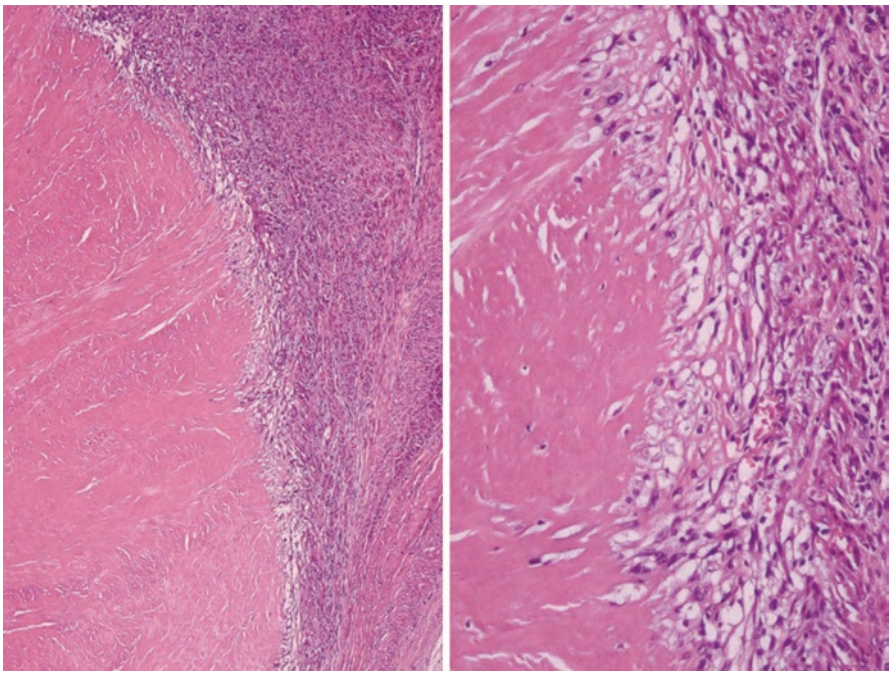

Fig. 1.9 Apoplectic leiomyoma features a necrotic area with extensive hyalinization (hyaline necrosis, left), while in the periphery there is granulation tissue adjacent to areas of smooth muscle with increased cellularity

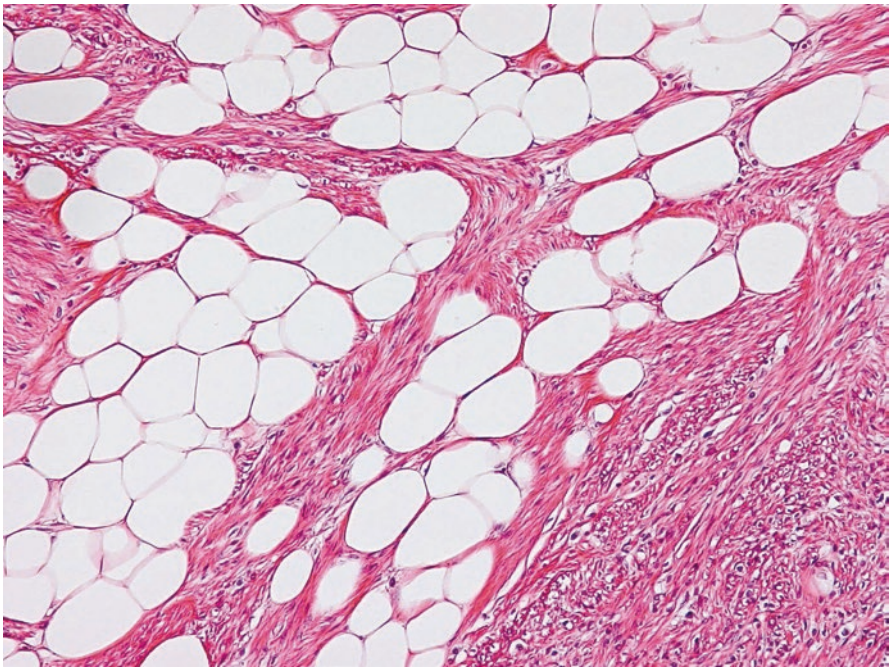

Fig. 1.10 Lipoleiomyoma is composed of a mixture of smooth muscle cells and mature adipocytes

1.3.2.7 Epithelioid Leiomyoma

Epithelioid leiomyoma, previously called leiomyoblastoma, is composed of polyhedral, ovoid, or round cells with an eosinophilic cytoplasm. The cells are arranged in sheets, cords, trabeculae, nests, or individually (Fig. 1.11). Clear-cell features may also be detected in these tumors. Coagulative tumor necrosis is absent, and the number of mitotic figures is minimal. It should be kept in mild that epithelioid leiomyosarcoma can be cytologically bland and shows low mitotic activity. At least six mitotic figures per 10HPF justify the diagnosis of epithelioid leiomyosarcoma. The majority of tumors regarded as extrauterine "epithelioid leiomyoma" or "leiomyoblastoma" previously represent epithelioid form of extraintestinal GIST.

1.3.2.8 Myxoid Leiomyoma

The appearance of myxoid change, characterized by the accumulation of Alcian blue-positive acidic mucin between tumor cells, in a smooth muscle tumor is an alarming sign that should prompt the pathologist to consider myxoid leiomyosarcoma (Fig. 1.12). As in epithelioid leiomyosarcoma, mitotic activity is minimal, with only one to two mitoses per 10HPF indicative of malignancy. The diagnosis of myxoid leiomyoma also requires that the tumor is well-demarcated and has a smooth border and that mitotic figures should be two or less per 10HPF.

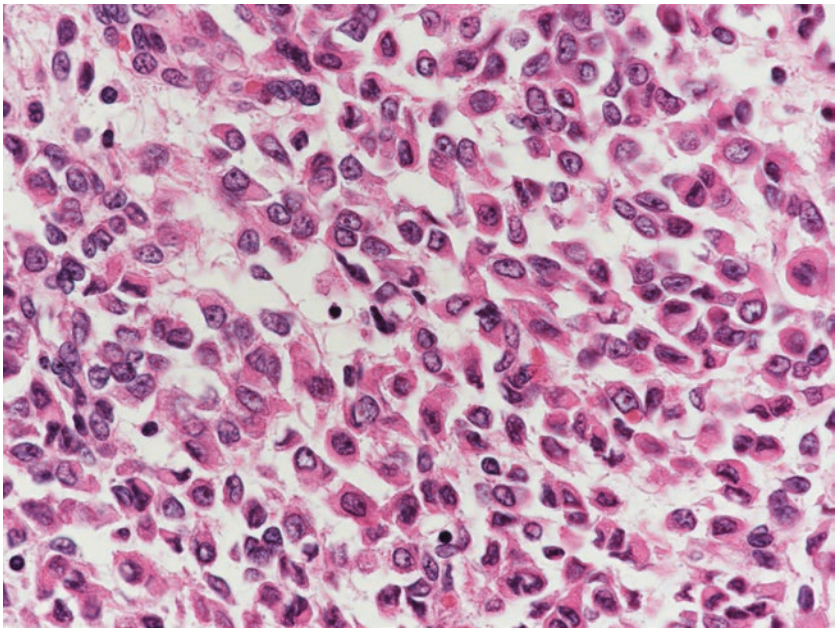

Fig. 1.11 In epithelioid leiomyoma, the neoplastic cells are round, ovoid, or polyhedral and have an abundant eosinophilic cytoplasm

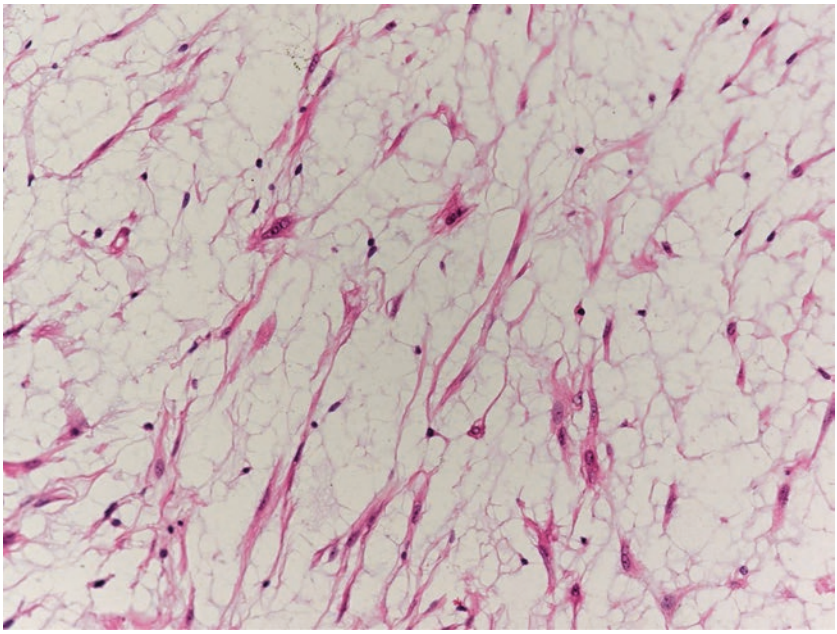

Fig. 1.12 Myxoid leiomyoma is characterized by the deposition of a bluish, wispy substance between the spindle cells

1.3.2.9 Dissecting (Cotyledonoid) Leiomyoma ("Sternberg Tumor")

Rarely, a leiomyoma will dissect the myometrium to extend outside the uterus, in which case it is referred to as dissecting leiomyoma. Extensive protrusion and marked hydropic changes may impart a resemblance to cotyledon of the placenta and thus is designated as "cotyledonoid" (Fig. 1.13a, b) [13]. Microscopically, dissecting leiomyoma usually shows a swirling pattern of growth with extensive liquefaction degeneration (Fig. 1.13c).

1.3.2.10 Diffuse Leiomyomatosis

The extensive involvement of the myometrium by numerous leiomyomatous nodules that result in uterine enlargement is referred to as diffuse leiomyomatosis [14]. These nodules are mostly microscopic and poorly demarcated. Except for the growth pattern, the morphology is similar to that of ordinary leiomyoma (Fig. 1.14).

1.3.2.11 Intravenous Leiomyomatosis

This rare variant is characterized by an extensive, intravenous smooth muscle proliferation (Fig. 1.15) [15, 16]. The tumor frequently extends to vessels outside the myometrium, including the inferior vena cava, the cardiac cavity, and, in rare cases, the pulmonary artery. Morphologically, these leiomyomas are bland and may show features

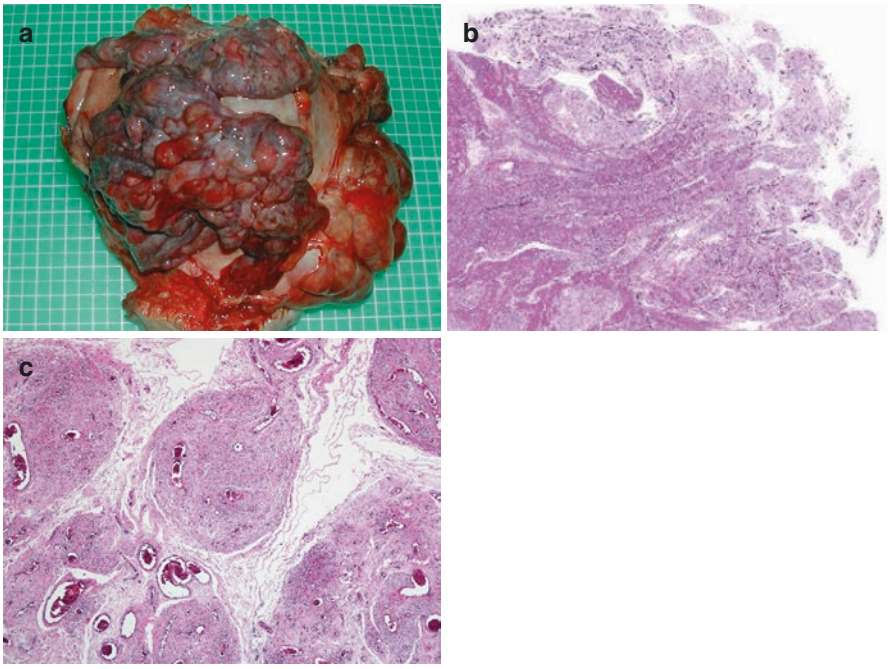

Fig. 1.13 Dissecting (cotyledonoid) leiomyoma. (**a**) A protruding mass on the uterine corpus, resembling placenta, is characteristic of the tumor's gross appearance. (**b**) Leiomyoma dissecting myometrium. (**c**) Liquefaction degeneration is seen between the remaining smooth muscle proliferation

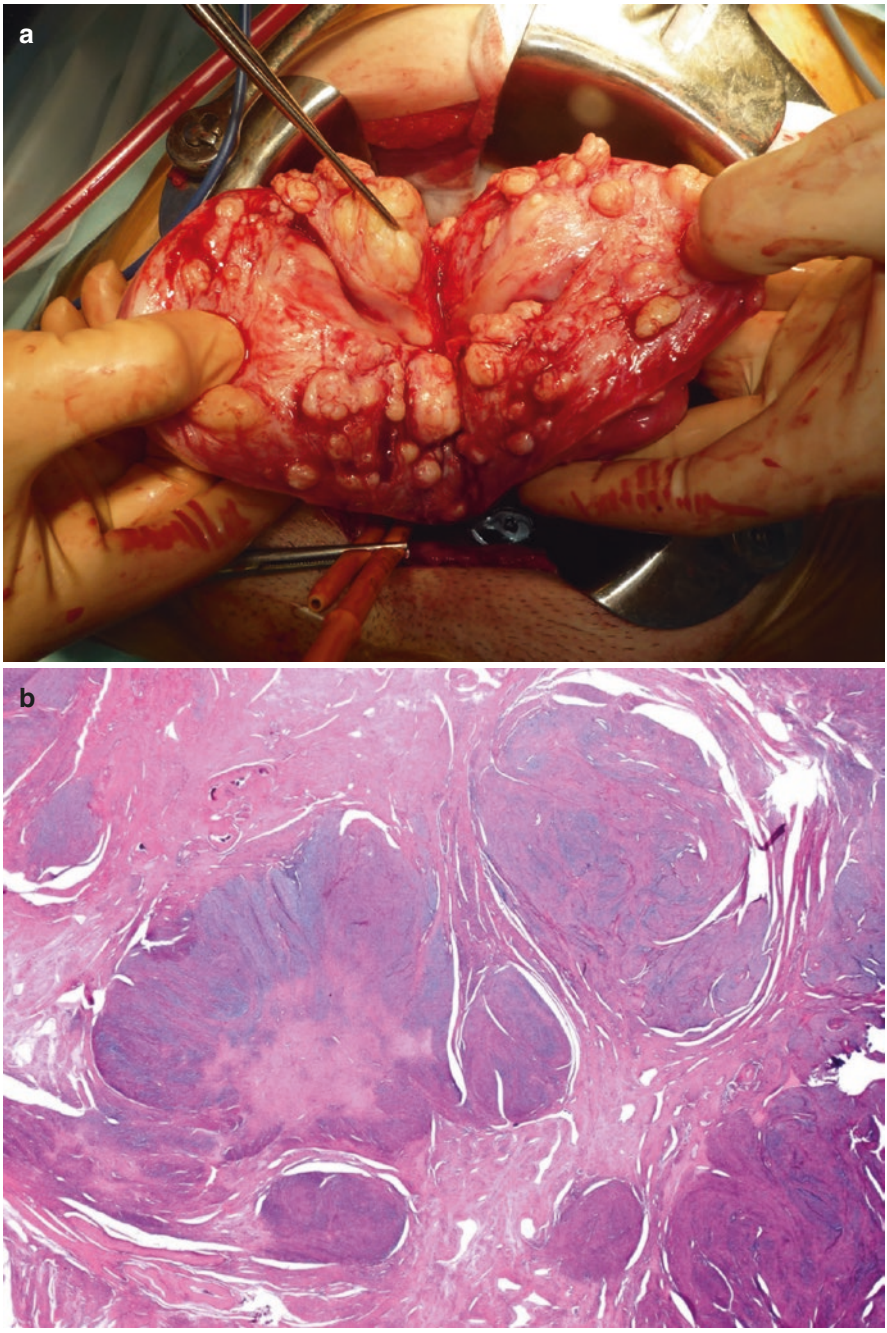

Fig. 1.14 Diffuse leiomyomatosis. (**a**) Multiple irregularly shaped nodules are evident in the myometrium. (**b**) The myometrium is involved by irregularly shaped but well-demarcated nodules with features of ordinary or cellular leiomyoma

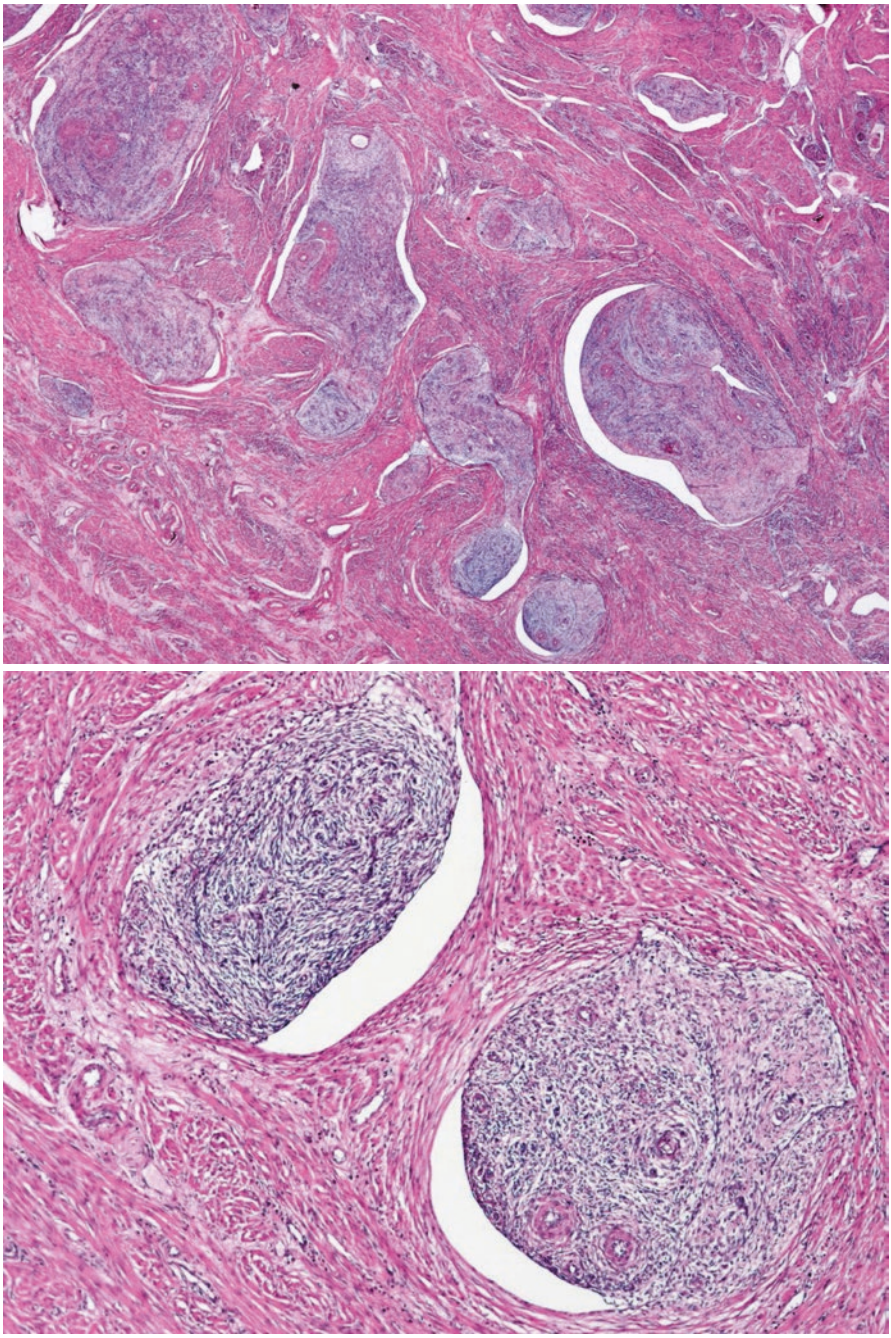

Fig. 1.15 In intravascular leiomyomatosis, smooth muscle cells proliferate within the muscular vessels

of cellular, epithelioid, lipomatous, or myxoid leiomyoma, or bizarre nuclei [16]. Since focal intravascular extension is also a feature of usual leiomyoma, this variant should be diagnosed based on the grossly visible worm-like appearance of the tumor. Rare cases of pulmonary metastasis have been reported as well [16, 17]. The clinical course is generally benign even in cases with metastasis [16, 17]. The pathogenesis of intravenous leiomyomatosis and benign metastasizing leiomyoma may be a vascular microinvasion of uterine leiomyoma in the initial phase of tumorigenesis [18].

1.3.2.12 Metastasizing Leiomyoma

Pulmonary nodules may be identified >10 years after a hysterectomy for uterine leiomyoma or in uncertain conditions [19]. Six pulmonary nodules measuring 1.8 cm in diameter are detected on average [19]. They show features of ordinary leiomyoma (Fig. 1.16a) and are thus well-demarcated and have a smooth border (Fig. 1.16b), but with pre-existing bronchiolar epithelium entrapped in the nodule (Fig. 1.16c). Some authors postulate that metastasizing leiomyoma is actually a slow-growing, low-grade leiomyosarcoma [19], but the term metastasizing leiomyoma has been retained because of the prolonged survival of patients after removal of the nodules and hormonal therapy.

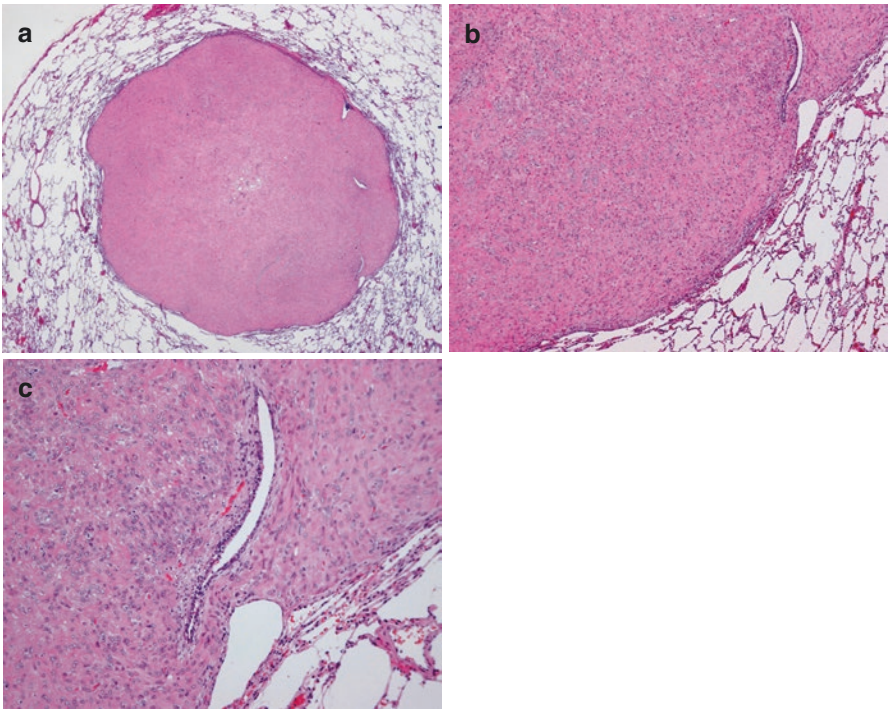

Fig. 1.16 Metastasizing leiomyoma. (**a**) A well-demarcated nodule with a smooth border is seen in the lung. (**b**) The smooth muscle proliferation is arranged in a fascicular pattern. (**c**) A proliferation of benign-looking spindle cells includes the entrapment of pre-existing bronchioles

1.3.3 Differential Diagnosis

Leiomyosarcoma, a malignant smooth muscle tumor, typically shows at least two of the following three features: (1) geographic coagulation tumor necrosis, (2) diffuse moderate and severe cytologic atypia, and (3) >10 mitotic figures per 10 HPF (Table 1.3) [20]. Hypercellularity and infiltrating growth are common. Epithelioid and myxoid variants can be pitfalls since the mitotic count is generally low. Thus, the criteria of five and two mitoses per 10HPF, respectively, and infiltrating growth should be employed in establishing the diagnosis of these leiomyosarcoma variants [21, 22]. Similar diagnostic criteria can be applied to distinguish diffuse leiomyomatosis, dissecting leiomyoma, intravenous leiomyomatosis, and metastasizing leiomyoma from leiomyosarcoma. Mimics of mitotic figures, including apoptotic bodies, karyorrhexis, aggregations of hematoxylin, and mast cells, should not be counted. Non-atypical mitosis may be seen in pregnant women or those on progesterone therapy. Infarct-type necrosis, also known as hyaline necrosis, is encountered in young women, especially in those who are pregnant or who are being treated with a gonadotropin-releasing hormone analog, such as leuprolide acetate, or by transarterial embolization. Torsion of a subserosal leiomyoma may result in the same morphology. In contrast to the extensive area of necrosis surrounded by a zone of gradual organization and marked by granulation tissue or hyalinization, geographic tumor necrosis is characterized by a spotty distribution and the irregular configuration of the necrotic areas, distinct borders, and preservation of the tumor vessels and surrounding neoplastic component. Immunohistochemical panels may be used to identify the tumor. The absence of estrogen receptor expression, a mutant pattern of p53 staining, p16 immunoreactivity, and an increased Ki-67 labeling index suggest the diagnosis of leiomyosarcoma, but the pathologist should be aware that in exceptional cases the tumor will be a leiomyosarcoma.

Endometrial stromal tumor, particularly stromal nodule and low-grade ESS, may be confused with cellular leiomyoma. The presence of both thick muscular vessels,

Table 1.3 Diagnostic criteria for ordinary leiomyosarcoma [20]

Coagulative necrosis	Cytologic atypia	Mitosis (/10 high-power fields)	Diagnosis
Present	Diffuse, moderate to severe	Any	Leiomyosarcoma
	None to mild	≧10 <10	Leiomyosarcoma Leiomyosarcoma (rule out infarcted leiomyoma)
Absent	Diffuse, moderate to severe	≧10 <5 5–9 or atypical mitosis	Leiomyosarcoma Atypical leiomyoma with low risk of recurrence STUMP
	None to mild	<5 ≧5	Leiomyoma Mitotically active leiomyoma
	Focal, moderate to severe	≧5 <5	STUMP Atypical leiomyoma

From: Blaustein's Pathology of the Female Genital Tract, 6th edition (2011)

in contrast to spiral artery-type small vessels, and a distinct interlacing fascicular pattern is indicative of leiomyoma. Immunohistochemical studies may be of limited value, because low-grade ESS occasionally shows smooth muscle differentiation and may thus be positive for smooth muscle markers, while leiomyoma may be diffusely and strongly positive for CD10, a marker of endometrial stromal cells [23]. Therefore, a constellation of findings, including growth pattern, cell morphology, and the results of immunohistochemistry using a panel of antibodies, is needed to establish the diagnosis.

Perivascular epithelioid cell tumor (PEComa) is a mimic of leiomyoma, although it is composed of rather spindle or epithelioid cells with clear or pale eosinophilic cytoplasm, showing vascular network. There may be extensive sclerosis. It can be associated with lymphangioleiomyomatosis and occasionally is associated with tuberous sclerosis. PEComa shows immunoreactivity for melanocytic markers including HMB45, Melan-A, and MiTF, as well as smooth muscle markers.

1.4 Hereditary Leiomyomatosis and Renal Cancer Syndrome (HLRCC)

This autosomal dominant disorder is associated with germline alterations in the fumarate hydratase gene, including point mutations and partial or complete deletions [24]. HLRCC is characterized by the coexistence of uterine and cutaneous leiomyomas and papillary renal cell carcinoma type 2 [25]. The type of renal cell carcinoma is diagnosed in approximately 30% of patients with this syndrome [24] and has an aggressive clinical behavior [26]. The patients tend to be younger than those with sporadic leiomyoma. Uterine tumor shows features of leiomyoma with bizarre nuclei. Prominent red or orange nucleoli with a perinuclear halo are considered to be a characteristic of HLRCC [7].

1.5 Extrauterine Leioyomatosis

Other than intravenous leiomyomatosis involving extrauterine vessels, in rare cases, including diffuse peritoneal leiomyomatosis and nodal leiomyomatosis [27], an extrauterine smooth muscle proliferation may be associated with uterine leiomyoma.

1.6 Smooth Muscle Tumor of Uncertain Malignant Potential (STUMP)

STUMP is defined as a smooth muscle tumor in which the risks of recurrence and metastasis are uncertain; thus, the term refers to a diagnostic category rather than a distinct entity. STUMPs include (1) leiomyoma, (2) leiomyosarcoma that cannot be recognized as such based on the current diagnostic criteria, and (3) true borderline smooth muscle tumors that are biologically intermediate between leiomyoma and

leiomyosarcoma. The third category appears to account for a minority of these cases. Indeed, limited sampling or limited experience may cause the pathologist to underevaluate leiomyosarcoma and instead diagnose STUMP. Therefore, the diagnosis of STUMP should be avoided as possible and is only rarely made.

From the clinical point of view, uterine smooth muscle tumor other than ordinary leiomyoma and leiomyosarcoma is classified as tumor with recurrent and/or metastatic potential and tumor with little or no recurrent and/or metastatic potential [2]. The former includes STUMP, intravenous leiomyomatosis, and benign metastasizing leiomyoma, whereas the latter leiomyoma with bizarre nuclei, mitotically active leiomyoma, cellular leiomyoma, dissecting leiomyoma, myxoid leiomyoma, epithelioid leiomyoma, and diffuse leiomyomatosis [2].

Morphologically, STUMPs are divided into tumors with prototypical smooth muscle differentiation and tumors with an unusual differentiation or uncharacteristic morphology, such as myxoid and epithelioid features. From the pathological point of view, ambiguity with respect to the nature of the necrosis, degree of differentiation, or mitotic count may prompt a pathological diagnosis of STUMP. For example, it may be difficult to distinguish coagulation necrosis from infarct-type necrosis. Epithelioid and myxoid leiomyosarcomas are diagnosed based on criteria that are different from those of the usual type of leiomyosarcoma. Tumors with a myxoid and epithelioid morphology with moderate to severe cytologic atypia and 5 or less and 2 more less mitotic figures per 10HPF, respectively, are categorized as STUMPs.

In a series from a single institution that included 16 cases of STUMP diagnosed by well-established criteria and with a follow-up interval of 21–192 months (mean, 80.8, and median, 51.5), Ip et al. reported that 2 patients (13%) had recurrent tumors in the pelvis, 51 and 15 months after the initial operation. Both patients were alive 74 and 48 months after recurrence, respectively [28]. The authors thus recommended that STUMP patients be followed every 6 months with a checkup of symptoms and general and pelvic examinations and every 12 months with imaging studies, including a chest X-ray to exclude pulmonary metastasis and pelvic ultrasonography, computed tomography, or magnetic resonance imaging to detect new lesions, for a minimum of 5 years, followed by annual surveillance for a further 5 years [2].

1.7 Prognosis and Predictive Factors

The clinical course of patients with the usual type of leiomyoma or its variants is benign, although a variety of complications may affect the quality of life in a subset of patients. In addition, variants such as intravenous leiomyomatosis may recur, including with inferior vena cava or intracardiac extension. Metastasizing leiomyoma also has an indolent clinical course but may cause respiratory failure due to the enlargement of the tumor.

Rare examples of leiomyosarcoma arising in leiomyoma have been described in the English medical literature [29]. Features of a bona fide leiomyoma may be identified in leiomyosarcoma, but the relationship between the two components is not well understood.

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Genetics and Genomics of Uterine Fibroids

2

Hiroshi Ishikawa and Makio Shozu

Abstract

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

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_2

Keywords
Chromosomal rearrangement · MED12 · HMGA2 · Whole-genome sequencing microRNA

2.1 Introduction

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

2.2 Genetic Backgrounds

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

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

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

2.3 Clonality

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

2.4 Genome-Wide Association Study

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

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

2.5 Chromosomal Rearrangements

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

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

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

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

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

2.6 Syndrome-Associated Fibroids

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

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

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

2.7 Genetic Driver Alterations of Uterine Fibroids

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

2.7.1 *MED12* Mutations

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

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

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

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

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

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

2.7.2 *HMGA2* Overexpression

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

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

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

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

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

2.7.3 Biallelic *FH* Inactivation

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

2.7.4 Alterations of *COL4A5* and *COL4A6*

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

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

2.8 Role of MicroRNAs in the Pathogenesis of Uterine Fibroids

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

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

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

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

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

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

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

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

Conclusions

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

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Molecular Pathogenesis of Uterine Fibroids

3

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Abstract

Researchers investigated aberrant gene expression in uterine fibroids to elucidate their molecular pathogenesis. Complex networks of multiple factors such as growth factors and WNT/β-catenin signaling have been shown to be involved in the pathogenesis of uterine fibroids. This chapter will focus on the current knowledge of molecular mechanism that are involved in the generation or development of uterine leiomyomas.

Keywords

Genetics · Molecular mechanism · Uterine fibroid

3.1 Origin of Uterine Fibroid and the Importance of Molecular Pathogenesis

It has yet to be determined which cell uterine fibroids originate. However, several studies suggested that each uterine fibroid originated from a transformed stem cell of the myometrium called a fibroid stem cell [1–4]. Asymmetric division results in a portion of cells undergo self-renewal with the majority producing daughter cells that become mature fibroid cells [2, 5, 6]. It was known that human uterine fibroid tissue contained fewer stem cells than normal myometrium [1, 7]. Furthermore, stem cells derived from fibroid tissue carry mutations in genes such as high-mobility group AT-hook 2 (HMGA2) [8–10] and mediator complex subunit 12 (MED12) [11, 12], suggesting that at least one genetic change initially transformed a myometrial stem cell to a fibroid stem cell. Although initiation of uterine fibroid development

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_3

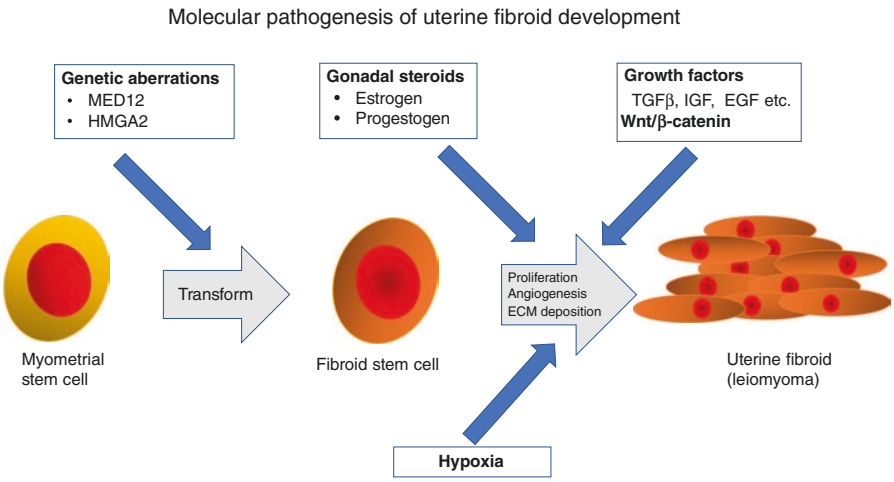

Fig. 3.1 Schematic representation of the molecular pathogenesis of uterine fibroid development. Modified from Tal R and Segars JH (2014) Human Reproduction Update 20 (2): 194–216

was considered to involve genetic alterations in myometrial stem cells causing transformation into fibroid stem cells, subsequent alterations in complex molecular signaling were also thought to be necessary for the development of uterine fibroids. Gonadal steroids, hypoxia, growth factors, and Wnt/β-catenin are involved in this process (Fig. 3.1).

3.2 Genetic Features

3.2.1 High-Mobility Group AT-Hook 2 and Mediator Complex Subunit 12

Mutations in chromosome 12q14-15 upstream of the HMGA2 gene promoter (12q15) have been observed in 7.5% of uterine fibroids, resulting in overexpression of full-length HMGA2 [7]. It is also known that uterine fibroids lacked Let-7 miRNA that suppressed HMGA2 [13]. In murine neural stem cells, HMGA2 was shown to suppress the production of p16Ink4a and p14Arf proteins that negatively regulated their self-renewal [14]. In fibroid cells, HMGA2 protected against senescence by downregulating p14ARF [9]. Aberrant expression in the Let7–HMGA2–p14ARF pathway may favor conditions for self-renewal of fibroid stem cells and protect from senescence [15].

The heterozygous somatic mutations that affected MED12 on the X chromosome occurred in approximately 70% of 225 fibroid tumors isolated from 80 patients [11]. The fact was confirmed in other studies [12, 13, 16, 17]. MED12 was known to regulate canonical WNT signaling [18–20]. The expression levels of WNT4, which is the regulator of β-catenin, increased in fibroids with MED12 mutations compared with

fibroids without the mutations [17]. Decreased levels of MED12 activated the TGF-β pathway, leading to activation of two signaling pathways: mothers against decapentaplegic homologue (SMAD) and mitogen-activated protein kinase (MAPK) [21]. Thus, MED12 mutations are involved in stem cell renewal, cell proliferation, and fibrosis via WNTβ-catenin activation as well as hyperactive TGF-β signaling [22–24]. Recently, silencing MED12 gene was reported to be reduced proliferation of human uterine fibroid cells mediated via Wnt/β-catenin signaling pathway [24].

3.3 Molecular Mechanism of Development of Uterine Fibroid

3.3.1 Estrogen

Uterine fibroids have been considered to be estrogen-dependent tumors [25–27]. Uterine fibroids appeared when women became sexually mature and then regress in postmenopausal women [27, 28]. No cases of uterine fibroids have been reported before puberty [28, 29]. Furthermore, gonadotropin-releasing hormone agonist (GnRHa) therapy in premenopausal women regressed fibroid size, regardless of the woman's age [30–32]. Based on these observations, it was thought that estrogen was associated with the development of uterine fibroids. Estrogen acts by binding to estrogen receptors (ERs). ERs are localized within the nucleus and/or on the cell membrane [33, 34]. Nuclear ERs are further subdivided into two types, ERα and ERβ [35, 36]. Both receptors have five domains including a DNA-binding domain and transcriptional activation domain [28], thus mediating effects via genomic mechanisms. The receptors were encoded by two distinct genes [37–40], localized within different tissues with ERα mainly in uterus and breast and ERβ expressed in a variety of organs [28, 35, 41]. Membrane estrogen receptors (mERs) included two nuclear estrogen receptors (Era1, ERβ) located on the plasma membrane, as well as membrane-specific receptors: G protein-coupled receptor 30 (GPR 30), ER-X, ERx, and Gq-mER [33, 34, 42]. Approximately 90–95% of ERs were localized in the cell nucleus with few located in plasma membrane and activated cell signaling via modulation of intracellular signaling cascades [33, 34].

Uterine fibroids were exposed to estrogens not only produced in the ovary but locally through conversion of androgens by aromatase within uterine fibroids [26, 43]. Cultured fibroid cells produced estrone from androstenedione, which was then converted to the more potent estradiol. The addition of androstenedione to cultured leiomyoma cells resulted in similar cellular proliferation profiles as culturing in estradiol [44]. The addition of aromatase inhibitors to the culture medium decreased proliferation of fibroid cells [44]. These data suggested that uterine fibroids were capable of producing estrogens which promoted the proliferation of themselves. Recently, it has been shown that uterine fibroids possessed relatively higher levels of hydroxysteroid 17-beta dehydrogenase 1 (17b-HSD type 1) as well as aromatase compared to adjacent uterine myometrium, presumably leading to higher estrogen levels observed in uterine fibroids [45], although the mechanism underlying

gonadotropin-independent expression of aromatase in fibroids is not well understood [46]. The estrogen effect on uterine fibroids was also mediated by two pathways, transcriptional activities through ERs in the nucleus [47, 48] and cell signaling activation through mERs on the plasma membranes [49]. Estrogen increased the expression of several genes thought to play a role in uterine fibroid pathogenesis, including growth factors, collagens, and sex hormone receptors, acting by gene transcription through ER. Some studies have reported that fibroids expressed more ERα and ERβ mRNA compared to normal myometrium [47, 48, 50]. Epigenetic regulation of ERα was also reported in uterine fibroids through DNA methylation [51]. Additionally, several studies have suggested that polymorphisms within the ERα gene may increase a women's susceptibility to uterine fibroids [52–54]. However, it was shown that estrogen regulated the expression of progesterone receptors (PR) and estrogen alone did not act as a mitogen in vivo [55]. Moreover, disruption of the estrogen signaling pathway by transfecting fibroid cells with an ER mutant that suppresses wild-type ER activity diminished both ER- and PR-gene expression [56]. These findings suggested that a more important role for estrogen was inducing PRs for progesterone. Progesterone has been shown to stimulate the growth of uterine fibroids through regulation of key genes that affected both apoptosis and proliferation. The role of progesterone on development of uterine fibroids is described later in this chapter.

On the other hand, there was evidence of rapid estrogen signaling through mERs in uterine fibroids. Uterine fibroids overexpressed GPR30 compared to normal myometrial tissue [49]. It has been demonstrated that estrogen exposure induced rapid and transient activation of the MAPK pathway and early downstream signal transduction events including rapid protein tyrosine phosphorylation of a subset of intracellular proteins, such as phosphoinositide 3-kinase (PI3K) and phosphoinositide phospholipase Cɣ (PLCɣ) [57]. This leads to secretion of platelet-derived growth factor (PDGF) involved in the proliferation of uterine fibroid cells [57]. In addition, it was demonstrated that estradiol rapidly increased levels of phosphorylated protein kinase C alpha (PKCα) in both immortalized uterine smooth muscle (UtSM) and uterine fibroid cell (UtLM) lines, although levels of phosphorylated extracellular signal-regulated kinase (ERK)(MAPK)1/2 was increased only in UtLM cells [58]. They also demonstrated a paradoxical effect of molecular and pharmacological inhibition of PKCα on ERK1/2 activation and cellular proliferation in UtLM and UtSM cells. PKCα inhibition decreased levels of phosphorylated ERK (MAPK)1/2 and proliferation in UtLM cells, although raised the levels in UtSM cells. Signaling of cAMP-PKA was rapidly activated only in UtSM cells cultured in estradiol and subsequently inhibited ERK (MAPK)1/2 activation and cellular proliferation. Based on this data, a model was proposed whereby the rapid activation of PKCα and cAMP-PKA signaling by estradiol played a central role in the maintenance of lower proliferation rates in normal uterine muscle through inhibition of the MAPK cascade [58]. This was confirmed when these pathways were altered to promote MAPK activation and proliferation in uterine fibroids. These studies demonstrated that estradiol signaling pathways through mERs contributed to development of uterine fibroids. The modulation of this estrogen effect has been

used as a strategy for therapies. It has been shown that suppressing estrogen production through continuous administration of GnRHa shrinks the size of uterine fibroids [30–32]. Therefore, estrogen contributed to the occurrence and progression of uterine fibroids through aberrant transcription of genes via nuclear receptors, as well as aberrant activation of cell signals mediated by membrane receptors.

3.3.2 Progestogen

While earlier studies focused on the role of estrogen in uterine fibroid development [59, 60], the significant role for progesterone has only recently been identified [29, 61]. Mitotic activities of fibroid cells were significantly higher during the secretory phase and not the proliferative phase [62]. Animal models supported the importance of progesterone on the development of uterine fibroids. A xenograft model generated by grafting human uterine fibroid tissue under the renal capsule of immunodeficient mice showed increased size of the xenograft following administration of estradiol and progesterone. Cell proliferation of fibroid cells and increased volume in the cellular and extracellular components of fibroid tissues was observed. The xenograft growth induced by estradiol and progesterone was blocked by the antiprogestin, RU486, and volume decreased significantly after discontinuation of progesterone. Furthermore, treatment with estradiol alone neither increased nor maintained the tumor size, emphasizing the critical role of progesterone rather than estrogen. The effect of estrogen on uterine fibroid development could be due to PR induction [55, 61].

Similar to estrogen receptors, PRs were localized, not only in the nucleus but also on the cell membrane. Nuclear PRs acted as transcriptional factors as well as nuclear estrogen receptors and can be further subdivided into two types, PRA and PRB [63, 64]. Both PR types were shown to be transcribed by the same gene. PRB underwent posttranscriptional modification becoming truncated to generate PRA, smaller by 164 amino acids [65]. Membrane PRs (mPRs) have also been identified in humans, as well as mERs, consisting of three members: mPRα, mPRβ, and mPRγ [66, 67].

The role of progestogen in uterine fibroid development has also been elucidated, although it was less understood, compared to estrogen. The effects of progesterone on the expression of growth factors (EGF, IGF-I) and apoptosis-related factors (TNFα, Bcl-2 protein) in cultured uterine fibroid cells have been analyzed [68–70]. Treatment with progesterone augmented EGF and Bcl-2 protein expression, although it inhibited expression of IGF-I and TNFα in cultured uterine fibroid cells. It was known that TNFα induced apoptosis in a variety of cell types and Bcl-2 protein was an anti-apoptosis gene. This study suggested that progesterone had dual actions on uterine fibroid growth. Firstly, it stimulated fibroid cell growth and survival through upregulating expression of EGF and Bcl-2 proteins, as well as downregulating TNFα expression. Secondly, it inhibited fibroid cell growth through downregulating IGF-I expression. This could explain why the size of uterine fibroids increased in some but decreased in other instances during administration of

progestogen and the reason why the size of some uterine fibroids did not increase during pregnancy despite increased circulating concentrations of sex steroid hormones. It has been demonstrated that a progestin, R5020, induced proliferation of fibroid cells in vitro [71]. In addition, R5020-induced phosphorylation on AKT and glycogen synthase kinase-3B (GSK3B) and API-59 (an AKT inhibitor) abrogated R5020-induced cellular proliferation. It was concluded, based on this study, that progesterone could induce proliferation of fibroid cells through activation of the AKT pathway. Although mPRs were localized in the uterus, there has been no evidence showing their role in uterine fibroid development.

Patients with symptomatic uterine fibroids were randomized into two groups, either receiving selective progesterone receptor modulator (SPRM) ulipristal acetate or placebo [72]. After 13 weeks of treatment, a significant reduction in the total volume of fibroids was evident in the treated groups. This study provides evidence that progesterone was involved in the proliferation of uterine fibroids.

3.3.3 Hypoxia

It has been shown that hypoxia was a key factor in tumor development, which promoted local invasion, vascularization, and metastatic spread as well as tumor growth [73–75]. The impact of hypoxia on gene expression was mainly due to the hypoxia-inducible transcription factor HIF-1 [76–78]. It was known that HIF-1 activated the development of malignant tumors by modifying glucose metabolism through increased glucose uptake and enhanced glycolysis, promotion of angiogenesis by increased expression of vascular endothelial growth factor (VEGF), and activation of the c-MET/HGF system which is related to cell proliferation, migration, and protection from apoptosis [79–81]. Consistent with the effects of HIF-1, the majority of original lesions and metastatic lesions of human cancers overexpressed HIF-1a [76, 82]. Thus, HIF-1 is believed to be a major regulator of cellular response under low oxygen concentration and associated with human tumor genesis and development. HIF-1 was shown to be a heterodimeric transcription factor composed of two subunits, a and b. While HIF-1b worked constitutively, HIF-1a was induced under hypoxic conditions, and inactivated and then degraded under normal oxygen concentration. The accumulation of HIF-1a led to heterodimerizing with HIF-1b, and the dimerized complex was then bound to hypoxia-responsive elements in target genes, resulting in transcriptional activation.

The origin of uterine fibroid is unknown, but accumulating evidence suggested that hypoxia was likely to be implicated in early cellular events that led to myometrial smooth muscle cell transformation into uterine fibroid. Areas of irregular zones with hyper cellularity, called myometrial hyperplasia (MMH), have been found in the myometrium. It was suggested that MMH were precursors to fibroid development [83–85]. A side population of myometrial cells (SP cells) was discovered in the uterus, with characteristics of myometrial stem cells, accounting for 1% of the total myometrial cell population [2]. Following transplantation into immunodeficient mice, SP cells formed larger fibroids with higher proliferation than normal

myometrial cells compared to myometrium that lacked SP cells, suggesting SP cells were crucial for fibroid development [3]. Interestingly, SP cells were shown to differentiate into mature myometrial cells only under hypoxic conditions in vitro, suggesting that hypoxia may be the trigger for SP cell transformation into fibroid cells. Since the menstrual cycle has been associated with periodic ischemic/hypoxic stress due to myometrial contractions, these hypoxic events may be the stimuli for SP cell differentiation in vivo.

Similar to other tumors, the tissue inside uterine fibroids has been revealed to be hypoxic. However, it has been shown that despite this extreme hypoxia, fibroids did not express HIF-1a, HIF-2a, and other hypoxia-related genes [86–88]. This indicates a lack of response to tissue hypoxia, although leiomyosarcomas, their malignant counterparts, expressed abundant HIF and related proteins [88]. The disturbed response to hypoxia and lack of HIF-1a expression may have contributed to features of fibroid pathophysiology, including their diminished ability to become vascularized, limited growth, and lack of invasion or metastasis. It has been demonstrated that loss of HIF-1a expression in tumors derived from embryonic stem cells resulted in a lack of formation of large blood vessels, impaired vascular function, downregulation of hypoxia-induced expression of VEGF, and decreased hypoxia-induced apoptosis [89]. Uterine fibroids also showed a diminished vasculature and decreased apoptosis when compared with leiomyosarcomas, which overexpressed HIF-1a.

The mechanism for why HIF-1a was not upregulated in uterine fibroids even with hypoxic conditions remains unclear. It was suggested that the serine/threonine kinase mammalian target of rapamycin (mTOR) played a crucial role in HIF-1a downregulation in uterine fibroids [87]. The mTOR pathway was shown to be involved in cell signaling pathways that promoted tumorigenesis via phosphorylation of cascade proteins that regulated protein synthesis, cell growth, and proliferation [90, 91]. The inhibition of mTOR directly downregulated HIF-1a protein expression, suggesting that HIF-1a expression was dependent on mTOR activity [92–94]. In pregnant rats, hypoxia occurred in the myometrium when the uterus reached a certain size, resulting in rapid downregulation of mTOR [84]. It was also suggested that downregulation of mTOR pathway was associated with hypoxic regulation of HIF-1a levels. Phosphorylated AKT (pAKT) and mTOR activity were very low in uterine fibroids as well as in the normal myometrium, although significantly increased levels of pAKT and activated mTOR were seen in leiomyosarcomas [95]. Therefore, low activity of the mTOR pathway was associated with HIF-1a downregulation in uterine fibroids.

Taken together, these data suggested that the differential expression of HIF-1a between uterine fibroids and leiomyosarcomas may be due to mTOR activity and that suppression of both mTOR and HIF-1a induced by mTOR pathway may play a crucial role in transformation of uterine fibroids and not malignant leiomyosarcomas.

Another explanation for reduced HIF-1a expression observed in uterine fibroids was the modified expression of hypoxia-inducible transcription factor early growth response-1 (EGR-1). It has been known that EGR-1 activated the promoter of HIF-1a in a cancer cell line by binding the promoter of HIF-1a [96]. EGR-1 induced

by hypoxia upregulated angiogenic factors involved in tumor growth and angiogenesis in cancer cells [97–99]. However, EGR-1 was significantly downregulated, resulting in reduced expression or lack of HIF-1a expression, in uterine fibroids [100, 101]. In uterine fibroids, expression of EGR-1 was reduced to 10% of levels when compared to the normal myometrium, providing further evidence for aberrant response to hypoxia in fibroid tumors.

3.4 Growth Factor

Growth factors are the general name for secreted proteins from specific cells [102]. It has been shown that growth factors work as regulators of various cytological and physiological processes and activate intracellular signal transduction by specifically binding to receptors on the plasma membrane of target cells. There are a variety of growth factors with individual growth factors existing as members of larger families of proteins with similar structure. The effect of representative growth factors on uterine fibroid development has been well investigated.

3.4.1 Transforming Growth Factor-β

It was shown that transforming growth factor-beta (TGF-β) belonged to the transforming growth factor superfamily composed of four different isoforms (TGF-β 1–4) [103]. After binding of TGF-β, the type 2 receptor kinase phosphorylates and activates the type 1 receptor kinase that activates a signaling cascade that then phosphorylates intracellular secondary messengers Smad2 and Smad3 [103–105]. The Smad complex translocates to the nucleus to act as a transcriptional regulator for responsive genes, mediating a wide range of TGF-β functions inducing differentiation, proliferation, apoptosis, angiogenesis, immune function, and synthesis of ECM components [103–105].

The TGF-β1, TGF-β2, and TGF-β3 isoforms and their receptors have been shown in the human myometrium and uterine fibroids [23, 106, 107]. TGF-βs, their receptors, and downstream signaling mediators such as Smad 2/3 complexes were overexpressed in uterine fibroids compared with the normal myometrium [108]. In vitro studies have shown that relatively low concentrations of TGF-β isoforms induced proliferation of human myometrial and uterine fibroid cells [109]. TGF-β3 has also been shown to increase the expression of ECM including fibronectin, collagen type 1, and versican, suggesting that TGF-β3 may stimulate fibroid growth through excessive ECM deposition and tissue fibrosis, characteristics of uterine fibroids [109–111]. It was demonstrated that retinoic acid decreased cell proliferation and ECM production in uterine fibroids via TGF-β3 pathway downregulation [112]. Most studies that investigated the role of TGF-β on uterine fibroid development focused on the contribution of TGF-β to fibroid cell proliferation and ECM production.

Sex steroid hormones may regulate the production of TGF-β in uterine fibroids [108]. Asoprisnil, steroidal selective progesterone receptor modulator (SPRM),

decreased TGF-β mRNA and protein expression, as well as TGF RII protein expression in uterine fibroids, but not myometrial cells [113]. Estradiol, MPA, and a combination of E_2 and MPA stimulated TGF-β1 production effecting DNA synthesis. Thus, the effect of sex steroid hormone may be via TGF-β [114].

3.4.2 Insulin-Like Growth Factor

It was shown that insulin-like growth factors (IGFs) are proteins with similar sequences and similar mitogenic effects as insulin [115–117]. Cells communicate with their environment through a complex system of insulin and IGFs. This complexity was because the system possessed two cell-surface receptors (IGF-1R and IGF-2R), two ligands (insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 2 (IGF-2)), and a family of six high-affinity IGF-binding proteins (IGFBP-1 to IGFBP-6), as well as associated IGFBP-degrading enzymes [115–117]. These systems have been examined to determine their relationship to uterine fibroid pathophysiology.

IGF-1 acted as both an endocrine and autocrine/paracrine factor [115–117]. Both myometrium and uterine fibroids contained higher amounts of IGF-1 compared to other growth factors [118, 119]. Thus, IGF-1 was involved in the progress of uterine fibroids. It was reported that there was no difference with regard to IGF-1 mRNA expression between fibroids and myometrium, whereas a fourfold difference was seen in mRNA expression between fibroids from the same patient [118]. After treatment with GnRH agonist, the reduction of fibroid volume was shown to be correlated to decreased levels of IGF-1 mRNA expression and decreased hormonal concentrations [118]. It was found that approximately 30% of uterine fibroids demonstrated dysregulation of IGFs [120]. This study looked at protein and mRNA transcriptional levels of IGF-1 and IGF-2 in uterine fibroid samples by immunohistochemistry, RT-PCR, and Western blotting. The protein and mRNA transcript levels of IGF-2 increased, although protein levels of IGF-1 increased without changes in the levels of mRNA transcription. IGF-1 signaling was regulated through a number of intracellular pathways, such as Ras/Raf/MAPK and the PI3K pathways. IGF-1 levels were correlated with phosphorylation levels of AKT, which was a key substance of the PI3K/Akt/mTOR signaling pathway and related to proliferation and cell growth of a variety of tumor types.

IGF-1R has been detected in the uterine fibroid at significantly greater levels than in myometrium [121, 122]. The endocrine activation of IGF-IR can be accomplished by IGF-1 and IGF-2 [120, 123]. The overexpression of IGF-1R or overabundance of the ligand induced tumor genesis through hyperactivation of IGF-1 signaling, leading to upregulation of the PI3K/Akt pathway [124]. Progesterone decreased expression of IGF-1mRNA in fibroid cells [68, 70], although IGF-R levels remained constant in any phase of menstrual cycle [123]. The highest amounts of IGF-1 were detected in uterine fibroid tissues during the proliferative phase [120, 123]. Thus, estrogen may be associated with the increased expression of the IGF system.

3.4.3 Epidermal Growth Factor

Epidermal growth factor (EGF) binds to the transmembrane EGF-receptor (EGF-R) to regulate proliferation, survival, and differentiation of cells [125]. Both EGF and EGF-R mRNA have been detected in myometrium and uterine fibroids and both proteins also existed in the myometrium and uterine fibroids [121, 126]. EGF possessed a mitogenic effect on DNA and protein syntheses in both cultured myometrium and uterine fibroid cells [127–129]. The importance of EGF in uterine fibroid growth was also supported by the fact that the selective EGF-R blocker blocked uterine fibroid cell proliferation in vitro [130, 131]. One study showed the staining signals of EGF in uterine fibroid tissue from the proliferative phase is significantly decreased compared with matched myometrium [121]. It has also been found that the addition of E2 reduces EGF expression but upregulates the expression of EGF-R in both myometrium and uterine fibroid cells [69, 132]. Progesterone upregulates EGF expression in uterine fibroid cells [69, 70, 132]. These suggested an important role for progesterone in EGF regulation. Indeed, it has been shown that selective progesterone receptor modulator (SPRM) was shown to downregulate EGF expression in uterine fibroids [113].

3.4.4 Heparin-Binding Epidermal Growth Factor

As the heparin-binding EGF (HB-EGF) protein was first detected in conditioned culture medium of immune cells [133], it was originally thought to act as an immune mediator [133]. However, in fact, HB-EGF has been revealed as a mediator of multiple physiological and pathological pathways [134]. HB-EGF possesses an EGF-like domain and thus binds to its cognate receptors, EGF-receptor 1 (HER1) and HER4 [134]. HB-EGF possesses a stronger affinity to the receptors in smooth muscle cells compared with EGF [134]. This suggested HB-EGF may work as a stronger mitogen for the development of uterine fibroids than EGF. HB-EGF mRNA expression and protein have been detected in both myometrial and uterine fibroids [135]. The EGF-R was also expressed in myometrial and uterine fibroids [121, 126]. HB-EGF protein expression decreased in uterine fibroids compared with myometrial tissue [136], although EGF-R was greater in uterine fibroids compared with myometrium.

It has been reported that addition of HB-EGF promoted proliferation and inhibited apoptosis in both cultured myometrial cells and fibroid cells in a dose-dependent manner via increased EGF-R expression [137]. However, increased proliferation was observed in myometrial cells at a much lower concentration of HB-EGF in culture medium compared to uterine fibroid cells in spite of the stronger affinity of HB-EGF to EGF receptors in uterine fibroid cells [137]. Taken together, the data suggested that HB-EGF may have a role in development of uterine fibroids, although the role is not as crucial as its role in maintenance and proliferation of myometrial cells.

3.4.5 Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) was shown to be a major regulator of angiogenesis, important in tumor development and physiological and pathological

angiogenesis [138, 139]. VEGF was shown to be a heparin-binding homodimeric protein consisting of six isoforms [140, 141]. VEGF acted as a mitogen for endothelial cells and its action mediated by binding to tyrosine kinase receptors, VEGFR-1 and VEGFR-2 (kinase domain-containing receptor: KDR/flk-1). VEGF mRNA and protein expression have been detected in both myometrium and uterine fibroids [142, 143]. The VEGF receptors, VEGFR-1 and VEGFR-2, were also expressed in myometrium and uterine fibroids [144]. VEGF expression has been reported to be higher in uterine fibroids compared with normal myometrium, suggesting that angiogenesis may be important for fibroid development and growth [145]. This was verified using a novel murine xenograft model for uterine fibroids that was pretreated with VEGF and cyclooxygenase-2 [146]. Furthermore, relatively greater expression of VEGF, VEGFR-1, and VEGFR-2 was shown in leiomyosarcoma compared to uterine fibroma [147], suggesting that the VEGF/VEGFR system was involved in uterine smooth muscle tumorigenesis.

3.4.6 Basic Fibroblast Growth Factor

The protein basic FGF (bFGF) was associated with mitogenesis and differentiation of mesodermal cells, including fibroblasts and SMCs [148]. Following binding to two major receptors, fibroblast growth factor type 1 receptor (FGFR-1) and type 2 receptor (FGFR-2), bFGF promoted angiogenesis and proliferation of vascular SMCs in response to vascular injury [149].

Protein and mRNA expression of both bFGF and receptors FGFR-1 and FGFR-2 have been identified in normal myometrium and uterine fibroid leiomyoma [150, 151]. Although bFGF works as a mitogen for both human uterine myometrial and uterine fibroid cells, fibroid cells are less responsive [152].

3.4.7 Platelet-Derived Growth Factor

PDGF is a dimer of five different isoforms: four homodimers (PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD) and a heterodimer PDGF-AB [153, 154]. All members of PDGFs enacted their cellular effects through activating two cell-surface receptor tyrosine kinases (PDGFR-a and PDGFR-b) [154]. The PDGF subunits A, B, and C bind to PDGFR-a with high affinity, while PDGF subunits B and D bind to PDGFR-b. PDGF acted as a mitogen and chemoattractant for both SMCs and fibroblasts. Its role in angiogenesis was established as it has been shown to lead to proliferation and migration of SMCs during vascular maturation. PDGF and PDGFR mRNA and their protein were shown to be expressed by normal myometrium and leiomyoma tissues [135, 155, 156]. It was reported that only mRNA expression of PDGF-C increased in uterine fibroids, although there were no differences in mRNA expression of PDGF-A, PDGF-B, and PDGF-D and their receptors between uterine fibroids and myometrium [157]. Furthermore, relatively greater PDGF-CC expression was shown in fibroid-derived SMCs [158]. PDGF promotes DNA synthesis as well as protein synthesis [128], increases collagen alpha1 expression [155], and modifies cell proliferation in normal myometrium and fibroid cells [155]. It was revealed that VEGF production could be

promoted by PDGF in human myometrial SMCs, suggesting that the higher expression of PDGF/PDGFR in uterine fibroids may be related to pathological angiogenesis in uterine fibroids [159].

3.5 Wnt-β Catenin

It has been shown that there are two Wnt signaling pathways, canonical and noncanonical [160]. The key molecule in the canonical Wnt signaling pathway is β-catenin. Without the stimulation of Wnt signaling, β-catenin made a complex with the scaffold protein Axin, the tumor suppressor gene product APC, and the kinases CKI and GSK-3β, and then β-catenin was degraded following ubiquitination [161, 162]. In the presence of Wnt ligands, Axin moved to the plasma membrane, and β-catenin was released from the complex. The accumulated β-catenin subsequently translocated into the nucleus and promoted transcription of Wnt target genes [161, 162].

There have been reports regarding the role of Wnt/β-catenin in fibroid development [17, 163]. It was shown that Wnt5b was overexpressed in smooth muscle cells derived from uterine fibroid compared with matched myometrial cells [163]. The aberrant secreted frizzled-related protein 1 (sFRP1), a modulator of Wnt signaling, was also reported to be expressed in uterine fibroids, suggesting a possible involvement of Wnt signaling on fibroid development. It was found that uterine fibroids that carried MED12 mutations overexpressed Wnt4 [17]. This study showed that estrogen along with MED12 mutations induced Wnt/β-catenin activation within fibroid-like lesions in murine models. A mouse model that expressed constitutively activated β-catenin in uterine mesenchyme driven by the expression of Cre recombinase had evidence of myometrial hyperplasia and developed mesenchymal tumors with 100% penetrance [164]. Furthermore, a critical paracrine role for the WNT/β--catenin pathway in estrogen-/progesterone-dependent tumorigenesis was demonstrated, which involved a leiomyoma side-population (LMSP) and differentiated myometrial or uterine fibroid cells [165]. Estrogen/progesterone treatment of mature myometrial cells induced expression of WNT11 and WNT16, which remained constitutively elevated in uterine fibroid tissues. Coculture of LMSP with mature myometrial cells with estrogen and progesterone selectively induced nuclear translocation of β-catenin in the LMSP cells leading to their proliferation. They also showed that WNT/β-catenin pathway inhibitors specifically blocked human uterine fibroid growth and proliferation. Thus, WNT/β-catenin was associated with the proliferation of LMSP cells and uterine fibroid growth.

In this chapter, we discussed the molecular mechanism of development of uterine fibroids. Treatment options for uterine fibroids include pharmacological treatments such as GnRH agonists, progestins, oral contraceptives, levonorgestrel intrauterine system, and selective progesterone receptor modulators, and invasive procedures such as myomectomy, uterine artery embolization, and focused ultrasound have been developed. However, all these treatments have side effects, and most of the treatment effects on uterine fibroids may be temporary or not consistent for patients. This is due to lack of a satisfactory medical treatment based on the

exact molecular pathophysiology underlying the development of uterine fibroids. Research regarding the molecular mechanism of uterine fibroid development will promise new treatment options, satisfying both patients and healthcare providers.

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Stem Cells and Uterine Fibroids

4

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Abstract

Although little is known on the origin of leiomyoma tumorigenesis, increasing evidence supports the hypothesis that leiomyomas arise from a stem cell population in the uterus. Recent articles on stem cells and their paracrine interactions with more specialized cell populations within leiomyomas may help to establish the missing link between the development of treatments designed to stop the growth of leiomyomas and therapies devised to eliminate them. Studies to identify leiomyoma stem or progenitor cell markers might offer new possibilities for understanding the origin of these tumors and perhaps aid the development of noninvasive treatments. Adult (or somatic) stem cells constitute a subset of cells residing in normal tissues. By undergoing asymmetric division, they retain their ability to self-renew while producing daughter cells that go on to differentiate and play a role in tissue regeneration and repair. The unique properties of the uterus to enlarge and remodel suggest the existence of uterine stem cell systems. Neoplastic stem cells or tumor-initiating cells, a subset of cells within a tumor, have the ability to reconstitute tumors. Leiomyomas appear to be monoclonal tumors derived from a single myocyte. Work in recent years has identified,

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_4

isolated, and characterized putative stem or progenitor cells in the myometrium and in leiomyomas. Here, we review the current literature on leiomyoma stem and progenitor cells and provide a new paradigm for understanding their pathology.

Keywords
Leiomyoma · Myoma · Stem cells · Adult stem cells · Neoplastic stem cells

4.1 Introduction

Uterine leiomyoma is one of the most common gynecologic tumors [1]. Clinically relevant leiomyomas are detectable by transvaginal sonography in approximately 50–80% of African-American women and 25–70% of Caucasian women [1–5]. Leiomyomas cause excess uterine bleeding, anemia, recurrent pregnancy loss, preterm labor, pelvic discomfort, and urinary incontinence in approximately 15–30% of patients [6, 7]. Due to these symptoms, surgical treatments such as hysterectomy and myomectomy are frequently performed [6, 8]. Despite their high prevalence, the cellular and molecular origins of uterine leiomyomas are not well understood. Recent studies suggest the involvement of epigenetic mechanisms, such as DNA methylation and microRNA and histone modification [9–14]. At the genetic level, several mutations, including germline mutations that cause fumarate hydratase deficiency, have been associated with leiomyoma formation [15].

Recently, recurrent somatic mutations in mediator complex subunit 12 (MED12) were identified through exome sequencing of 18 uterine leiomyomas from Finnish patients [16]. Further analysis of a total of 225 tumors from 80 patients revealed that a striking 70% of uterine leiomyomas display MED12 mutations, making it the most frequently altered gene in patients with leiomyoma. All the identified mutations are clustered in an evolutionarily conserved region of the gene: exon 2 and the intron 1-exon 2 junction. This finding has been validated in several studies representing various ethnic groups [17–23], and the data suggest that aberrant functioning of the encoded region contributes to tumorigenesis. In the MED12 mutation study, leiomyoma size is inversely correlated with the presence of MED12 mutations [17]. An elevated incidence of chromosomal abnormalities in leiomyomas due to translocations, deletions, and duplications of chromosomes 6, 7, 12, and 14 has been reported [24]. These data were confirmed by whole-genome sequencing, suggesting that the rearrangements in leiomyomas have a common origin [25].

An adult stem cell is thought to be an undifferentiated cell, existing among differentiated cells in a tissue or organ. The adult stem cell can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. After completion of embryonic development, undifferentiated adult stem cells persist throughout the body [26, 27]. Analogously, tumor-initiating cells are a subset of cells within a tumor cell

population that retain the ability to reconstitute tumors [28]. Leiomyomas are thought to be monoclonal tumors arising from the myometrium [29, 30]. Several recurrent genetic aberrations, such as chromosome 12 trisomy, deletions in 7q, and mutations in genes coding for MED12 or the high mobility group AT-hook 2, have been reported in leiomyomas [5, 16, 31, 32]. As in other diseases, these genetic abnormalities occurring in adult stem cells are thought to play pivotal roles in the pathogenesis of leiomyomas (Fig. 4.1).

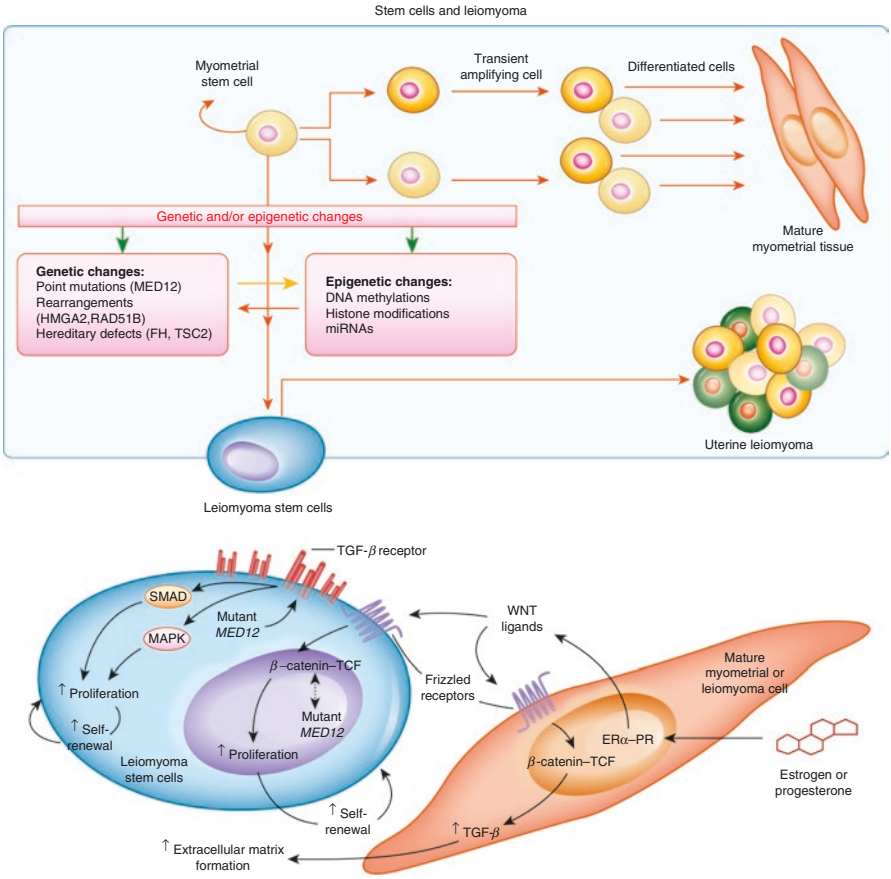

Fig. 4.1 Schematic representation of stem cells in leiomyomas. The myometrial stem cell resides in its niche and renews itself. This cell also gives rise to a progenitor cell through asymmetric cell division. Genetic/epigenetic abnormalities transform myometrial stem cells into leiomyoma stem cells. Estrogen receptor α (ERα) and progesterone receptor (PR) levels are high in mature myometrial and leiomyoma niche cells compared with stem cells. Thus, estrogen and progesterone signals reach leiomyoma stem cells through hormone receptors in mature niche cells in a paracrine fashion. Estrogen and progesterone might increase secretion of wingless-type (WNT) ligands from the surrounding ERα or PR-positive niche cells. In both cell types, WNT, acting through the frizzled family of receptors, activates the T-cell factor pathway and leading to excessive formation of extracellular matrix. Parts of this figure were modified from reference with permission [3]

4.2 Leiomyoma Stem Cells

Leiomyomas are thought to be monoclonal tumors [29, 30]. It has been proposed that each leiomyoma originates from a single transformed adult stem cell of the myometrium in an ovarian steroid-dependent manner [33]. Mas et al. (2012) characterized the side population (SP) of human leiomyomas, analyzing its clonogenic activity under hypoxic conditions. Microarray analysis detected 100 upregulated and 53 downregulated genes in the leiomyoma SP (LMSP) compared with leiomyoma fragments. In addition, LMSP cells lack typical muscle markers and hormone receptors, indicating that they are not yet committed to a specific lineage. These cells show the in vitro and in vivo ability to differentiate into mesenchymal lineage cell types and to form tissue-like leiomyomas in animal models [34]. Ono et al. showed that the LMSP accounts for 1% of tumor cells and displays the characteristics of tumor-initiating stem cells [35]. The growth of the LMSP in vivo drives tumor expansion, and this growth is estrogen- and progesterone-dependent, even though the LMSP expresses remarkably lower estrogen receptor (ESR) and progesterone receptor (PGR) levels than mature myometrial or leiomyoma cells [36–38]. Chang et al. studied the differences between leiomyomas and normal myometrium tissue with regard to innate growth capacity by evaluating the colony-forming ability and the mesenchymal stem cell markers based on CD90 expression and SP cells. They also studied the differentiation status by CD90 expression patterns after in vitro culture. They found that leiomyoma cells form fewer mesenchymal stem cell colonies and display less Hoechst dye-excluding SP cell activity versus cells isolated from normal myometrial tissue [39].

Originally, leiomyoma stem cells were isolated using the Hoechst dye-exclusion technique for SP. However, the SP technique is expensive, very sensitive to minor staining variations, and is detrimental to cell survival, making further study of leiomyoma cells difficult [40]. As a solution to these pitfalls, Yin et al. reported a novel way of isolating leiomyoma stem cells using the cell surface markers cluster of differentiation (CD)34 and CD49b [41, 42]. Cell sorting, using antibodies to these cell surface proteins, reveals three distinct cell populations: CD34+/CD49b+, CD34+/CD49b−, and CD34−/CD49b−. The CD34+/CD49b+ cells are highly enriched with stem cells, whereas the other two groups do not contain any stem cells. Moreover, genes specific to stem cells such as Kruppel-like factor 4 (KLF4), NANOG, and octamer-binding transcription factor 4 (OCT4) are overexpressed in CD34+/CD49b+ cells, further suggesting that these are indeed stem cells. Interestingly, CD34+/CD49b− cells have intermediate levels of these stem cell factors compared with CD34−/CD49b− cells. Additionally, ESR1 and PGR are significantly underexpressed in CD34+/CD49b+ cells, consistent with prior studies on SP, and CD34+/CD49b− cells again show intermediate expression levels, between CD34+/CD49b+ and CD34−/CD49b− cells. Mas et al. demonstrated that Stro-1+/CD44+ can be used as surface markers to enrich a subpopulation of myometrial, leiomyoma stem, and progenitor cells [43]. It is likely that CD34+/CD49b+ and Stro-1+/CD44+ cells represent a similar population, considering the published data thus far.

Estrogen- and progesterone-dependent in vivo growth of human leiomyoma tissue requires the presence of these multipotent leiomyoma stem cells [34, 35]. The growth of leiomyoma stem cells requires the presence of mature myometrial or leiomyoma cells with higher levels of steroid receptors and their ligands; this statement is based on the postulate that steroid-hormone action on leiomyoma stem cells is mediated via mature myometrial cells (tumor initiation) or mature leiomyoma cells (growth maintenance) in a paracrine fashion. It is likely that paracrine interaction with surrounding ESR1- or PGR-positive niche cells supports self-renewal of leiomyoma stem cells (Fig. 4.1).

Recently, Mas et al. showed that the cervix is a hypoxic "niche." The pool size of these myometrial and leiomyoma stem cells also responds to environmental cues and expands in response to developmental environmental exposures that promote leiomyoma development. They reported that expansion of leiomyoma stem cells occurs prior to the development of tumors, in response to an environmental exposure that increases the risk of developing leiomyomas. The presence of leiomyoma stem cells correlates with leiomyoma risk factors in women [44].

In recent years, advances in stem cell biology have made it clear that most tissues are extremely plastic and renew through adult stem or progenitor cells. Leiomyomas are known to have a clonal origin, but so far the initiating event remains unknown. Several theories have attempted to explain leiomyoma pathogenesis, and recently a role for uterine stem cells has been proposed. One possible explanation for the development of leiomyomas is the dysregulation of mesenchymal stem cell activity.

4.3 Paracrine Sex Steroid Action on Leiomyoma Stem Cells

In a mouse xenograft model, transplanted cells containing leiomyoma stem cells mixed with myometrial cells grow into substantially larger tumors and have higher proliferation indices under the influence of estrogen and progesterone than transplanted cells containing only differentiated leiomyoma cells with myometrial cells [35]. Perhaps most interestingly, leiomyoma stem cells have minimal to no ESR and PGR expression yet respond to estrogen and progesterone stimulation with tumor expansion. Additionally, leiomyoma stem cells cannot induce proliferation or tumor growth without the presence of differentiated leiomyoma or myometrial cells. These observations have led us to hypothesize that leiomyoma stem cells rely on paracrine signaling from surrounding mature myometrial and leiomyoma cells to facilitate estrogen and progesterone action.

The wingless-type (WNT) and β-catenin pathway was recently proposed as a possible mechanism for paracrine interaction between leiomyoma stem and differentiated cells. A study showed that mature myometrial cells secrete WNT ligands in response to estrogen and progesterone treatment, resulting in upregulation of β-catenin signals. Additionally, inhibiting WNT binding or β-catenin in leiomyoma cells results in significantly decreased tumor growth [45, 46]. Selective overexpression of the β-catenin gene in the uterine mesenchyme during embryonic

development and in adults gives rise to leiomyoma-like tumors in the uteri of female mice [47]. In those mutant mouse uteri, Tanwar et al. observed dysplastic lesions in the myometrium with 100% penetrance. Signaling by WNT and β-catenin appears to play a role in adult stem cell function in the myometrium and in uterine leiomyoma tissue. Selective deletion of the murine β-catenin gene from the uterine mesenchyme during embryonic development significantly reduces uterine size and leads to the replacement of myometrial cells by adipocytes, thus completely disrupting normal myometrial smooth muscle differentiation and regeneration [48]. These findings collectively suggest that signaling by WNT and β-catenin plays a role in adult stem cell function in the myometrium and in uterine leiomyoma tissue. These findings indicate that β-catenin plays a key role in stem cell renewal and in differentiation to the smooth muscle phenotype observed in myometrial and leiomyoma tissues [49]. Moreover, activated β-catenin induces expression of transforming growth factor β3 (TGFB3), which was shown to induce proliferation and extracellular matrix formation in human leiomyoma tissue [47, 50]. These results suggest that there might be pleiotropic effects on uterine function and tumorigenesis resulting from dysregulated WNT and β-catenin signaling [47].

Given that WNT signaling controls cell fate decisions throughout development and in adult stem cells, we further posit that the relevant target of β-catenin-mediated tumorigenesis is a progenitor cell that gives rise to leiomyomas, rather than a mature smooth muscle cell. Thus, a paracrine role for the WNT ligand is suggested. The WNT ligand probably originates from mature myometrial or leiomyoma smooth muscle cells in response to estrogen and progesterone. The WNT ligand acts on adjacent leiomyoma stem-like tumor-initiating cells to stimulate self-renewal and proliferation, eventually leading to tumor growth (Fig. 4.1) [51].

There are other pathways that mediate paracrine signaling. For example, it was discovered that progesterone leads to expansion of stem cells in breast cancer via downregulation of miR-29 and subsequent upregulation of KLF4 [52]. These results are interesting for leiomyoma in light of the findings that steroid-hormone suppression of miR-29 is necessary for leiomyoma growth [53].

4.4 Challenges and Future Directions

Leiomyoma development and progression depend on a number of variables: steroid hormones, growth factors, cytokines, chemokines, and extracellular matrix components. Everything, however, depends on the triggering of an initial tumor-initiating event. Current evidence supports the role of putative stem and progenitor cells, found in the human uterus, as contributors to the onset of leiomyoma formation. A thorough characterization of leiomyoma stem cells is a prerequisite for understanding the mechanisms underlying the altered physiology and remodeling of the uterus. Analogously, a better understanding of the pathogenesis of myometrium-derived tumors, including leiomyomas, adenomyosis lesions, leiomyosarcomas, and mixed Müllerian tumors, is required. Defining the cellular and functional properties is a necessary first step to achieve this broad goal. A number of laboratories have been

able to isolate a small population of leiomyoma cells consistent with undifferentiated tumor-initiating cells or adult stem cells. This is a groundbreaking development, because it shifts the therapeutic target away from the bulk of the leiomyoma to a small population of cells. We predict that increasing our understanding of leiomyoma stem cells will transform the field of uterine biology and medicine.

Conflict of Interest The authors declare that no conflicts of interest exist.

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Epigenetics and Uterine Fibroids

5

Ryo Maekawa and Norihiro Sugino

Abstract

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

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

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

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_5

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

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

5.1 Introduction

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

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

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

5.2 Epigenetics

5.2.1 DNA Methylation

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

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

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

5.2.2 Histone Modifications

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

5.2.3 Noncoding RNAs

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

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

5.3 Aberrant DNA Methylation in Uterine Fibroids

5.3.1 Aberrant Expression of DNA Methyltransferases (DNMTs)

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

5.3.2 Genome-Wide DNA Methylation Profiles of Uterine Fibroids

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

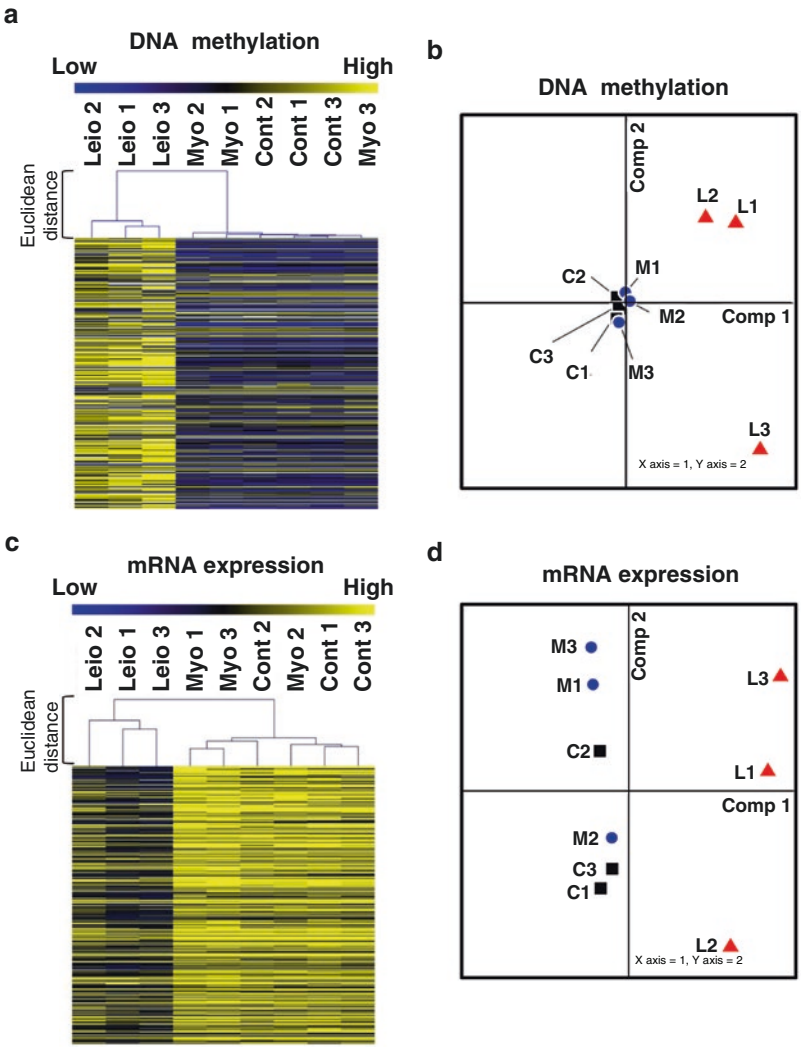

Fig. 5.1 DNA methylation profiling and mRNA expression profiling of fibroids and myometrium with and without fibroids. Fibroids (L1, L2, and L3), myometrium with fibroids (M1, M2, and M3), and myometrium without fibroids (C1, C2, and C3) were compared using hierarchical clustering analyses and principal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

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

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

5.3.3 Aberrantly Methylated Genes in Uterine Fibroids

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

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

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

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

5.3.4 Aberrantly Enriched DNA Hypomethylation in X Chromosome in Uterine Fibroids

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

5.3.5 Estrogen Receptor Alpha and Its Target Genes

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

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

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

[a]Microarray mRNA expression statuses of leiomyoma relative to adjacent myometrium are shown

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

5.4 Noncoding RNAs and Uterine Fibroids

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

5.4.1 Aberrantly Expressed MicroRNAs in Uterine Fibroids

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

Table 5.2 MicroRNAs dysregulated in uterine fibroids

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

Table 5.3 Target genes of microRNAs and lncRNAs

microRNAs	Expression in uterine fibroids	Targets	Class of expression	Targets expression	Status	Reference
miR-21	Upregulated	PLAG1[a], PPARa[a], TIMP3[a], MAPK10[a], BRD1[b], and SKI[b]	mRNA	Downregulation	Predicted	Wang (2007) [45][a], Zavadil (2010) [42][b]
let-7	Upregulated	HMGA2[a] and Ki67[b]	Protein	Downregulation	Confirmed	Wang (2007) [45][a] and Zavadil (2010) [42][b]
miR-194-1	Upregulated	EGFR	Protein	Downregulation	Predicted	Zavadil (2010) [42]
miR-199a-2	Downregulated	TGF-alpha	Protein	Upregulation	Predicted	Zavadil (2010) [42]
miR-200a/200b	Downregulated	MAF, CTBP2, BCL2, CITED2, LASS6, PHF21A, TSC22D1, ATXN1, JUN, and NFIB	mRNA	Upregulation	Predicted	Zavadil (2010) [42]
miR-200a	Downregulated	CYP1B1 and CTBP2	mRNA	Upregulation	Confirmed	Zavadil (2010) [42]
miR-200c	Downregulated	ZEB1, ZEB2, TIMP2, FBLN5, VEGFA, and IL8	mRNA and/or protein	Upregulation	Confirmed	Chuang (2014) [48] Chuang (2012) [50]
miR-93	Downregulated	F3, IL8, CGF, and PAI-1	mRNA	Upregulation	Confirmed	Chuang (2012) [49]
miR-29b	Downregulated	COL1A1, COL2A1, COL4A1 and COL7A1	mRNA	Upregulation	Predicted	Wang (2007) [45]
Long noncoding RNAs (lncRNAs)	**Expression in uterine fibroids**	**Targets**	**Class of experiment**	**Targets expression**	**Status**	**Reference**
Intergenic10	Upregulated	ADAM12	mRNA	Upregulation	Confirmed	Guo (2014) [44]

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

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

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

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

5.4.2 Steroid Hormone and MicroRNAs in Uterine Fibroids

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

5.4.3 Chromosomal Abnormality and Deletion of MicroRNAs

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

5.4.4 Racial Differences in MicroRNA Expression in Uterine Fibroids

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

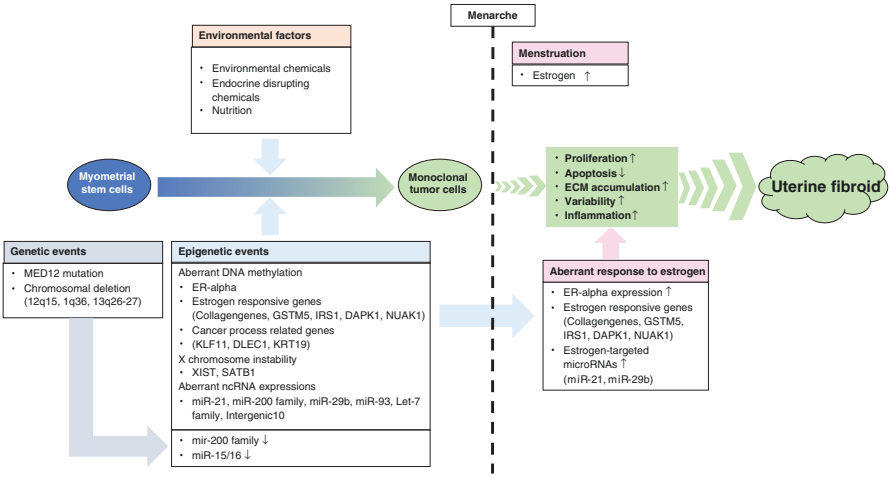

Fig. 5.2 Hypothesis of the pathogenesis and development of uterine fibroids

5.4.5 Long Noncoding RNAs and Uterine Fibroids

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

Conclusion

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

ncRNAs, e.g., miR-21 and genes of the miR-200 and let-7 families, also may have important roles in tumorigenesis of uterine fibroids. Genomic alteration such as chromosomal deletion is also involved in the aberrant expression of microRNAs including miR-200 family genes and miR-15/16. After menarche, the tumor cells aberrantly respond to estrogen and gradually proliferate and differentiate to form uterine fibroid nodules. Aberrant DNA methylation of the promoters of ER-alpha-target genes, e.g., COL4A1, COL6A3, DAPK1, and NUAK1, is responsible for the aberrant response to estrogen. In addition, the responses to estrogen and progesterone could be modified by dysregulated microRNAs such as miR-21 and miR-29b. Some epigenetic factors such as microRNAs and lncRNAs may affect the size of uterine fibroids. Moreover, there are differences in epigenetic statuses including DNA methylation and microRNAs between races. These findings suggest that epigenetic status can explain not only the pathogenesis but also diverse characteristics of uterine fibroids.

Taken together, these facts suggest that aberrant epigenetic modifications contribute to the pathogenesis of uterine fibroids and may lead to the development of new strategies for treatment.

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Biomarkers of Uterine Fibroids

6

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Abstract

Although uterine leiomyomas, or fibroids, are benign tumors, surgery has been the main mode of therapy for them. Because women who wish to retain the uterus for future pregnancies are increasing in number due to an increase in the average age of childbearing, therapy with molecular-targeted agents is desired. The development of effective agents requires a better understanding of the molecular mechanisms involved in the onset and development of leiomyomas. In addition, because uterine leiomyosarcomas, which, unlike leiomyomas, are malignant, occur in a similar location and have similar shapes, differentiating leiomyomas from leiomyosarcomas is needed to retain the uterus. Therefore, in this chapter, we focus on the pathological diagnostic markers that appear to be involved in the mechanisms of the onset and development of leiomyomas and the differential diagnostic markers to distinguish them from leiomyosarcomas. To identify the pathological diagnostic markers of leiomyomas, previous studies have used cytogenetic status, mRNA, microRNA and protein expression, and DNA methylation patterns. In recent years, genome-wide sequencing studies have associated leiomyomas most frequently with somatic mutations of the *MED12* gene and less frequently with several other genomic alterations. To identify candidate markers for differential diagnosis, several studies have used microRNA expression, omics, and immunohistochemical analyses. Here, we outline the above findings and describe our recent application of leiomyoma-specific marker genes that have aberrant DNA methylation, to distinguish leiomyomas from leiomyosarcomas.

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_6

Keywords
Diagnostic biomarkers · Uterine leiomyoma · Uterine leiomyosarcoma

6.1 Introduction

Uterine leiomyomas, or fibroids, are tumors that are derived from uterine smooth muscle cells and are most common in gynecologic neoplasms [1]. More than 30% of reproductive-age women suffer from leiomyomas [1]. Although leiomyomas are benign, they cause severe pelvic pain, menorrhagia, dysmenorrhea, anemia, infertility, and miscarriage [1, 2]. Therefore, the quality of life of women with leiomyomas is significantly impaired. Surgery has long been the main mode of therapy for leiomyomas. For many women who have completed childbearing, hysterectomy is an attractive option to eliminate such problems. However, in recent years, the average age of marriage is increasing due to changes in women's lifestyle. Women with leiomyomas who wish to retain the uterus for future pregnancies are also increasing in number. Thus, there is a need for a therapy with molecular targeted agents. To develop effective prophylactic and therapeutic agents, it is necessary to elucidate the molecular mechanisms involved in the onset and development of leiomyomas. To clarify the molecular mechanisms, identification of pathological diagnostic markers for leiomyomas is also important.

Uterine smooth muscle neoplasms have malignant uterine leiomyosarcomas in addition to benign leiomyomas. Because leiomyomas and leiomyosarcomas occur in a similar location and have similar shapes, differentiating them can be difficult in some cases. Leiomyosarcomas are high-grade tumors whose 5-year survival rate is less than 50% and metastasize to the lungs or liver in the early stages [3, 4]. The risk factors of leiomyosarcomas are not clear. Conventional chemotherapy and radiotherapy have little effect at prolonging survival, leaving hysterectomy as the only option [3, 5–7]. Leiomyosarcomas are diagnosed based on three histological findings using light microscopy: increased activity of mitosis, cytological atypia, and the presence of coagulative necrosis [8, 9]. However, because histological diagnoses lack objectivity, the diagnostic results of a given specimen may differ depending on the facilities and individuals that make the diagnosis. Smooth muscle tumors occur in histologically different types such as the smooth muscle tumors of uncertain malignant potential (STUMPs) and atypical leiomyomas (ALMs), which are more difficult to diagnose [9, 10]. In addition, in rare cases, uterine smooth muscle neoplasms diagnosed as benign leiomyomas metastasize [11, 12]. Thus, there is a need for objective differential diagnosis of leiomyomas and leiomyosarcomas, especially for women who wish to retain the uterus for future pregnancies.

In general, a biomarker is defined as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [13]. Biomarkers can roughly be classified according to their purposes as follows: (1) diagnostic

markers to diagnose the patients with a disease or abnormal condition, (2) prognostic markers to predict the course of disease, (3) pharmacodynamic markers to elucidate the mechanism of action of the agents, (4) predictive markers to predict the effect of a therapeutic intervention, (5) monitoring markers to monitor the clinical response to therapeutic intervention, and (6) safety/toxicity markers to evaluate safety or toxicity. In this chapter, we focus on pathological diagnostic markers (Sect. 6.2) that appear to be involved in the mechanisms of the onset and development of leiomyomas and differential diagnostic markers (Sect. 6.3) to distinguish leiomyomas from leiomyosarcomas. We outline recent findings and also describe our recent work with a differential diagnostic marker.

6.2 Pathological Diagnostic Markers of Uterine Leiomyomas

Because a leiomyoma is a monoclonal tumor derived from a single cell [14, 15], cytogenetic (Sect. 6.2.1) and genomic alterations are likely to become useful biomarkers. Genomic alterations include somatic mutations in the *mediator complex subunit 12* (*MED12*) gene (Sect. 6.2.6) [16], which currently appears to be the most reliable biomarker of leiomyomas. In recent years, whole genome sequencing studies have found multiple genomic alterations (Sect. 6.2.7), which would be used to classify leiomyomas into several subgroups. Several molecular biological studies have comprehensively investigated the expression of mRNAs (Sect. 6.2.2), microRNAs (Sect. 6.2.3), and proteins (Sect. 6.2.4) to elucidate the mechanisms of the onset and development of leiomyomas or to characterize the tumor. In addition, a few laboratories including our own have identified leiomyoma-specific aberrant genes (Sect. 6.2.5) by using genome-wide DNA methylation analyses (DNA methylome) combined with transcriptome. These studies could provide useful pathological diagnostic markers for leiomyomas. In the following sections, we outline each of the above studies.

6.2.1 Cytogenetic Aberrations in Uterine Leiomyomas

Cytogenetic aberrations are found in approximately 40–50% of leiomyomas and are nonrandom and leiomyoma-specific [17, 18]. Major cytogenetic aberrations are translocation of chromosomes 12 and 14 (t(12:14) (q15; q23~24)), loss of the long arm of chromosome 7 (del(7)(q22q32)), and rearrangement of the short arm of chromosome 6 (6p21) [17, 18]. These three aberrations are detected in 20%, 17%, and 5%, respectively, of the specimens with chromosomal abnormalities. Some of the candidate genes that are influenced by the aberrations include *HMGA2* located at 12q15; *RAD51B* located at 14q23~24; *ORC5*, *PCOLCE*, *TRIP6*, and *CUX1* located at 7q22; and *HMGA1* located at 6p21 [18]. In addition, low-frequency cytogenetic aberrations specific to leiomyomas have been detected in the X chromosome and in chromosomes 1, 2, 3, 10, and 13 [17, 18].

6.2.2 Transcriptomes of Uterine Leiomyomas

Several microarray studies have examined mRNA expression in uterine leiomyomas and normal myometrium since 2002 [19–21]. Arslan et al. compared the transcriptomes of leiomyoma and adjacent normal myometrium in three leiomyoma cases. Among 9673 genes, 14 genes were upregulated, and 66 genes were downregulated in leiomyomas [21]. A comparative analysis including their study and 7 previous studies revealed 8 prominent genes identified by at least 5 different studies, 11 genes identified by 4 studies, and 12 genes identified by 3 studies (Table 6.1) [21]. These genes appeared to be involved in retinoic acid production, IGF2 metabolism, TGFB signaling, and ECM formation [21]. However, the expression profiles were largely inconsistent among the eight studies. The discrepancies could have been due to differences in the specimens (race, age, or phase of the menstrual cycle), transcriptome platform, and method of data analysis.

6.2.3 MicroRNA Expression in Uterine Leiomyomas

Two studies used microarrays to compare microRNA (miRNA) expression in leiomyomas and adjacent normal myometrium. Wang et al. examined the expressions of 206 relatively well-characterized miRNAs in 48 cases and identified 24 miRNAs that increased and 21 miRNAs that decreased in leiomyomas (Table 6.1) [22]. Marsh et al. examined the expressions of 454 miRNAs in ten cases and identified 19 miRNAs that increased and 27 miRNAs that decreased in leiomyomas (Table 6.1) [23]. However, the two sets of data were largely inconsistent, mainly because of differences between the analyzed miRNAs.

6.2.4 Protein Expression (Proteome) in Uterine Leiomyomas

The proteomes in leiomyomas and normal myometrium were reported from two research groups using different analysis methods. Lemeer et al. quantitatively analyzed more than 7000 proteins using GeLC-MS/MS and identified 49 differentially expressed proteins that could completely distinguish leiomyomas from myometrium using principal component analysis (PCA) and hierarchical clustering (HCL) [24]. The proteins with increased expression in leiomyomas included kinases and ubiquitin ligases, while the proteins with decreased expression included the integrins. The authors, who were particularly interested in kinases, quantified the expressions of 200 kinases (the kinomes), 9 of which were specific to leiomyomas (Table 6.1) [24]. Ura et al. identified 24 upregulated proteins in leiomyomas by combining two-dimensional gel electrophoresis with mass spectrometry [25]. Twelve of the identified proteins were associated with the metabolic processes of leiomyomas. The authors reported that 13 of 24 strongly expressed proteins were also found to be strongly expressed in the study by Lemeer et al. [24] (Table 6.1) [25].

Table 6.1 Genes that are potential pathological diagnostic markers for leiomyomas identified in various analyses

Analysis	Notes	# genes	Genes	Reference
Transcriptome	Common in at least five studies	8	*ADH1*, *ATF3*, *CRABP2*, *CYP61*, *DPT*, *GRIA2*, *IGF2*, *MEST*	[21]
	Common in four studies	11	*ALDH1*, *CD24*, *CTGF*, *DCX*, *DUSP1*, *FOS*, *GAGEG1*, *IGFBP6*, *PTGDS*, *PTGER3*, *TYMS*	
	Common in three studies	12	*ABCA*, *ANXA1*, *APM2*, *CCL21*, *CDKN1A*, *CRMP1*, *EMP1*, *ESR1*, *FY*, *MAP3K5*, *TGFBR2*, *TIMP3*	
miRNA array	Top10 genes (Wang T. et al.)	10	*let-7*, *miR-21*, *miR-23b*, *miR-27a*, *miR-30a*, *miR-29b*, *miR-32*, *miR-144*, *miR-197*, *miR-212*	[22]
	Top20 genes (Marsh E. et al.)	20	*miR-542-3p*, *miR-377*, *miR-582*, *miR-376b*, *miR-493-5p*, *miR-323*, *miR-34a*, *miR-196b*, *miR-335*,*miR-542-5p*, *miR-642*, *miR-150*, *miR-203*, *miR-139*, *miR-29b*, *miR-486*, *miR-451*, *miR-109*, *miR-29c*, *miR-498*	[23]
Proteome	Common in two studies	13	TUBB, KRT1, LASP1, ACTC1, MDH1, LDHB, CCT5, PDIA3, KRT9, AP1M2, GOT1, FABP5, CRABP2	[24, 25]
	Kinome data (Lemeer S. et al)	9	ADCK4, CDK5, CSNK2B, DDR1, EPHB1, MAP2K2, PRKCB, PRKG1, RPS6KA5	[24]
DNA methylome and transcriptome	Common in two studies	12	*POPDC2*, *TFAP2C*, *SFRP1*, *THBS2*, *GRAMD3*, *NUAK1*, *CORIN*, *HATTIP2*, *EFEMP1*, *TMEM173*, *CFB*, *CRIM1*	[27, 29]
Whole genome sequence and transcriptome	*MED12* mutation, Top20 genes	20	*RAD51B*, *PLP1*, *GARNL3*, *KIAA1199*, *LAMP5*, *MMP11*, *ADAM12*, *POPDC2*, *CPA3*, *THSD4*, *CACNA1C*, *MMP16*, *CNTROB*, *NHSL2*, *KCNAB3*, *UNC5D*, *HPGDS*, *PCP4*, *WBSCR17*, *RUNDC1*	[41]
	FH biallelic inactivation, Top20 genes	20	*AKR1B10*, *TKT*, *PDK1*, *SLC7A11*, *G6PD*, *PIR*, *GCLM*, *SRXN1*, *ENTPD7*, *TNFRSF21*, *SLC6A6*, *NQO1*, *BNIP3*, *RNF128*, *MGAT5*, *PGD*, *FAM46C*, *AEBP1*, *SESN3*, *ABCC3*	
	HMGA2 translocation, Top20 genes	20	*HMGA2*, *IGF2BP2*, *CCND2*, *IL11RA*, *C19orf38*, *PLAG1*, *GRPR*, *PAPPA2*, *PLA2R1*, *TBX3*, *CBLN4*, *GPR20*, *GPR22*, *QPRT*, *PAWR*, *MB21D2*, *CCND1*, *WIF1*, *EGFR*, *AVPR1A*	
	COL4A5-COL4A6 deletion, Top20 genes	20	*IRS4*, *NSG1*, *MXRA8*, *FBLN1*, *PCSK2*, *DPYD*, *SPATA6*, *CTNNA3*, *TMEM55A*, *PCDHB8*, *SCG2*, *SLAIN1*, *PLAGL1*, *PARM1*, *LIX1*, *RHOB*, *TGFBR3*, *HIST1H4H*, *COL4A5*, *PCDHB2*	

6.2.5 Leiomyoma-Specific Aberrant Genes Identified Using DNA Methylome and Transcriptome

In recent years, our laboratory has studied genome-wide DNA methylation in leiomyomas based on the hypothesis that epigenetic mutations would be associated with the onset and development of leiomyomas (see Chap. 5 for details) [26–28]. In an analysis of the DNA methylomes and transcriptomes of leiomyomas and adjacent normal myometrium in three leiomyoma cases, we identified 120 aberrantly methylated and expressed genes specific to leiomyomas [27]. The DNA methylomes were examined with an Infinium HumanMethylation450 BeadChip (Illumina) capable of analyzing more than 480,000 CpG sites. Many of these genes were cancer-related, 22 of which were target genes of ESR1. In another analysis of DNA methylomes (including about 27,000 CpG sites) and transcriptomes of leiomyomas and adjacent normal myometrium in 18 leiomyoma cases, Navarro et al. identified 55 aberrant genes specific to leiomyomas [29]. Although 12 of these genes were also identified in our study [27] (Table 6.1), the other genes were inconsistent in the 2 studies. The discrepancy may have been due to differences in the specimens (Japanese vs African-American), methylome platform, and analytical method.

6.2.6 Somatic Mutations of the *MED12* Gene in Uterine Leiomyomas

Somatic mutations in the *MED12* gene were first detected by a research group at the University of Helsinki in 2011 by whole exon sequencing (exome sequencing) in uterine leiomyomas [16]. The authors reported that the all mutations occurred in exon 2 of *MED12* and were detected in about 70% (159/225) of leiomyoma specimens of Caucasian (Finnish) women [16]. Subsequent analyses of the *MED12* mutation in different races and regions including North America, South Africa, and Asia revealed that the mutations were present in 37–85.5% (average 64%) of the leiomyoma specimens regardless of race [30–32]. In our unpublished data, 71% (32/45) of leiomyoma specimens from Japanese women had *MED12* mutations. All *MED12* mutations reported to date were missense point mutations or in-frame insertions/deletions (indels), which were predicted to result in in-frame transcripts. The frequency of point mutations is highest in codon 44; such mutations account for about 70% of all of *MED12* mutations. More recently, mutations in exon 1 of *MED12* were reported to occur at low frequency (occurring in 0.6% of leiomyoma cases) [33]. To date, neither point mutations nor indels common to leiomyomas without *MED12* mutation have been found by the exome sequencing [34].

MED12 mutations have also been investigated in histologically different types of smooth muscle tumors and other tissue tumors. *MED12* mutations in leiomyosarcomas were detected in 2–30% (average 8%) of the specimens [10, 30, 35]. In STUMP and ALM cases, *MED12* mutations were detected at frequencies of 10% and 12%,

respectively [10, 30]. *MED12* mutations were detected at low frequencies (5% or less) in other tissue tumors including the colorectal tumors, prostate cancers, breast cancers, ovarian cancers, hematologic malignancies, and endometrial polyps [30, 36]. The only non-uterine tumor in which *MED12* mutations have been found frequently is benign breast fibroadenomas (59%) [30, 37]. These results suggest that *MED12* mutations are common in hormone-sensitive benign tumors.

6.2.7 Other Genomic Alterations in Uterine Leiomyomas

Deficiency of the *FH* gene has been shown to be the cause of heredity leiomyomatosis and cell cancer (HLRCC). In addition, biallelic loss of *FH* occurs at low frequency (1.3%) in non-hereditary leiomyomas [38]. To identify genomic alterations specific to leiomyomas, the Helsinki group sequenced the whole genomes of 16 leiomyomas with *MED12* mutations, 4 leiomyomas with *FH* mutations, and 18 leiomyomas without either alterations (double (−) leiomyomas) [39]. Unlike exome sequencing, whole genome sequencing (WGS) can detect the structural aberrations of chromosomes. Complex chromosomal rearrangements (CCRs) occurred frequently (78% (14/18)) in double (−) leiomyomas, while they occurred at low frequency in leiomyomas with *MED12* (19% (3/16)) and *FH* (0% (0/4)) mutations [39]. In the double (−) leiomyomas, translocations of *HMGA2* were detected in nine specimens, and rearrangements of *COL4A5-COL4A6* were detected in three specimens [39]. The authors suggested that each of the genomic alterations in *MED12*, *FH*, *HMGA2*, and *COL4A5-COL4A6* is a driver mutation, i.e., a mutation that is causally implicated in tumorigenesis, because all studies to date found the four genomic alterations to be mutually exclusive [39–42]. In addition, in the HCL using their transcriptome data (Table 6.1) [41], the leiomyomas with each of the four alterations clustered separately, suggesting that the alterations would classify the leiomyomas into four subtypes with distinct pathological pathways [39–41].

6.3 Differential Diagnostic Markers to Differentiate Uterine Leiomyomas and Uterine Leiomyosarcomas

Although uterine leiomyosarcomas, like leiomyomas, are monoclonal tumors [15], no cytogenetic alteration specific to leiomyosarcomas has so far been identified due to their complicated and frequent chromosomal aberrations [43]. In this section, we describe exome sequencing to identify genomic mutations specific to leiomyosarcomas (Sect. 6.3.1) and studies of molecules differentially expressed in leiomyomas and leiomyosarcomas. The latter include studies of microRNAs (Sect. 6.3.2) and omics (Sect. 6.3.3), immunohistochemical analyses (Sect. 6.3.4), and our recent application of leiomyoma-specific marker genes having aberrant DNA methylation (Sect. 6.3.5).

6.3.1 Genomic Mutations in Uterine Leiomyosarcomas

Exome sequencing of 19 leiomyosarcoma specimens by the Helsinki group revealed an average of 373 somatic mutations (range 240 to 779) per specimen [44]. However, mutations in only six genes (*TP53*, *ATRX*, *MED12*, *ESIP2*, *ABCA13*, and *ANKRD26*) were common to more than three leiomyosarcoma specimens (Table 6.2). Their frequencies were 33% (6/19) at *TP53*, 26% (5/19) at *ATRX*, 21% (4/19) at *MED12*, and 16% (3/19) at the other 3 genes [44].

Table 6.2 Genes that are potential differential diagnostic markers of leiomyomas and leiomyosarcomas identified in various analyses

Analysis	Notes	# genes	Genes	Reference
Exome sequence		6	*TP53*, *ATRX*, *MED12*, *ESIP2*, *ABCA13*, *ANKRD26*	[44]
miRNA array	Leiomyosarcoma specific	27	*miR-150*, *miR-508*, *miR-495*, *miR-139*, *miR-329*, *miR-650*, *miR-196b*, *miR-30b*, *miR-30c*, *miR-455*, *miR-29b*, *miR-202*, *miR-29c*, *miR-148a*, *miR-26b*, *miR-125a*, *miR-193a*, *miR-24*, *miR-106b*, *miR-18a*, *miR-21*, *miR-19a*, *miR-663*, *miR-370*, *miR-15b*, *miR-630*, *miR-490*	[10, 45]
	Leiomyoma specific	19	*miR-292*, *miR-326*, *miR-144*, *miR-203*, *miR-184*, *miR-324-5p*, *miR-154*, *miR-136*, *miR-212*, *let-7e*, *miR-10a*, *miR-10b*, *miR-26a*, *miR-34a*, *miR-126*, *miR-100*, *let-7a*, *miR-145*, *miR-23a*	[10, 22]
Transcriptome (omics)	Myometrium and leiomyoma specific	4	*PGR*, *RIC3*, *DPP6*, *ACKR1*	[46]
	Myometrium specific	4	*CYP4B1*, *MS4A2*, *STEAP4*, *LCN6*	
	Leiomyoma specific	1	*SMPX*	
	Leiomyosarcoma specific, Top 6 genes	6	*CDCA2*, *FOXD1*, *TICRR*, *TTK*, *MELK*, *KIF4A*	
DNA methylome (omics)		2	*NPAS4*, *PITX1*	[46]
Immunohistochemistry		3	PSMB9 (LMP2), CNN1 (calponin h1), CCNB1 (cyclin B1)	[49–51]
DNA methylation		10	*ALX1*, *CBLN1*, *CORIN*, *FOXP1*, *GATA2*, *IGLON5*, *NPTX2*, *NTRK2*, *PRL*, *STEAP4*	[54]

6.3.2 MicroRNA Expression in Uterine Leiomyomas and Leiomyosarcomas

Danielson et al. compared the expressions of miRNAs submitted to the miRNA database (miRBase version 9.2) in leiomyosarcomas, leiomyomas, and normal myometrium (ten specimens each). They found 72 miRNAs predominantly expressed in leiomyosarcomas and 6 miRNAs predominantly expressed in leiomyomas [45]. Further, to characterize the different types of smooth muscle tumor including STUMPs and ALMs, the same research group selected the 27 leiomyosarcoma-specific and 19 leiomyoma-specific miRNAs (Table 6.2) [10] based on miRNA expression profiles reported previously [22, 45]. The selected miRNA expression profiles of different types of smooth muscle tumor revealed that STUMPs resembled leiomyomas and ALMs resembled leiomyosarcomas [10].

6.3.3 Omics Analysis Using Genomic, Epigenomic, and Transcriptomic Profiling in Uterine Leiomyomas and Leiomyosarcomas

Miyata et al. conducted an omics analysis using three cases of leiomyosarcomas, leiomyomas, and normal myometrium based on three analyses of the chromosomal copy number, transcriptome, and DNA methylome [46]. The chromosomal aberrations occurred at very high frequency (66.7–89.5%) in leiomyosarcomas but at low frequency (0.7–8.9%) in leiomyomas, indicating that the normality of chromosomes itself may become a biomarker. The transcriptome revealed 54 highly expressed genes that were specific to various tissues: 4 genes specific to both the normal myometrium and leiomyomas, 4 genes specific to the normal myometrium, 1 gene specific to the leiomyomas, and 45 genes specific to the leiomyosarcomas (Table 6.2) [46]. DNA methylome obtained with the Infinium HumanMethylation450 BeadChip indicated that the genes that were hypermethylated in the leiomyosarcomas belonged to the protocadherin family and genes targeted by the polycomb SUZ12. The authors also proposed two genes as candidates for DNA methylation markers of leiomyosarcomas: *PITX1*, whose promoter was hypomethylated, and *NPAS4*, whose promoter was hypermethylated (Table 6.2) [46].

6.3.4 Immunohistochemical Staining in Uterine Leiomyomas and Leiomyosarcomas

Increased MKI67 and TP53 expression and decreased ESR1 and PGR expression have previously been proposed as immunohistochemical markers for leiomyosarcomas [47, 48]. In recent years, Hayashi et al. reported the LMP2 (PSMB9), calponin h1 (CNN1), and cyclin B1 (CCNB1) proteins as more reliable immunohistochemical markers (Table 6.2) [49–51]. LMP2 is a subunit of the immune proteasome. In the *Psmb9* knockout mice, leiomyosarcoma-like tumors

developed in about 40% of the uteri within 12 months of birth [52, 53]. Calponin h1 is expressed specifically in smooth muscle cells and may play a role in smooth muscle contraction. Cyclin B1 is essential for the progression of metaphase in the cell cycle and plays an essential role in carcinogenesis. LMP2 and Calponin h1 were highly expressed in leiomyomas and normal myometrium, whereas they were detected rarely (15% (8/54) and 3% (1/32)) in leiomyosarcoma specimens [49, 50]. In contrast, cyclin B1 was highly expressed in almost all leiomyosarcoma specimens (97% (31/32)), but not in leiomyomas or normal myometrium [51].

6.3.5 Leiomyoma-Specific Marker Genes Based on DNA Methylation

As mentioned in Sect. 6.2.5, we previously identified 120 aberrantly methylated and expressed genes specific to leiomyomas based on the transcriptome and DNA methylome of three cases of leiomyoma and adjacent normal myometrium [27]. A PCA showed clear differences in DNA methylation between the leiomyomas and normal myometrium, but did not show clear differences in mRNA expression (see Chap.) [27]. Thus, profiling by DNA methylation is better than profiling by mRNA expression at defining cell identity. These results prompted us to search for marker genes specific to leiomyomas based on DNA methylation. We first selected 33 of the 120 leiomyoma-specific aberrant genes, confirmed their DNA methylation levels with 10 paired specimens of leiomyoma and adjacent normal myometrium, and identified 12 genes that were hyper- or hypomethylated in at least 70% of the leiomyoma specimens as leiomyoma-specific marker genes (Fig. 6.1a) [54]. Among the 12 leiomyoma-specific marker genes, we attempted to select the most appropriate combination of the marker genes that best differentiate between leiomyomas and normal myometrium and found that a combination of 10 of the genes completely differentiated them in the HCL based on the gene methylation profile of the 18 paired specimens (Fig. 6.1b) [54]. As shown in Fig. 6.1b, the DNA methylation profiles of the marker genes in the 18 normal myometrium specimens were highly homogeneous. Although the DNA methylation profiles of the leiomyoma specimens are not as homogeneous, it was possible to classify them into three subclusters (SC1, SC2, and SC3 in Fig. 6.1b). Interestingly, all four of the leiomyoma specimens that did not have a *MED12* mutation were in SC2 (Fig. 6.1b) [54]. Next, we investigated whether our HCL system can differentiate between leiomyomas and leiomyosarcomas. DNA methylation levels of the marker genes were measured in 12 uterine leiomyosarcoma specimens (LMS_1 to 12, gray boxes) and 2 leiomyosarcoma cell lines (SKN and RKN, gray boxes). When combined with the HCL, 8 of 12 leiomyosarcoma specimens (LMS_1, 3, 4, 6, 9, 10, 11, and 12) and the 2 leiomyosarcoma cell lines formed a cluster (leiomyosarcoma in Fig. 6.1c). The other four leiomyosarcoma specimens (LMS_2, 5, 7, and 8) clustered in the SC2 sub-cluster of the leiomyoma group (Fig. 6.1c) [54]. Thus, 10 (71%) out of the 14 leiomyosarcomas clustered together, indicating that our HCL system can differentiate between leiomyosarcomas and leiomyomas with approximately 70% accuracy.

6.4 Concluding Remarks

To date, the most significant innovation in pathological diagnostic markers of uterine leiomyomas is the discovery of *MED12* mutations, which are detected in about two thirds of leiomyoma specimens regardless of race. Furthermore, a study of transgenic mice expressing a mutant form of *Med12* in the uterus suggested that the mutated MED12 protein was one of the factors driving the onset of leiomyomas [55]. Therefore, *MED12* mutations seem to play an important role not only as pathological diagnostic markers but also for elucidating the mechanisms of the onset and development of leiomyomas. Although the genomic alterations of the *FH*, *HMGA2*, and *COL4A5-COL4A6* genes remain unproven as the actual driver mutations, the distinct transcriptional profiles of leiomyomas with each alteration suggest that each alteration appears to be involved in the onset or development of each leiomyoma subtype. To date, in the analysis of mRNA, miRNA, protein expression, and DNA methylation patterns, the low commonalities among the studies, which confounded the identification of leiomyoma-specific features, have been thought to be mainly attributed to differences in the specimens (race, age, or phase of the menstrual cycle), analysis platforms, and analytical methods. As mentioned above, the low commonalities may also be due to different leiomyoma subtypes with distinct pathological pathways. In the future, researchers need to take the leiomyoma subtypes into account when attempting to identify leiomyoma-specific markers and to elucidate the molecular mechanisms of the onset and development.

Immunostaining by the LMP2, calponin h1, and cyclin B1 was shown to be useful for differential diagnosis of uterine leiomyomas and leiomyosarcomas [49–51]. Although those biomarkers have not yet been used in practice, the authors have started large-scale clinical studies with several clinical research facilities in order to confirm their reliability and specificity [51]. However, it might be difficult to objectively differentiate the staining results in some cases. A combination of immunostaining and miRNA expression profiles or our HCL system should provide more reliable criteria for differential diagnosis of leiomyomas and leiomyosarcomas. Leiomyomas, which are associated with multiple genomic alterations, have subtypes with distinct pathological pathways. Similarly, leiomyosarcomas are expected to have various subtypes and distinct pathological pathways. Some leiomyosarcomas may have arisen from preexisting leiomyomas [56–58]. It has also been pointed out that some cases of leiomyosarcoma with *MED12* mutation may have arisen from a leiomyoma precursor with *MED12* mutation [36, 44]. In our study, all of the leiomyosarcoma specimens that were clustered into the leiomyoma group were included in SC2 sub-cluster, indicating that the DNA methylation profiles of the marker genes are similar to those of leiomyomas without *MED12* mutations [54]. Since DNA methylation profiles are specific to cell types and could reflect the origin of the cells [59, 60], these results suggest that some cases of uterine leiomyosarcomas were derived from leiomyoma cells without *MED12* mutations. To obtain more reliable diagnostic criteria, we need to clarify the nature of leiomyosarcoma itself including the pathological subtypes. Additional exosome sequencing, WGS, and omics analyses of leiomyosarcomas would provide clues about their nature.

a
ALX1
DUSP6
IGLON5
STEAP4
CBLN1
FOXP1
NPTX2
PART1
CORIN
GATA2
NTRK2
PRL
M
L
Methylation (%)
Case1
Case2
Case3
Case4
Case5
Case6
Case7
Case8
Case9
Case10

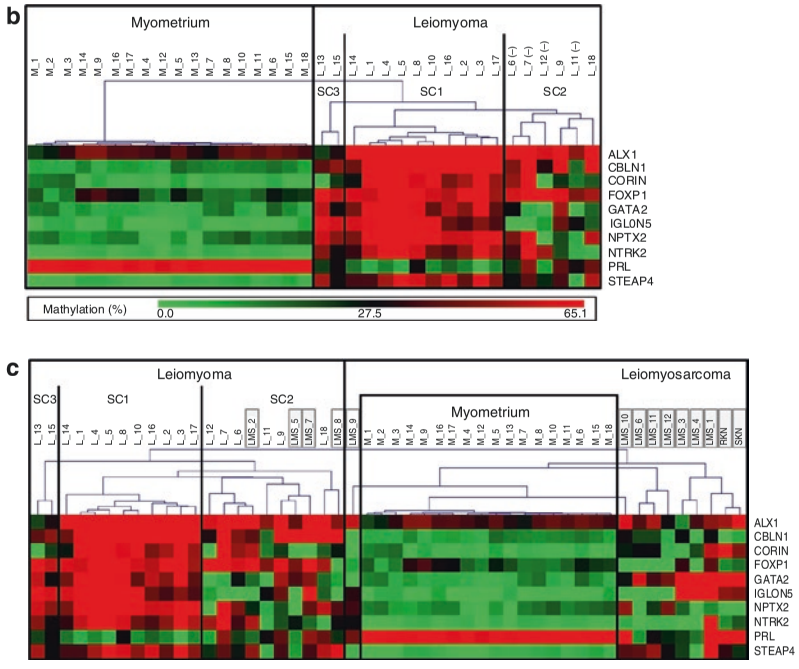

Fig. 6.1 Hierarchical clustering using uterine leiomyoma-specific marker genes based on DNA methylation and its clinical application to differential diagnosis of uterine leiomyosarcomas [54]. (**a**) Methylation levels of 12 leiomyoma-specific marker genes in 10 paired specimens of leiomyomas and adjacent normal myometrium. Gray and black bars indicate the percentages of DNA methylation in the myometrium (M) and leiomyoma (L) specimens, respectively. (**b**) Hierarchical clustering of 18 paired specimens of leiomyoma (L_1 to 18) and normal myometrium (M_1 to 18) based on DNA methylation level of the 10 leiomyoma-specific marker genes (Table 6.2). DNA methylation profiles of the leiomyoma specimens were classified into three sub-clusters (SC1, SC2 and SC3). Leiomyoma specimens without *MED12* mutations are shown as (−). (**c**) Hierarchical clustering of leiomyomas and leiomyosarcomas. The DNA methylation profiles of each sample of l2 leiomyosarcoma specimens (LMS_1 to 12, gray boxes) and two sarcoma cell lines (SKN and RKN, gray boxes) were subjected to the hierarchical clustering with the 18 paired specimens of the leiomyoma (L_1 to 18) and normal myometrium (M_1 to 18). LMS_1, 3, 4, 6, 9, 10, 11, and 12, SKN, and RKN were classified into a separate cluster (leiomyosarcoma). LMS_2, 5, 7, and 8 were classified into the leiomyoma subgroup (SC2)

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Current Medical Treatments for Uterine Fibroids

7

Izumi Kusuki and Jo Kitawaki

Abstract

Uterine fibroids are benign tumors with a high prevalence rate and are considered the most common benign tumors in women of reproductive age. Surgical or medical treatment is necessary when symptoms, such as abnormal uterine bleeding, pelvic pain, and infertility, occur. Regarding hormonal therapies, a large number of GnRH agonist therapies have been employed. Current alternative treatments for the improvement of abnormal uterine bleeding and reduction of fibroid volume include GnRH antagonists, aromatase inhibitors, progestin, and selective progesterone receptor modulators, and their therapeutic effects have been studied.

Keywords

Uterine fibroid · Medical treatment · Selective progesterone receptor modulator

7.1 Introduction

Uterine fibroids are the most common benign tumors in women of reproductive age and occur in 70–80% of this population [1]. The main symptoms of uterine fibroids are abnormal uterine bleeding, pelvic pain, and infertility [2]. Approximately, 20–50% of patients with fibroids have related symptoms such as excessive menstrual bleeding, pelvic pressure, and increased uterine size [3].

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_7

Asymptomatic patients or those with negligible symptoms are often not treated because there is insufficient evidence to support treatments in such patients. However, management is needed if the symptoms are notable or infertility occurs. The management of uterine fibroids is roughly divided into surgical therapy and conservative therapy. For surgical therapy, simple hysterectomy serves as a radical surgery and the enucleation of uterine fibroids as a function-preserving surgery. For uterine submucosal myomas with a high degree of protrusion into the uterine cavity, the removal of submucosal myomas is performed with hysteroscopy. Conservative therapy includes medical therapy, uterine artery embolization [4], and magnetic resonance imaging-guided focused ultrasound surgery [5]. The treatment protocol for uterine fibroids is determined based on age, size, and occurrence site of the fibroids, degree of symptoms, and presence of infertility, and the preservation of fertility, follow-up observation, conservative treatment, and surgical treatment (radical surgery or function-preserving surgery) are performed individually.

Medical therapy for uterine fibroids is centered on hormonal therapy. Uterine fibroids are hormone-dependent tumors, and estrogen is involved in its proliferation. Moreover, progesterone and progesterone receptors, which are highly expressed in leiomyoma cells, also play a major role in fibroid proliferation [6]. The main purpose of medical therapy is to reduce tumor volume and improve the symptoms associated with uterine fibroids. Furthermore, it aims to preclude surgery, reduce surgical difficulty after preoperative administration, or prevent recurrence after uterine fibroid enucleation. GnRH agonists have been indicated for uterine fibroids for many years. Currently, there are alternative hormonal therapies such as GnRH antagonists, aromatase inhibitors, progestin preparations, and selective progesterone receptor modulators (SPRMs).

7.2 GnRH Agonist

GnRH is a peptide hormone consisting of ten amino acids and is secreted into the pituitary portal vein from the hypothalamus and acts on gonadotropin-secreting cells of the anterior pituitary to secrete LH and FSH. GnRH agonists are synthetic peptides stabilized through the alternation of amino acids at the 6-position. They are not susceptible to the action of degrading enzymes and have a greater affinity for GnRH receptors than GnRH. The half-life of natural GnRH is very short, but the GnRH agonist has a very long half-life [7]. GnRH agonists are frequently used as preoperative treatments, and consensus regarding their evaluation has been reached, especially for laparoscopic enucleation [8] and hysteroscopic resection of the submucosal myoma node [9]. The main effect of the GnRH agonist on uterine fibroids is the reduction of fibroid volume, improvement of uterine bleeding, and normalization of hemoglobin [10]. Long-term administration of GnRH agonists causes a hypoestrogenic state, ovarian deficiency symptoms, and bone demineralization, as side effects such as osteoporosis, mood discomfort, and depressive symptoms increase. Therefore, the administration of GnRH agonists should generally not exceed 6 months, and long-term management by GnRH agonist is not possible

(if necessary add-back therapy is recommended [11]). It has also been demonstrated that the fibroids return to their original size within a short time after treatment stops [12, 13]. Thus, administering a GnRH agonist in a random fashion to patients with uterine fibroids is not recommended, and it is necessary to consider its effective use in long-term treatment planning.

7.3 GnRH Antagonist

GnRH antagonists are derivatives of GnRH and compete with endogenous GnRH to inhibit the secretion of gonadotropin. Flare-up does not occur with these drugs unlike with GnRH agonists. The early onset of the reduction of uterine fibroid volume and improvement of uterine bleeding are recognized effects of GnRH antagonist treatment [14]. They are advantageous for the maintenance of bone density for improved adjustment of the suppression of blood E2 concentration, and their clinical application for uterine fibroids is expected. However, studies of the therapeutic effect of GnRH antagonists on symptomatic uterine fibroids have been limited to observational studies [15]. Moreover, GnRH antagonists are expensive and require daily injections, so they are unsuitable for long-term use. GnRH antagonists have no significant advantage over GnRH agonists, and their use for uterine fibroids is limited.

7.4 Aromatase Inhibitor

Aromatase inhibitors inhibit aromatase, which is an estrogen synthase that converts androgen to estrogen and suppresses the production of local estrogen. It is used to treat postmenopausal breast cancer. Letrozole and anastrozole are clinically used. In uterine fibroids, the expression of aromatase is enhanced locally, and it is thought that letrozole, one of the aromatase inhibitors, reversibly inhibits aromatase and inhibits the local proliferation of estrogen, reducing the size of uterine fibroids, with expected symptom improvement [16]. In an RCT of letrozole, the uterine fibroid significantly shrunk in 3 months, but its effect was not significantly different compared to that of the GnRH agonist [17, 18]. Aromatase inhibitors contribute to the alleviation of symptoms and are considered to have little influence on the hormonal state of the whole body compared to GnRH agonists, causing no change in E2, T, FSH, and LH values in blood [16]. Therefore, for short-term use, aromatase inhibitors should preclude the side effects of low estrogen levels. At present, its use is limited.

7.5 Levonorgestrel Intrauterine System

The levonorgestrel intrauterine system (LNG-IUS) was developed for contraception and has been reported as effective in improving hypermenorrhea caused by uterine fibroids [19]. In a report, the amount of menstrual blood decreased significantly in

comparison with the low-dose combined oral contraceptive preparation [20], and uterine fibroid shrinkage was not obtained after LNG-IUS treatment [21]. Moreover, for heavy menstrual bleeding associated with fibroid or submucosal fibroids, the expulsion rate of LNG-IUS from the uterine cavity is high and requires attention [19].

7.6 Selective Progesterone Receptor Modulators

The influence of progesterone and progesterone receptors on uterine fibroids has been clarified. SPRMs are synthetic ligands for progesterone receptors and act as agonists or antagonists against progesterone receptors (PR). A report showed that progesterone stimulates proliferative activities in leiomyoma cells but not in normal myometrial cells [22]. Similarly, it has been shown that SPRMs promote the induction of apoptosis in leiomyoma cells without affecting normal myometrial cells by changing signals to PR [23]. SPRMs may be effective for the preoperative management of uterine fibroids and for avoiding surgery. Since SPRMs block progesterone action, chronic SPRM administration may cause endometrial hyperplasia. Endometrial changes due to SPRMs are benign and are called progesterone receptor modulator-associated endometrial changes (PAECs). Endometrial glands show dilatation and cystic changes but lack mitotic activity; changes are reversible and are distinguishable from endometrial hyperplasia [24].

Mifepristone (RU-486) and ulipristal acetate (CDB-2914) are currently used clinically (Fig. 7.1). Both act antagonistically to the action of PR and are believed to reduce uterine fibroids by an antiprogestin action. Mifepristone is a synthetic ligand derived from 19-nortesterone and is one of the first developed SPRMs with PR antagonist activity. It is mainly used for emergency contraception and abortion drugs; however, an observational study showed that it has the ability to suppress the growth of uterine fibroids [25]. The administration of mifepristone 50 mg/day resulted in a 49.0 ± 9.2% reduction in uterine fibroids and improvement in uterine

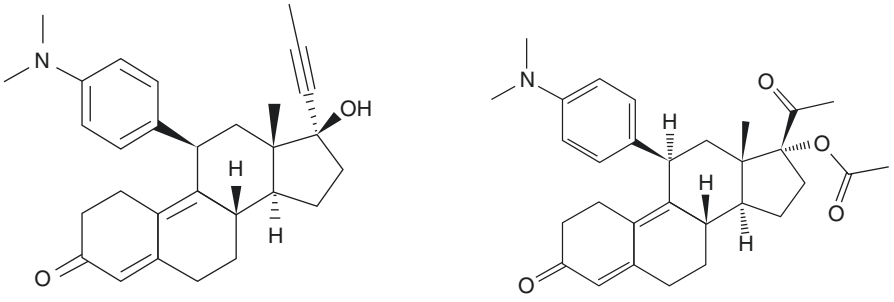

Fig. 7.1 Structure of SPRMs

bleeding. Even a small amount of mifepristone (5 or 25 mg/day) demonstrated a fibroid-reducing effect. Moreover, a prospective study and RCT reported the reduction of uterine fibroids and menstrual blood volume [26] and the suppression of pelvic pain [27], respectively. Mifepristone was evaluated as a cost-effective therapy for fibroids with minimal side effects. However, a recent meta-analysis reported that mifepristone contributed to the improvement of excessive menstruation, but another study reported that this effect was not observed [28].

Ulipristal is a synthetic steroid derived from 19-norprogesterone, which has a high affinity for PR-A and PR-B, and works as an antagonist and partly as an agonist [29, 30]. A study showed that UPA enhanced the expression of VEGF, IGF-1, and MMPs and selectively inhibited the proliferation of leiomyoma cells without affecting normal uterine myometrial cells. In Europe and Canada, the preoperative administration of ulipristal for uterine fibroids has been licensed. Ulipristal has been found to be effective in reducing uterine fibroids, decreasing menstrual blood flow, and improving quality of life with fewer side effects. The effect of ulipristal on uterine fibroids has been clinically studied. In an RCT of 18 patients conducted in 2008, the ulipristal-treated group (10 and 20 mg/day) had significantly reduced fibroids after 3 months compared to the placebo-treated group. A decline in estradiol levels was not observed in all groups [31].

An RCT demonstrated the effect of ulipristal on uterine fibroids [32]. The effect of ulipristal was well studied in the European phase III studies PGL 4001 (ulipristal) Efficacy Assessment in Reduction of Symptoms Due to Uterine Leiomyomata (PEARL). PEARL I compared ulipristal (5 and 10 mg/day) and placebo in 242 patients with uterine fibroids with excessive menorrhagia for 13 weeks. In this study, 73% of the group receiving 5 mg of ulipristal and 82% of the group receiving 10 mg had amenorrhea, but in the placebo group, only 6% had amenorrhea. In the ulipristal group, 92% showed improvement in menstrual volume after 13 weeks compared to 19% in the placebo group. In addition, a significant reduction in uterine fibroids was obtained compared to the placebo group [33]. In PEARL II, a double-blinded noninferiority trial of ulipristal (5 and 10 mg/day) and GnRH agonist (depot leuprolide acetate 3.75 mg/month) for 13 weeks was performed with 307 patients having uterine fibroids with excessive menstruation. Menstrual volume decreased in all three groups. The median of the period until amenorrhea was shorter in the ulipristal than in the GnRH agonist group (UPA 5 mg, 7 days; UPA 10 mg, 5 days; GnRH agonist, 21 days). The uterus and fibroid volume also decreased in all three groups, but the GnRH agonist had a higher reduction ratio (total volume of the three largest myomas, ulipristal 5 mg −36%, ulipristal 10 mg −42%, GnRH agonist −53%; uterine volume, ulipristal 5 mg −20%, UPA 10 mg −22%, GnRH agonist −47%). The incidence of hot flashes was higher in the GnRH agonist group (ulipristal 5 mg, 11%; ulipristal 10 mg, 10%; GnRH agonist, 40%). It was concluded that ulipristal had a uterine bleeding inhibitory effect equivalent to that of the GnRH agonist but with a significantly lower occurrence of hot flashes [34]. Prolonged administration of SPRM has been suggested to result in the increase of endometrial hyperplasia and cancer. However, recently, many reports

have precluded the association between SPRMs and atypical endometrial hyperplasia. In PEARL III and IV, long-term ulipristal trials were conducted, but the incidence of PAEC remained unchanged, and no endometrial hyperplasia or endometrial cancer occurred [35].

Conclusion

Although surgical therapy and drug therapy are mainly regarded as treatments for uterine fibroids, it is necessary to examine therapeutic strategies individually according to symptoms, size, site of occurrence, patient age, and the desire for pregnancy. GnRH agonists are frequently used as hormone therapy for uterine fibroids and have been well evaluated; however, long-term management with GnRH agonists alone is difficult, and the evaluation of new hormonal therapies is expected. Among these, ulipristal has few side effects while improving uterine fibroids and symptoms. The future accumulation of clinical data regarding these therapies is required.

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Diagnostic Imaging for Uterine Fibroids, Adenomyosis, and Uterine Sarcomas

8

Aki Kido

Abstract

The main role of imaging for uterine mesenchymal tumors is to make an accurate diagnosis of each tumor, especially differentiating malignant tumors from benign ones. Because of the superior spatial resolution of MR imaging compared to ultrasound and computed tomography, detailed information related to the disease is often obtained from MR imaging. For uterine leiomyoma, or fibroid, it is necessary to know the variation of image findings of leiomyoma such as edema, myxoid change, and several variants of leiomyoma. Because most of the degenerated leiomyomas and variants show high signal intensity on T2-weighted image of MRI, a key sequence for diagnosing uterine tumor is T2-weighted image. That knowledge of variation is important for differentiation from sarcomas, especially leiomyosarcoma and low-grade endometrial stromal sarcoma. Typical features of leiomyosarcoma are hemorrhage and necrosis within the tumor. They are reflected as irregularly unenhanced lesion with high signal intensity on T1-weighted image. In cases of adenomyosis, hemorrhagic foci or dilated cystic glands within the heterotopic endometrial tissue are visualized as cystic lesions both on US and MRI. Adenomyosis also receives hormonal influence by hormonal therapy or pregnancy. Those changes were visualized using MR imaging.

Keywords

MRI · Uterine fibroid/leiomyoma · Uterine sarcoma

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_8

8.1 Selection of Imaging Modalities

For uterine mesenchymal diseases including uterine leiomyoma, adenomyosis, and sarcomas of various kinds, the most required role of imaging is to make an accurate diagnosis of sarcoma, distinguishing it from benign diseases. The initial imaging modality for the diagnosis is transabdominal sonography (TAS) or transvaginal sonography (TVS). However, because of the limited spatial resolution of ultrasound, it is not easy to differentiate uterine leiomyoma from adenomyosis or sometimes even from uterine sarcomas [1]. Magnetic resonance (MR) imaging is an objective, noninvasive modality for diagnosing mesenchymal diseases. Among many MR sequences, T2-weighted image (WI) is the key for the diagnosis, followed by T1-WI, gadolinium-enhanced T1-WI, and diffusion weighted image (DWI). Gadolinium-enhanced T1-WI is expected to be helpful for diagnoses in some circumstances, such as uterine sarcomas for visualization of necrotic lesions within the tumor. When using contrast materials, it is necessary to check renal function because of the increased risk of nephrogenic systemic fibrosis (NSF) in patients with reduced renal function [2]. Computed tomography (CT) is superior for obtaining images of a wide area of the body with short scan time, but its tissue resolution is limited in gynecological organs with radiation exposure. Fluorodeoxyglucose-positron emission tomography (FDG-PET) is also used for whole-body evaluation, screening of metastases, and recurrence of malignant tumors. Recently, reports of several studies of the relevant literature have described the usefulness of differentiating uterine sarcomas from leiomyoma [1]. It is necessary to select adequate modalities depending on the clinical objective.

8.2 Uterine Leiomyoma

8.2.1 Usual Leiomyoma

When using ultrasound, uterine leiomyomas are visualized as hypoechoic round lesions, but they are not easily differentiated from adenomyosis [3]. On CT, leiomyomas are isodense with muscle and are sometimes accompanied by scattered or dense calcification. Furthermore, CT does not allow adequate differentiation from other mesenchymal tumors because of its poor soft tissue contrast. In contrast, MRI enables accurate assessment of the location of leiomyomas, such as the cervix, corpus, and fundus or submucosal, intramural, and subserosal, in addition to the number and size of leiomyomas by superior soft tissue contrast and multiplanar orientation. On MRI, leiomyomas typically appear as well-circumscribed masses that are sharply demarcated from the surrounding myometrium with distinct low signal intensity relative to that of the myometrium on T2-WI and low signal intensity on T1-WI [4, 5] (Fig. 8.1). These characteristic signal intensities are induced by extensive hyalinization, which occurs in more than 60% of uterine leiomyomas [6, 7]. On DWI, recently widespread MR sequence reflecting water diffusibility, a typical leiomyoma shows low signal intensity because of the “T2 blackout effect” [8].

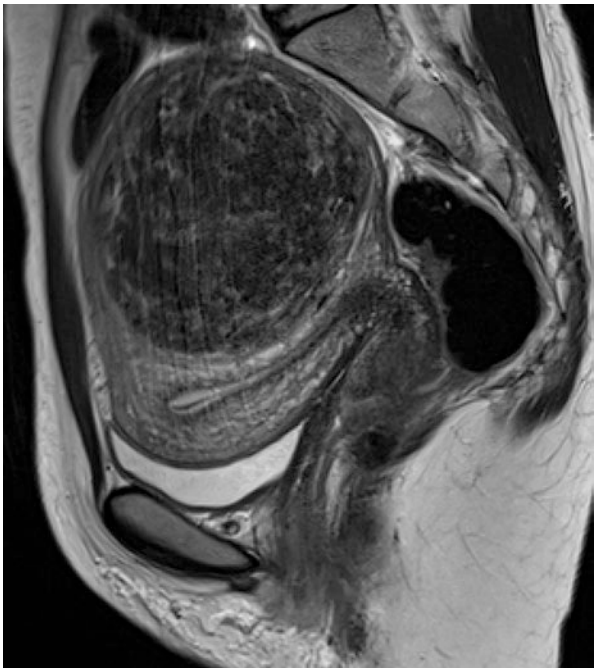

Fig. 8.1 On sagittal T2-weighted image, leiomyoma is visualized a sharply demarcated mass with distinct low signal intensity relative to normal uterine myometrium. High signal intensity scattered within the myometrium is reflecting edema

By the "T2 blackout effect," hypointensity on DWI reflects low signal intensity on T2-WI. It is also related to the lower apparent diffusion coefficient (ADC) values, which represent the degree of diffusion of water molecules quantitatively [9, 10]. A pseudocapsular layer of leiomyoma is sometimes visualized as high signal rim on T2-WI, which corresponds to markedly dilated lymphatic vessels, dilated veins, edema, or a combination of these features attributable to local vascular congestion by the presence of the leiomyomas [11]. Another characteristic finding of leiomyoma is bridging vascular sign, which is uterine vessels that supply the pedunculated leiomyoma traversing the vascular pedicle of the fibroid [12]. Subserosal leiomyoma is often difficult to differentiate from adnexal lesion, especially fibroma or fibrothecoma and gastrointestinal stromal tumor (GIST), which also shows a low-signal-intensity tumor on T2-WI. Because pedunculated subserosal leiomyomas might undergo torsion, which can lead to emergent abdominal pain, it is an important aspect of this differentiation [13]. This flow void can be recognized as more than 80% in case of larger leiomyoma, more than 7 cm, but not detected as less than 3 cm [12, 14]. T1-WI is more sensitive than T2-WI in detecting flow voids surrounding uterine leiomyomas [14]. Lateral growth of subserosal leiomyomas might extend between the folds of the broad ligament by displacing the bladder or rectum. It exhibits similar appearance with retroperitoneal tumors such as neurogenic tumors of schwannoma and paraganglioma [7, 15]. MR can also delineate the prolapsing leiomyoma by showing the stalk and its uterine attachment, which is useful information for determining the surgical approach [16]. The prolapsed lesion

sometimes appears as a lobulated mass with heterogeneous signal intensity on the T2-WI and poor enhancement, called *broccoli sign* [17].The degeneration of leiomyoma is usual. Hyalinization, explained above, is the most common type. Another typical degeneration is edema and red degeneration. Edema is a common histopathologic condition, observed in about 50% of leiomyomas. The edema is visualized as a speckled pattern with high signal intensity on T2-weighted images [7]. It is frequently observed at the periphery but can be scattered throughout the leiomyoma [7, 11]. Cystic degeneration, which is considered as an extreme sequela of edema, appears as clearly delineated cystic lesions with fluid signal intensity (Fig. 8.2). Red degeneration is common during pregnancy and is associated with progestational therapy [18]. It often occurs as acute abdomen probably because it is hemorrhagic venous infarction of leiomyoma [19]. Therefore, MRI shows a completely unenhanced lesion with a hyperintense rim on T1-WI and hypointense rim on T2-WI, corresponding with dilated vessels filled with red blood cells at the lesion periphery [19]. A notably large area of hemorrhage is also observed in about 10% of leiomyoma [6]. Because the appearance of leiomyoma varies widely as a result of that degeneration, differentiation from sarcoma can often be difficult (Fig. 8.3). Details of the differentiation between uterine sarcomas and degenerated leiomyoma are discussed later.

Hormonal therapy using gonadotropin-releasing hormone (GnRH) analog is one option for fertility-preserved treatment [20]. Histologically, leiomyomas treated with GnRH analogs show reduction in cellularity and an increase in hyaline degeneration [21, 22]. This degeneration might decrease both the volume and signal intensity on T2-WI. Oguchi et al. show higher signal intensity on T2-WI, with a

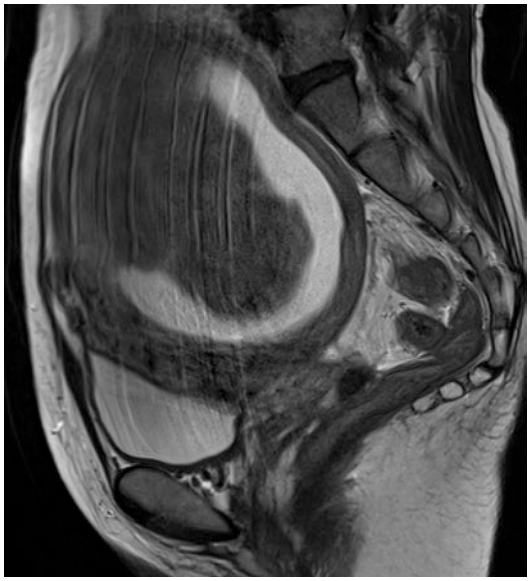

Fig. 8.2 On sagittal T2-weighted image, leiomyoma is located at the posterior wall of the uterus. Most of the fibroid shows typical low signal intensity, but the periphery of the fibroid showed fluid signal intensity, indicating cystic degeneration of leiomyoma

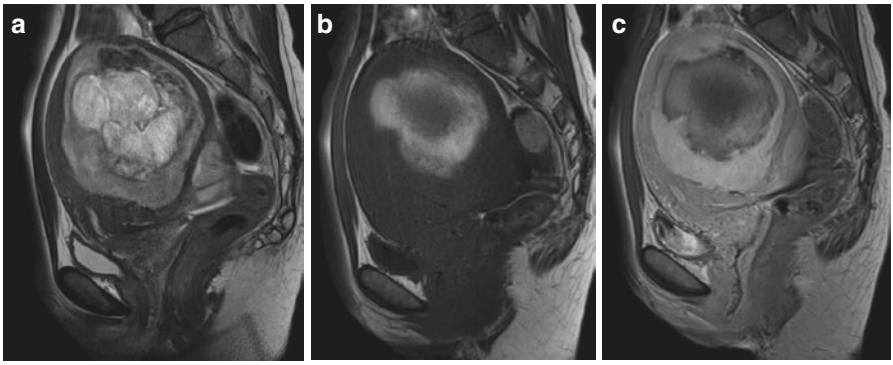

Fig. 8.3 (**a**) Sagittal T2-weighted image, (**b**) sagittal T1-weighted image, (**c**) sagittal contrast-enhanced T1-weighted image. Large tumor is located at the posterior wall of the uterus. The tumor margin is clear, but the signal intensity within the tumor shows heterogeneously high signal on T2-WI. High signal intensity on T1-WI shows hemorrhage within the tumor, and unenhanced lesion with irregular margin indicates hemorrhagic necrosis of the tumor. These findings indicated the suspicion of uterine sarcoma, but it was pathologically diagnosed hemorrhagic degeneration of leiomyoma after operation

higher reduction rate by hormonal therapy, possibly correlated with increased cellularity in high-signal leiomyoma on T2-WI [23].

Uterine artery embolization (UAE) has been reported as an effective alternative to surgical therapy for leiomyoma [24–26]. Long-term results and volume reduction depend mainly on the post-UAE degree of devascularization of the leiomyoma on contrast-enhanced T1-WI [27, 28]. After 6 months of UAE, cases with complete fibroid infarction, in other words completely unenhanced fibroid, were more likely not to have enhancing lesions at 3-year follow-up than those with incomplete infarction [29]. In contrast, incomplete fibroid infarction might engender the regrowth of uninfarcted fibroid tissue and symptom recurrence [29].

Differentiation is necessary for adenomatoid tumors and adenomyosis after hormonal therapy. Adenomatoid tumors are rare tumors originating from the mesothelium, usually arising in the genital tract [18]. Although it is not encapsulated pathologically, it often appears as a well-defined low-signal-intensity tumor on T2-WI similar to uterine leiomyoma [30, 31]. In cases with cystic component or indistinct boundaries, it can be differentiated [30, 31].

8.2.2 Variation of Morphological Features

Several variations of morphological features exist for leiomyoma. Those characteristics are reflected by MR imaging.

Cellular leiomyoma: Cellular leiomyoma has significantly more cellularity than the surrounding myometrium [32]. Hypercellularity suggests a diagnosis of

leiomyosarcoma, but cellular leiomyoma lacks tumor cell necrosis and has moderate to severe cytological atypia [6]. As Oguchi et al. described, the signal intensity of the leiomyoma on T2-WI on MRI increased according to increased cellularity [23]. For that reason, cellular leiomyomas usually show higher signal intensity on T2-WI than ordinary leiomyomas with strong early enhancement during dynamic enhanced MR imaging [8, 33] (Fig. 8.4). Hypercellularity also led to increased signal intensity on DW images, as well as decreased ADC values. It led to difficulties for differentiating uterine sarcomas [8, 34].

Lipomatous leiomyoma: Pathologically, leiomyomas that contain a substantial amount of fat are called lipoleiomyomas. They are often observed in elderly women [6]. Imaging findings show signal intensity and density resembling those of subcutaneous fat, which can be interpreted as a hyperechoic mass on ultrasound, low density in CT, and high signal intensity on T1-WI and T2-WI. Fat tissue can be confirmed using fat-suppression techniques [7, 35].

Myxoid leiomyoma: Myxoid degeneration appears as abundant amorphous myxoid materials presenting between smooth muscle cells, sometimes with cystic change and difficulty in differentiation from myxoid leiomyosarcoma [6, 7]. During MR imaging, the myxoid area shows very high signal intensity on T2-WI and enhances well except for small foci of mucinous lakes or clefts [7]. Delayed and prolonged enhancement is observed because of the presence of a myxoid stroma [36].

Intravenous leiomyomatosis: Intravenous leiomyomatosis has two origins: from the venous wall with many sites of attachment to the vein walls and from uterine leiomyoma infiltrating intravenously and spreading into the venous cavity [6]. The tumor grows from the internal iliac vein and extends into the heart through the inferior vena cava, which might cause a fatal obstruction, but leiomyosarcoma showed a similar course of development [37, 38]. US images usually demonstrate vascularized thrombi within the pelvic veins and IVC [39]. On MR imaging, several

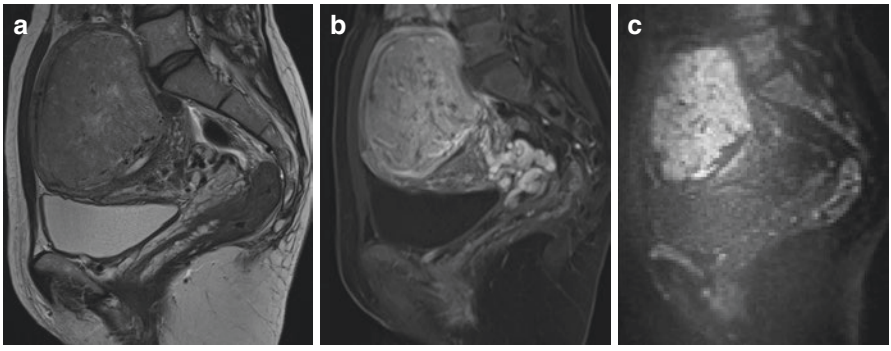

Fig. 8.4 (**a**) Sagittal T2-weighted image, (**b**) sagittal contrast-enhanced T1-weighted image, (**c**) diffusion-weighted image. Typical MR appearance of cellular leiomyoma. The tumor is located at anterior wall to fundus of the uterine myometrium. The tumor shows homogenously high signal intensity on T2-WI and well enhanced as normal myometrium. High signal intensity on DWI shows restricted diffusion compared to usual fibroids

reports have described heterogeneously high signal intensity on T2-WI accompanying well or prominent enhanced tumor continuing from the uterine myometrium [40, 41].

Cotyledonoid dissecting leiomyoma: Dissecting leiomyoma is an unusual growth pattern of smooth muscle. It features the dissection of compressive tongues of smooth muscle into the surrounding myometrium and occasionally into the broad ligament and pelvis [6]. When edema and congestion are prominent, it resembles placental tissue, and is therefore called cotyledonoid dissecting leiomyoma. On MR imaging, it shows higher signal intensity on T2-WI than uterine myometrium, often because of hydropic changes. It resembles intravenous leiomyoma in cases with extrauterine extension along the broad ligament [18, 42].

Diffuse leiomyomatosis: Diffuse leiomyomatosis is also an unusual growth pattern of smooth muscle. Innumerable small leiomyomas produce symmetric enlargement of the uterus [6] because the leiomyomas replace most of the uterine parenchyma, and it is difficult to recognize normal myometrium on T2-WI [7].

Metastasizing leiomyoma: Metastasizing leiomyoma spreads hematogenously from benign tumors and often affects the lung [43]. CT findings of pulmonary nodules vary from solitary subcentimetric lesions to multiple lesions simulating pulmonary metastases from malignant tumors, sometimes with cavitation [39].

8.2.3 Uterine Peristalsis and Leiomyoma

Recent rapid development of improved hardware has enabled the evaluation of effects of uterine leiomyoma on uterine movement on US and MRI. That movement is a kind of uterine contraction: so-called uterine peristalsis. It is defined as rhythmic and subtle contractions of the inner myometrium with a frequency of 2–3 contractions per minute [44, 45]. Its behavior, such as frequency and direction of the wave, changes according to the menstrual cycle depending on the role of cycle phases. That is, the direction of contractions is usually retrograde (cervix to fundus) during the mid-cycle to transform the sperm, anterograde during menstruation to discharge menstrual blood, with no movement during the luteal phase for preservation of the gestational sac [44, 45]. On US, uterine peristalsis is observed as rhythmic and subtle wave-like endometrial movements associated with contractions of the inner myometrium [44, 45]. On MR, it is visualized as low-intensity conduction within the subendometrial myometrium (often within the junctional zone (JZ)), usually associated with a stripping movement of the endometrium [44–47].

The effect of leiomyoma on uterine peristalsis has not been established clearly, but some evidence has been published related to the disturbance of normal peristaltic wave propagation in the inner myometrium by leiomyoma and its relation to fertility [48, 49]. In patients with symptomatic leiomyoma, their respective peristaltic frequencies were significantly lower than in women without leiomyomas during the periovulation phase [49]. However, the presence and frequency of uterine peristalsis increased after UAE, probably because of the recovery of normal myometrial contraction by decrease of the leiomyoma [50]. Another report

describes patients with intramural leiomyoma, showing more frequent peristalsis during the mid-luteal phase, in other words, the implantation period, and resulting in a lower pregnancy rate than that of patients in the lower frequent peristaltic group [51]. However, myomectomy will lead to a favorable outcome in these fibroid patients both in terms of uterine peristalsis and fertility. In patients with fibroid presenting hyperperistalsis during the mid-luteal phase, peristalsis became normalized in most cases after myomectomy and resulted in successful pregnancy in 40% of patients [52]. The presence of uterine leiomyoma might induce abnormal uterine peristalsis in some patients, leading to infertility. Myomectomy might improve fertility in such patients [52].

8.3 Uterine Sarcomas

8.3.1 Leiomyosarcoma

Leiomyosarcoma, the most common uterine sarcoma, accounts for about one-third of uterine sarcoma cases and 1–2% of all uterine malignancies [6, 18]. On MR imaging, differentiation from uterine leiomyoma, especially from degenerated leiomyoma, is always challenging. It has been tried by many researchers, but efforts are continuing. Detailed discussion related to differentiation of uterine sarcomas and leiomyoma will be presented later (Sect. 8.3.3).

On MR imaging, leiomyosarcoma is observed as a large heterogeneous tumor on T2-WI with an irregular contour [53–56]. It often includes a hemorrhagic or necrotic area within the tumor and produces higher signal intensity on T1-WI and heterogeneous enhancement on enhanced T1-WI [57]. On DWI, the solid area shows high signal intensity with decreased ADC values, probably reflecting higher cellularity of the tumor [8, 57, 58]. However, these findings were also applicable to degenerative and cellular leiomyomas [8, 55–58]. For the assessment of post-therapy surveillance of uterine sarcoma, CT and PET are useful to detect distant metastasis and local recurrence [59].

8.3.2 Endometrial Stromal Sarcoma (ESS)

Low-grade ESS, the second most common uterine malignant mesenchymal tumor, affects younger people than leiomyosarcoma: with mean age of 42–53 years or younger [6, 18]. Because it occurs sometimes in women in their 20s and 30s, accurate diagnosis is important to preserve fertility [18]. Its MR findings are variable from polypoid endometrial mass resembling endometrial polyps to extensive myometrial lesions with high signal intensity at T2-WI, often protruding from intramyometrial or parametrial veins [18, 32]. Because pathological gross findings are known to show three growth patterns within the myometrium, MR findings also reflect those tendencies, which were well-circumscribed nodular tumors, poorly demarcated nodules permeating to the myometrium, and diffusely thickened myometrium

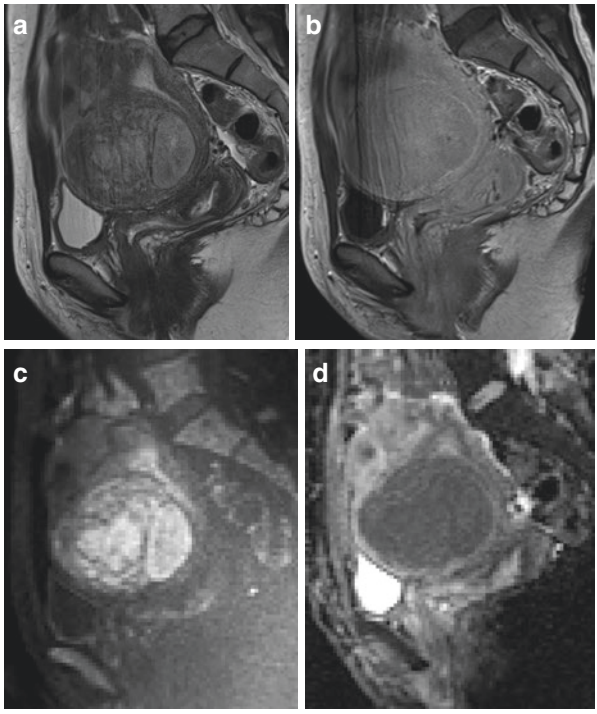

Fig. 8.5 (**a**) Sagittal T2-weighted image, (**b**) sagittal contrast-enhanced T1-weighted image, (**c**) diffusion-weighted image, (**d**) ADC map. A case of low-grade endometrial stromal sarcoma. The tumor is located at lower portion of anterior wall of the uterine myometrium, and its margin is relatively well-demarcated. The characteristic imaging finding of bands of low signal is visualized on T2-WI and DWI. Significant restricted water diffusion by DWI and ADC map indicates malignant tumor

without definitive tumors [6, 60]. The characteristic imaging finding is known to be the presence of bands of low signal intensity within the area of myometrial invasion, corresponding to the bundles of preserved myometrial fibers on pathology [60] (Fig. 8.5). Therefore, this finding can be observed only in cases existing within the myometrium, not in cases of polypoid endometrial masses without any readily apparent myometrial invasion [60]. In cases with well-demarcated tumor, a characteristic low-intensity rim can be found on T2-WI [61]. This structure corresponds to fibrous tissue layers and/or a part of myometrium with decreased free water caused by tumor expansion [61]. Another characteristic finding is continuous extension of the tumor into adjacent structures along the fallopian tubes, the surrounding uterine ligaments, or parametrial veins [18, 60]. Tumor extension occurred through the uterine wall rather than through direct extension through the fallopian tube from the endometrial cavity. This finding is visualized clearly by DWI, even within the veins [60, 62].

8.3.3 Differentiation Between Degenerated Leiomyoma and Various Sarcomas

As described above, differentiation of uterine sarcomas from uterine leiomyoma, especially from degenerated leiomyoma, is always difficult in clinical practice. US

and CT are not helpful for that differentiation because of their low contrast resolution, except for detecting the presence of invasion into adjacent organs. Considerable overlap occurs even in the MR findings including signal intensity and enhancement characteristics of uterine sarcomas and degenerative/cellular leiomyomas [56]. This background might lead to unexpected uterine sarcomas in cases treated by morcellation.

Morphologically, ill-defined margins with distortion of the uterine architecture and presence of nodular borders are important findings [63, 64]. As for the signal intensities on MR imaging, combined assessment of several sequences such as T1-WI, T2-WI, and DWI are necessary for differentiation. One objective criterion reported in the literature is the presence of intratumoral hemorrhage within necrotic foci, demonstrated as hyperintense signal areas on T1-WI with central unenhanced area(s) on contrast-enhanced T1-WI, reflecting pathological coagulative necrosis within the tumor [55, 64]. Regarding T2-WI and DWI, some researchers have tried to produce a decision tree to find uterine sarcoma from other uterine mesenchymal tumors [8, 57]. When we see uterine myometrial tumors, high signal intensity on T2-WI is the first step to suspect uterine sarcoma and degenerated/cellular leiomyoma [8, 55, 57, 58]. In other words, ordinary leiomyoma always shows distinct low signal intensity on T1 and T2-WI [8, 57]. After excluding ordinary leiomyoma, tumors with low signal intensity on DWI with b-value of 1000 s/mm^2 can be diagnosed as degenerated leiomyoma [8, 34, 57]. Degenerated leiomyoma shows either high or low signal intensity on DWI depending on the degree of degeneration and the nature within the tumor, such as edema and myxoid change. However, sarcoma always shows high signal intensity on DWI because of increased cellularity of the tumor [8, 34, 57]. The next step to consider is ADC values, but it is still in discussion. Its cutoff value is variable depending on the report. Thomassin-Naggara et al. demonstrated that an ADC value lower than 1.23 was predictive of malignancy, with a positive predictive value of 92%. Namimoto et al. reported that a value of less than 1.05 will be the threshold [57, 58]. Tamai et al. described that "The ADC values of uterine sarcomas were lower than those of degenerated leiomyomas without any overlap; however, they were overlapped with those of cellular leiomyomas" [8]. Recent progress of mathematical analysis of imaging has developed a new evaluation factor to quantify tumor heterogeneity [65]. Texture analysis extracts local variations within pixel intensities using mathematical methods to visualize information related to the tumor microenvironment [65]. Significantly more heterogeneity was found in leiomyosarcoma than in uterine leiomyomas [64].

Another technique for differentiation is fluorine 18 (^{18}F) fluorodeoxyglucose (FDG)-PET. FDG-PET generally reveals malignant lesions because of their higher glucose-accumulating metabolism, but glucose also accumulates in uterine leiomyoma, especially in premenopausal women [66]. Recently, PET with 16a-18F-fluoro-17b-estradiol (18F-FES), the most bioactive type of estrogen, became available for assessing the presence of regional estrogen receptor (ER) expression in normal organs or within diseased organs and tumors [67, 68]. Because the expressions of ER and progesterone receptors (PRs) were found to be significantly fewer in uterine leiomyosarcoma than in leiomyoma, 18F-FES accumulation is weaker in uterine

sarcomas than in uterine leiomyoma [67, 68]. In contrast, significantly higher uptake for FDG was found in patients with sarcoma than in patients with leiomyoma [67, 68]. Combined assessment of FDG and FES findings might lead to accurate diagnosis of uterine malignancy [67, 68].

8.4 Adenomyosis

8.4.1 Imaging Findings of Adenomyosis

Adenomyosis is a common condition sometimes encountered at the time of another imaging examination of the objective without symptoms. The role of imaging is to make a correct diagnosis, to ascertain the extent and depth of myometrial penetration, and to monitor the treatment response. The initial imaging modality for diagnosing adenomyosis is transvaginal sonography. On ultrasound, adenomyosis is visualized as decreased echogenicity or heterogeneity of the myometrium [69, 70]. Pathologically, the decreased echogenic area corresponds to smooth muscle hyperplasia. The heterogeneous area corresponds to a mixture of echogenic endometrial tissue and surrounding hypoechoic smooth muscle [3]. Almost half of the cases had accompanying myometrial cysts, which are hemorrhagic foci or dilated cystic glands within the heterotopic endometrial tissue [3]. Those cysts are usually small, less than 5 mm, but sometimes extensive hemorrhage occurs and produces large cystic spaces.

MR imaging is also an accurate, noninvasive modality for diagnosing adenomyosis with high sensitivity (78–88%) and specificity (67–93%). These diagnostic values are almost the same as those of ultrasound [71–73]. On MR imaging, adenomyosis is visualized as an ill-defined low-signal-intensity area on T2-WI. It can be recognized as diffuse or focal widening of JZ. In cases of maximal JZ thickness of 12 mm or greater, adenomyosis was diagnosed with high accuracy. Occasionally, a low signal area accompanies embedded bright foci on T1 and T2-WI. Pathologically, areas of low signal intensity on T2-WI correspond to smooth muscle hyperplasia, and bright foci on T2-WI represent islands of ectopic endometrial tissue and cystic dilatation of glands [4, 74, 75] (Fig. 8.6). Although the endometrium within adenomyosis only contains the basal layer of the endometrium, adenomyosis exhibits a functional response to ovarian hormones [6]. When hemorrhage occurs within these ectopic endometrial tissues, embedded foci of high signal intensity can be observed on T1-WI images [74]. In rare cases, extensive hemorrhage occurs. It is visualized as blood-filled cystic spaces on MR [76]. This condition, called adenomyotic cyst, might be difficult to differentiate from hemorrhage of leiomyoma [76, 77]. Pseudowidening of the endometrium, which is visualized as linear striations of increased signal intensity radiation from endometrium to myometrium on T2-WI, is another characteristic finding of adenomyosis [3] (Fig. 8.7). These striations represent direct invasion of the basal endometrium into the myometrium. Sometimes we encounter adenomyosis localized at the posterior subserosal area without affecting the uterine inner layer on MR images. Adenomyosis of these types is regarded as

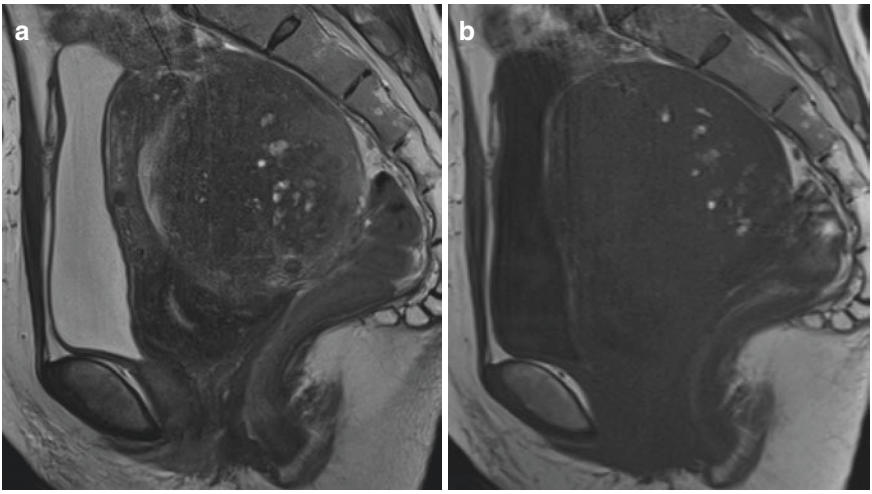

Fig. 8.6 (**a**) Sagittal T2-weighted image, (**b**) sagittal T1-weighted image. Typical case of adenomyosis. The posterior wall of the uterus is diffusely thickened and shows low signal intensity on T2-WI. Small embedded bright foci on both T1-WI and T2-WI are visualized indicating hemorrhagic endometrial tissue

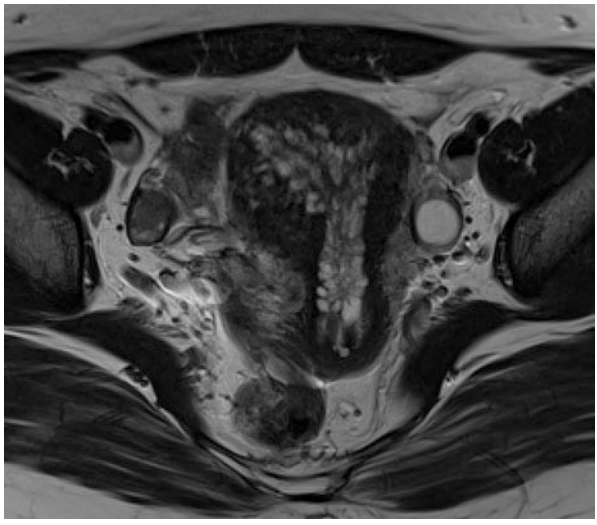

Fig. 8.7 Axial T2-weighted image of a case of adenomyosis with pseudowidening of the endometrium. Pseudowidening is characteristic finding of adenomyosis, representing direct invasion of the basal endometrium into the myometrium

invading from pelvic endometriosis and as a variant of pelvic endometriosis [78, 79]. These types of adenomyosis are often accompanying posterior cul-de-sac endometriosis (92.2%), and ovarian endometrioma (66.7%), all of which represent pelvic endometriosis [79]. It is suspected that posterior cul-de-sac endometriosis induces utero-rectal adhesion (i.e., posterior cul-de-sac obliteration) and then invades anteriorly into the uterus [79, 80].

The appearance of adenomyosis might change after hormonal therapy using GnRH agonist. In patients responding well to GnRH agonist therapy, demarcated changes of the lesion can be observed, resembling a leiomyoma [81]. Those cases with demarcated changes tend to accompany high-signal-intensity foci on T2-WI before therapy [81]. These high signal spots representing ectopic endometrial gland resolved after the therapy in most of the cases [81]. Decidualization of the endometrium within adenomyosis might occur in some cases [82, 83]. On MR imaging, bright foci within the lesion are more dilated than the nonpregnant condition on T2-WI caused by decidual reaction of endometrial tissue [83]. After childbirth, adenomyosis shows hemorrhage, probably attributable to the rapid decrease of large amounts of blood flow [83]. It is suspected that adenomyosis might be in a more or less ischemic condition, in contrast to normal myometrium, which recovered its blood flow naturally.

Adenocarcinoma can arise from endometrial tissue of adenomyosis, although it is rare. Reports of imaging findings are few and are not specific from a discrete mass to irregularly shaped lesions on T2-WI [84, 85]. Clinically, it is not easy to find a tumor during the early stage because of the lack of invasion of eutopic endometrium [84]. Therefore, MR imaging including DWI might facilitate early detection of the tumor.

8.4.2 Pseudolesions

Two normal conditions are similar to adenomyosis on MR imaging: the uterus in menstrual phase and myometrial contraction [84]. Because the uterus in those conditions shows strong contraction squeezing out the blood, the uterus shows a focal low-signal-intensity area similar to JZ thickening on T2-WI [86, 87]. In the menstrual phase, especially just after the start of bleeding, 60–80% of women show thickening of JZ focally or diffusely, involving more than half of the myometrium [86]. Sustained myometrial contraction is observed as transient focal masses of low signal intensity on T2-WI, bulging into the endometrium and continuing for several minutes [87]. Therefore, they can be differentiated by checking the menstrual cycle or transient change of uterine myometrial morphology in other sequences during the examination.

8.4.3 Unusual Growth Patterns of Adenomyosis

Adenomyoma is circumscribed nodular mixing of smooth muscle, the endometrial gland, and stroma [6]. It might be located within the myometrium or might grow as a polyp [6]. On MR imaging, it shows a well-demarcated tumor with small or large cavities caused by hemorrhage [88]. When adenomyoma manifests as a polypoid mass within the endometrial cavity, it is called an adenomyomatous polyp or polypoid adenomyoma, included among endometrial polyps pathologically.

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Role of Epithelial-Mesenchymal Transition in Human Adenomyosis: A New Insight into Its Pathogenesis

9

Khaleque N. Khan

Abstract

The exact pathogenesis of adenomyosis is still elusive. Among different reported concepts, direct invagination of gland cells from basalis endometrium deep into myometrium is the most widely accepted opinion in the development of adenomyosis. To address the mechanistic basis of this accepted concept, we investigated the role of hepatocyte growth factor (HGF) and estrogen in the occurrence of epithelial-mesenchymal transition (EMT) in human adenomyosis. Biopsy specimens from endometrium to myometrium were collected after hysterectomy from women with and without adenomyosis. The relationship between HGF and E-cadherin (epithelial cell marker)/N-cadherin (mesenchymal cell marker) was examined using endometrial epithelial cells (EECs) and tissues by qRT-PCR and immunohistochemistry. The gene and protein expressions of two transcriptional repressors of E-cadherin, SLUG and SNAIL, were examined using Ishikawa cells. HGF downregulated *E-cadherin* and upregulated *N-cadherin* mRNA expression in EECs, and an inverse relationship between HGF and E-cadherin was observed in basalis endometria of women with adenomyosis. HGF induced morphological changes and promoted migration of EECs. Ishikawa cells exhibited upregulation of *SLUG/SNAIL* gene expression in response to HGF and estrogen with an additive effect between them. HGF- and estrogen-promoted *SLUG/SNAIL* gene expression was significantly abrogated after pretreatment of cells with anti-HGF antibody or ICI 182720, an estrogen receptor antagonist. Our findings suggested that HGF either alone or in combination with estrogen may be involved in gland invagination deep into myometrium by inducing EMT in women with adenomyosis.

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_9

Keywords
Adenomyosis · Basalis endometrium · HGF · EMT · Pathogenesis

9.1 Introduction

Adenomyosis is defined by the intramyometrial presence of gland and stroma derived from basalis endometrium surrounded by reactive hyperplastic or hypertrophic myometrium causing either focal or diffuse enlargement of the uterus [1, 2]. Adenomyosis occurs most likely during the fourth and fifth decades of life and after the completion of childbearing activity. However, recent development of diagnostic tools such as transvaginal ultrasonography and magnetic resonance imaging has disclosed that adenomyosis may occur in women of younger ages. The differences between endometriosis and adenomyosis are origin of endometrium (functionalis or basalis) and anatomic site of the lesion (outside or inside of the uterus) [2].

The clinical symptoms of adenomyosis are dysmenorrhea, menorrhagia, chronic pelvic pain, and subfertility and need proper treatment. Hysterectomy is the definitive treatment. In cases in which adenomyosis is located focally in either one of the uterine wall, uterine-preservation surgery such as adenomyomectomy is an alternative surgery that is applicable to those who desire to preserve their reproductive potential [3].

The exact physiopathology and pathogenesis of adenomyosis are still elusive. According to published literatures, there are different proposals for the pathogenesis of adenomyosis, such as de novo metaplasia of Müllerian remnants, invagination through vascular or lymphatic channel, and mechanical tissue injury or stress reaction at the endo-myometrial interface [1, 2, 4] (Fig. 9.1). The most widely accepted

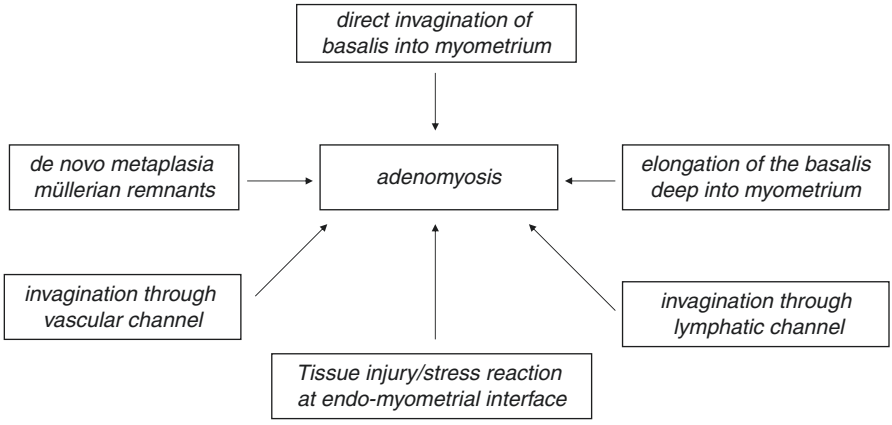

Fig. 9.1 Represents different concepts on the pathogenesis of human adenomyosis

opinion is that adenomyosis develops as a downgrowth and invagination of the basalis endometrium into the myometrium [1]. However, a possible mechanism of gland invagination from basalis endometrium deep into underlying myometrium is unknown. Moreover, information on the biological differences between functionalis and basalis endometria, the two compartments of human endometrium, is still lacking.

Epithelial-mesenchymal transition (EMT) and its converse, mesenchymal-epithelial transition (MET), are concepts first defined by Elizabeth Hay some 45 years ago [5]. Once epithelial cells become competent to respond to EMT-inducing signals, these signals can promote disruption of the intercellular adhesion complexes and loss of the apicobasal polarity of the epithelial cells, a prime feature crucial for cells to leave the epithelium and achieve migration potentiality. Since, basalis endometrium and inner myometrium (junctional zone) are closely apposed without any intervening basement membrane, events of EMT might occur here.

E-cadherin is a multigene family of transmembrane glycoproteins and normally acts as an "adhesive zipper" that means E-cadherin maintains tight cell-cell or cell-matrix contact for epithelial cells [6]. It has been demonstrated that epithelial cells, potentially invasive in human endometriosis, lack the expression of E-cadherin [7]. Studies with breast cancer, bladder cancer, and hepatoma cells indicated that overexpression of hepatocyte growth factor (HGF) may downregulate cadherin-mediated cell-cell adhesion with consequent migration and invasion into mesenchymal cells [8]. Recent reports demonstrated that estrogen-induced epithelial-mesenchymal transition (EMT) of epithelial cells may contribute to the development of adenomyosis or may be involved in metastatic potential of cancer cells [9, 10]. As an estromedin and pleiotropic growth factor, we speculated that similar to estrogen, HGF might play a similar role in EMT and in the invasion of gland cells into myometrium in women with adenomyosis.

A number of transcriptional repressors of E-cadherin such as SLUG (a Snail-related zinc-finger transcription factor), SNAIL (another Snail-related zinc-finger transcription factor), SIP-1/Zeb-2, E12/E47, and Twist have been reported [10–12]. They are found to interact with the proximal E-boxes of E-cadherin promoter and induce DNA hypermethylation or histone deacetylation thereby causing decreased expression of E-cadherin [11, 12]. An inverse correlation between E-cadherin and SLUG/SNAIL expression has been described in different cell systems [10–12]. Ectopic expression of SLUG/SNAIL in epithelial cells caused EMT by the aid of migratory and invasive behaviors [11, 12]. The expression pattern of SLUG/SNAIL in epithelial cells in response to HGF and the additive effect between HGF and estrogen on their expression are not clearly described. We performed a series of experiments to establish that as an estromedin (regulated by estrogen) and pleiotropic growth factor, HGF might play a role in inducing EMT, and if it is true, HGF-induced EMT might be involved in the pathogenesis of adenomyosis.

9.2 Biological Differences Between Functionalis and Basalis Endometria

While endometriosis originates from the functionalis endometrium, origin of adenomyosis is from the basalis endometrium. Therefore, it is important to know the biological/functional differences between these two compartments of endometrium. Basalis endometrium can be distinguished from functional endometrium by defined histological criteria: (1) constant appearance of basalis throughout the menstrual cycle, (2) glands of basalis appear weakly proliferative, (3) basalis cells lack secretory change and the stroma is spindled and non-decidualized, and (4) 1 mm area from the endo-myometrial junction as an additional criteria independent of menstrual cycle [13, 14].

Considering biological differences, ER and PR expression was equally observed in gland cells and stromal cells of both functional endometrium and basalis endometrium. While there was no remarkable difference in the expression of ER and PR in these two layers of endometrium in the proliferative phase, a weak expression of ER and PR was observed in the basal layer during the secretory phases of women with adenomyosis and control women [15]. A strong expression of Ki-67 was observed in functionalis endometrium of proliferative phase and a weak expression in basalis endometrium throughout the menstrual cycle. The TUNEL-positive apoptotic cells were least in the proliferative phase, modest in the secretory phase, and highest in the menstrual phase as observed in the functionalis endometrium. A few TUNEL-positive apoptotic cells were observed in the basalis endometrium. A similar pattern of activated caspase-3 immunoexpression was found in the functionalis and basalis endometrium throughout the menstrual cycle [15]. These findings confirmed that the two compartments of endometrium are biologically different. A mixture of blood and tissues from the necrotic functionalis endometria are the constituents of the antegrade flow of menstruation. The surviving basalis endometria consequently may suffer a constant tissue stress reaction in response to cyclic breakdown of functionalis endometria.

9.3 Stress Reaction at the Endo-myometrial Interface

When we consider cyclic shedding of functionalis endometrium, cyclic tissue breakdown during menstrual phase may induce a constant stress reaction on or cause tissue injury at the endo-myometrial junction. In fact, as a marker of tissue stress reaction, we previously demonstrated higher expression of human heat shock protein 70 (HSP70) in eutopic/ectopic endometria and peritoneal fluid of women with endometriosis during the secretory phase and the menstrual phase of the cycle [16, 17]. Increased tissue expression of stress-reactive proteins including HSP70 may also occur at the endo-myometrial junction as a result of repeated curettage after abortion or after multiple pregnancies that have been traditionally considered as high-risk factors for adenomyosis [1].

By immunohistochemical analysis, we observed a strong immunoexpression of HSP70 in basalis endometrium (gland+stroma) as well as in inner myometrium in women with adenomyosis than in control women. A similar moderate to strong HSP70 expression was found in glands cells and myometrium of both diffuse and focal adenomyotic lesions. A significantly higher quantitative-histogram (Q-H) score of HSP70 immunoreaction was found at the endo-myometrial junction (EMJ) of women with adenomyosis than in control women and in adenomyotic lesions of women with diffuse adenomyosis than in focal adenomyosis [18]. This indicates that an area of endo-myometrial interface may suffer from a constant tissue stress reaction in women with adenomyosis.

9.4 Effect of HGF on Basal EECs and E-Cadherin/N-Cadherin Expression

Expression of c-Met, a receptor of HGF, in endometrial epithelial cells (EECs) has already been described in a separate study [19]. A cell separation/scattering effect on cytokeratin-positive pre-confluent EECs was observed after treatment with HGF (at 50 and 100 ng/mL). A time-dependent increase in the mRNA expression of *E-cadherin* in basal EECs (HGF-untreated) was found with maximum gene expression at 24 and 48 h. This could be associated with cell-cell contact of EECs in response to overexpression of *E-cadherin.* Treatment with HGF on pre-confluent EECs significantly decreased mRNA expression of *E-cadherin* (epithelial cell marker) and induced concomitant increase in the mRNA expression of *N-cadherin* (mesenchymal cell marker) [18]. These differential expressions of E-cadherin and N-cadherin with consequent cell separation are the basic features of HGF-induced EMT. In fact, downregulation of E-cadherin resulting in the disruption of cell-cell contact is one of the components of EMT.

9.5 Expression of HGF, E-Cadherin/N-Cadherin in Functionalis and Basalis Endometria

A similar pattern of immunoexpression of HGF and E-cadherin was observed in gland cells of both functionalis and basalis endometria derived from control women. While no staining of N-cadherin was observed in either of these two layers, Vimentin (another mesenchymal cell marker) expression was increased. The protein expressions of HGF/E-cadherin/N-cadherin/Vimentin were equally observed in the functionalis layer of the endometrium derived from women with diffuse adenomyosis. However, a stronger expression of HGF and mild expression of E-cadherin was found in the basalis endometrium of women with diffuse adenomyosis. Comparing to expression of N-cadherin, a stronger expression of Vimentin was observed in functionalis/basalis endometrium [18].

A similar pattern of immunoexpression of HGF and E-cadherin was observed in the functionalis and basalis endometria derived from women with focal adenomyosis. While no difference in the expression of HGF and E-cadherin was observed in the functionalis and basalis endometria of contralateral side (no lesion) and functionalis layer of ipsilateral side (with lesion), a decreased expression of E-cadherin and moderate expression of HGF was observed in the basalis endometrium of ipsilateral side. That was confirmed by Q-H score analysis that showed a significantly more decreased expression of E-cadherin in the basalis layer than in the functionalis layer of the endometrium on the ipsilateral side [18]. It was interesting to observe that inverse relationship between HGF and E-cadherin was lost once adenomyotic lesion develops. This might be due to the hyperplastic or hypertrophic changes of surrounding smooth muscle cells causing realignment of migrating endometrial cells into glandular structure.

A constant tissue stress insult as manifested by higher expression of HSP70 in the basalis endometrium or in the inner myometrium may trigger the very early switch of EMT by higher expression of HGF and consequent lower expression of E-cadherin in the basalis endometrium. In fact, biological events of EMT at tissue level and morphological recognition of adenomyosis by image are different. We clinically diagnose adenomyosis by subjective and objective findings of each patient. Therefore, failure to diagnose adenomyosis by image cannot exclude the absence of biological events (stress reaction/EMT) at the tissue level. A silent variable degree of cyclic tissue stress reaction may occur at the endo-myometrial junction even in control women. Some of these women may time-dependently develop adenomyosis to be recognized either by histology or by image. We believe that the cause-effect of EMT in adenomyosis is still an open question for future investigation.

9.6 Tissue Concentration of HGF at the Endo-myometrial Junction

We collected tissue specimens from endo-myometrial interface after hysterectomy of women had operated for uterine myoma (control) and for focal and diffuse adenomyosis. These tissue specimens were cut into minute pieces and homogenized using Polytron homogenizer and tissue concentration of HGF was measured by ELISA. A significantly higher tissue content of HGF was found at the endo-myometrial interface derived from ipsilateral side of focal adenomyosis and anterior/posterior walls of diffuse adenomyosis when compared with similar tissues derived from control women or from contralateral endometrium of focal adenomyosis [18]. The gland cells, stromal cells, smooth muscle cells of the endometrium and myometrium as well as different immune cells have been reported to produce and secrete HGF in tissues and body fluids of women with different reproductive diseases [20, 21].

9.7 Effect of HGF/Estrogen on Changes in Cell Morphology and Cell Migration

We examined two other key cellular components of EMT in response to HGF and E_2 such as cell migration and cell morphological change. Boyden's chamber assay revealed that HGF was able to significantly migrate EECs from upper chamber to lower chamber with a dose of both 50 and 100 ng/mL. The expression of ER and PR in endometrial and endometriotic cells has been described previously [22, 23]. In addition to significant single effect of E_2, an additive between HGF and E_2 was observed in cell migration. This effect of HGF and E_2 on cell migration was significantly abrogated after pretreatment of EECs with anti-HGF antibody or ICI 182720, an ER antagonist. Treatment with non-immune mouse IgG + HGF did not influence this migration. We found that HGF (100 ng/mL) induced a sequential and time-dependent change in EECs morphology, from cobblestone-like appearance (at 0 h) to a mesenchymal phenotype (elongated spindle-shaped cells) at 48 h [18]. Estrogen had similar effect on morphological change of EECs. These findings give us further information that in addition to function as estromedin growth factor (regulated by E_2) [22] or as a pleiotropic growth factor [24] in endometriosis, HGF also exhibits its potential capacity to induce EMT in adenomyosis.

9.8 Effect of HGF and E_2 on SLUG and SNAIL Expression in Ishikawa Cells

Ishikawa cells are a well-differentiated endometrial adenocarcinoma cell line that retains phenotype of endometrial epithelial cells and displays apical adhesiveness and a similar expression profile of different molecules to endometrium, under the control of estrogen and progesterone [25, 26]. In fact, different transcriptional repressors of E-cadherin (SLUG/SNAIL/Twist) are known to express in Ishikawa cells [27]. We used Ishikawa cells to investigate expression pattern of SLUG and SNAIL in response to HGF and estrogen. Both basal and estrogen (E_2)-stimulated and ER-positive Ishikawa cells displayed c-Met (HGF receptor) expression. Individual treatment of Ishikawa cells with HGF and E_2 upregulated mRNA expression of *SLUG* and *SNAIL* comparing to HGF/E_2-untreated cells. In addition to individual significant effect, an additive effect between HGF and E_2 was observed in the overexpression of *SLUG* and *SNAIL* mRNA. The individual effect of HGF and E_2 was more prominent in the expression of *SLUG* gene comparing to *SNAIL* gene. HGF- and E_2-promoted *SLUG* and *SNAIL* mRNA expression were significantly suppressed after pretreatment of cells with anti-HGF antibody or ICI 182720, an ER antagonist [18].

9.9 Expression of E-Cadherin and SLUG/SNAIL in Functionalis and Basalis Endometria

A similar pattern of E-cadherin and SLUG immunostaining was observed in both functionalis and basalis endometria derived from contralateral side (no lesion) and in functionalis endometrium derived from ipsilateral side (with lesion) of focal adenomyosis. In contrast, a mild expression of E-cadherin and moderate expression of SLUG was found in the basalis endometrium derived from ipsilateral side (with lesion) of focal adenomyosis [18]. SNAIL immunostaining was not observed in the functionalis or basalis endometria of either side of focal adenomyosis. Comparing to functionalis layer, a significantly decreased Q-H scores of E-cadherin was observed in the basalis layer with higher expression of SLUG in the ipsilateral side [18]. Similar to HGF and E-cadherin expression, no obvious difference in the expression of E-cadherin and SLUG was observed in gland cells of adenomyotic lesions. These results demonstrated that overexpression of SLUG with consequent downregulation of E-cadherin in response to HGF and estrogen may be involved in the disruption of cell-cell contact. This HGF-mediated cell separation effect together with increased cell migration/invasion and cell morphological change in the endometrium may be responsible for the gland invagination deep into the myometrium in women with adenomyosis.

9.10 Summary and Perspective

We demonstrated here that there are some fundamental biological differences between functional endometria and basal endometria of women with adenomyosis. Less ovarian steroid response and less apoptosis of cells of basal endometria indicate that there is substantial survival of cells in this compartment of endometria across the phases of the menstrual cycle. When there is mechanical tissue injury or tissue stress reaction at the endo-myometrial interface, these surviving cells of basal endometria may escape deep into myometrium resulting in the development of adenomyosis.

In an attempt to explore the mechanistic basis of this phenomenon, here we demonstrated a possible involvement of HGF-induced EMT in the pathogenesis of adenomyosis. A tissue stress insult may explain higher expression of HSP70 in the basalis endometrium or in the inner myometrium and may trigger the very early switch of EMT by higher expression of HGF and consequent lower expression of E-cadherin in the basalis endometrium. Unlike all previous studies, we used isolated epithelial cells and full thickness endometrium to myometrial tissues derived from women with both focal and diffuse adenomyosis after hysterectomy.

We found that higher tissue concentrations of HGF at the endo-myometrial interface triggered a sequential cascade of EMT such as cell migration and cell

morphological changes (EECs) to a mesenchymal phenotype. Our findings of overexpression of HGF (EMT-inducing signal) and downregulation of E-cadherin with consequent disruption of tight cell-cell contact of EECs and resulting migration of EECs may be the basic components in the event of EMT in adenomyosis. The effect of HGF may be further supported by the action of local estrogen. Here we found that less expression of E-cadherin in response to HGF was well corresponded with migration through porous filters of EECs. Previous reports calculated the invasion capacity of EECs in relation to their motility taking into account that invasion also depends on motility [28, 29].

The event of HGF in EMT was mediated by upregulation of SLUG/SNAIL (transcriptional repressors of E-cadherin) in Ishikawa cells and downregulation of E-cadherin, an epithelial cell marker, and upregulation of N-cadherin/Vimentin, two mesenchymal cell markers. According to published literatures, lower tissue expression of E-cadherin and increased tissue expression of Vimentin or N-cadherin dictates a transitional cascade from an epithelial cell phenotype to mesenchymal cell phenotype [8, 21]. In other words, a phenomenon of EMT that was reported to be involved in the metastatic invasion of cancer cells [8] might also work in the pathogenesis of adenomyosis. We found that HGF either alone or in combination with estrogen was able to induce morphological changes of EECs from cobblestone appearance to spindle-shaped fibroblast-like cells. The functional specificity of HGF and E_2 was confirmed by their neutralizing effect on EECs motility. These findings give us further information that in addition to function as estromedin growth factor (regulated by E_2) [22] or as pleiotropic growth factor [23] in endometriosis, HGF also exhibits its potential capacity to induce EMT in adenomyosis.

It was interesting to observe that inverse relationship between HGF and E-cadherin was lost once adenomyotic lesion develops. This might be due to the hyperplastic or hypertrophic changes of surrounding smooth muscle cells causing realignment of migrating endometrial cells into glandular structure. A diagrammatic representation of the cascade of EMT in response to HGF and estrogen that may result in the development of adenomyosis is shown in Fig. 9.2.

We conclude that similar to estrogen [9], HGF may be involved in gland invagination deep into myometrium by inducing EMT at the endo-myometrial junction in women with adenomyosis. Since there is no basement membrane in between basal endometrium and inner myometrium (EMJ), the process of EMT can be better initiated here in response to tissue stress insult and/or local tissue inflammation and higher tissue content of HGF. Estrogen-induced EMT was found to be abrogated after treatment with raloxifene, a selective estrogen receptor modulator [9]. The findings of our current hypothesis warrant further investigation to examine the pattern of changes in the biological events of EMT in adenomyosis after treatment with estrogen-suppressing agent. Further studies are needed to strengthen our current findings.

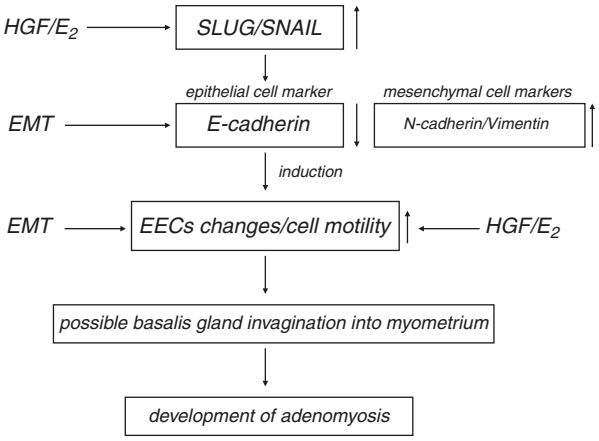

Fig. 9.2 A diagrammatic representation showing hepatocyte growth factor (HGF)- and estrogen (E_2)-induced pathways in the occurrence of epithelial-mesenchymal transition (EMT) in human adenomyosis. Upregulation of *SLUG and SNAIL*, two transcriptional repressors of E-cadherin, in response to HGF and E_2 is associated with decreased expression of E-cadherin (epithelial cell marker) and increased expression of N-cadherin/Vimentin (mesenchymal cell markers) causing disruption of tight cell-cell contact. These cellular events in endometrial cells and in intact tissues trigger morphological changes of endometrial epithelial cells (EECs) to a mesenchymal phenotype and induced increased cell migration, two essential components of EMT that was induced by HGF and E_2 either alone or in combination. All these events of EMT may be involved in the invagination of glandular epithelial cells from basalis endometrium deep into the myometrium and finally result in the development of adenomyosis

Acknowledgment This work was supported in part by Grants-in-Aid for Scientific Research (grants 21592101 and 24592474) from the Japan Society of the Promotion of Science (to K.N.K.).

I gratefully thank Dr. Akira Fujishita and Dr. Koichi Hiraki of Saikeikai Nagasaki Hospital and Dr. Michio Kitajima of Nagasaki University Hospital for their kind assistance in sample collection and Prof. Masahiro Nakashima for his experimental advice. K.N.K. designed and supervised the project and edited/wrote the complete draft.

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Adenomyosis and Subfertility

10

Yasushi Hirota and Yutaka Osuga

Abstract

Although it had been controversial whether adenomyosis causes infertility, current better imaging techniques have enabled nonsurgical diagnosis of adenomyosis and the studies investigating relationship between adenomyosis and subfertility. Recent studies focusing on the relationship between adenomyosis and subfertility have indicated that adenomyosis is associated with infertility and poor pregnancy outcome. Here we presented the current evidence suggesting the effects of adenomyosis on infertility and other pregnancy outcome.

Keywords

Adenomyosis · Infertility · Implantation · Miscarriage · Cytoreductive surgery

10.1 Introduction

Uterine adenomyosis is defined as a gynecological disease with the presence of ectopic endometrial epithelium and stroma in the myometrium, and its strict diagnosis is based on histological examination. Previous studies based on histological examination using hysterectomy specimens showed that adenomyosis is observed more often in multiparous than nulliparous women [1]. From these studies, the connection between adenomyosis and subfertility was not fully revealed. Thus, it has

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_10

been controversial whether adenomyosis causes infertility. Recent advancement of imaging technology such as ultrasonography and magnetic resonance imaging has enabled nonsurgical diagnosis of adenomyosis. According to these current better imaging techniques, clinical diagnosis of adenomyosis can be easily made in the patients who want to conceive. Thus, advancement of imaging techniques has improved our ability to identify the presence of adenomyosis and to investigate a possible association with infertility. Recently, both magnetic resonance imaging and ultrasound are noninvasive tests with equivalent accuracy in diagnosing adenomyosis [2]. According to the lifestyle change in women of today, more women tend to want first pregnancy in their late thirties to forties when the symptoms of adenomyosis often appear and adenomyosis is often observed in women who wants first baby. Due to these social settings, adenomyosis patients concurrently with subfertility may be increasing. Although no direct epidemiologic evidence about the relationship between adenomyosis and subfertility is present, there were several recent reports focusing on this issue, indicating that adenomyosis occurs even in young women and is associated with both pelvic endometriosis and infertility [3–8]. In this chapter, we have described the current evidence suggesting the role of adenomyosis on subfertility.

10.2 The Presence of Adenomyosis Is Associated with Infertility

T2-weighted MR image of the uterus in women of reproductive age detects three different layers: the endometrium with a signal of high intensity, the intermediate subendometrial area with a signal of low intensity called junctional zone (JZ) myometrium, and the outer myometrium with a medium-signal intensity [9]. The junctional zone myometrium lacks a recognizable protective layer or membrane and imposes endometrial glands into direct contact with the outer myometrium. The thickness of JZ myometrium in women with healthy uterus is ≤5 mm, and the myometrial thickness in adenomyosis patients is significantly increased [10, 11].

A recent literature has proposed the classification of simple JZ hyperplasia and adenomyosis according to the thickness of JZ (zone thickness ≥8 mm but <12 mm, and ≥12 mm, respectively) [12], although this classification remains to be officially discussed. From the recent common viewpoint, JZ thickness greater than 12 mm could be utilized as the basis for adenomyosis diagnosed by MRI [13]. Importantly, JZ thickness was the best predictive factor of repeated implantation failure in women with IVF [14], suggesting that adenomyosis may impair embryo implantation in IVF cycles. Supporting to this notion, a systematic literature review and meta-analysis showed that women with adenomyosis had a significant reduction in rates of clinical pregnancy and live birth, and a significant increase in first trimester miscarriage rate on IVF/ICSI outcome compared to

Table 10.1 Clinical outcome in IVF/ICSI patients undergoing frozen embryo transfer with and without symptomatic adenomyosis at our IVF center

	Symptomatic adenomyosis		
	+	–	*P* value
Number of patients	94	1211	–
Number of transferred embryos	122	1380	–
Age of patients	37.6 ± 3.8 (mean ± SD)	37.6 ± 3.9 (mean ± SD)	N.S. (Student's *t* test)
Implantation rate	10.7% (13/122)	21.4% (295/1380)	*<0.05* (Chi-squared test)
Clinical pregnancy rate	7.4% (7/94)	23.1% (280/1211)	*<0.05* (Chi-squared test)
Miscarriage rate	71.4% (5/7)	27.9% (78/280)	*<0.05* (Chi-squared test)

Data were retrospectively collected from the patients who underwent frozen embryo transfer at the IVF center in the University of Tokyo Hospital from 2008 to 2015. *N.S.* not significant

those without adenomyosis [15]. In our unpublished observation, symptomatic adenomyosis patients undergoing frozen embryo transfer (FET) reduced both rates of clinical and ongoing pregnancy and increased miscarriage rate compared to patients undergoing FET without symptomatic adenomyosis (Table 10.1). Taken together, there might be a strong association between adenomyosis and implantation/pregnancy outcome in IVF/ICSI patients. It remains unclear what kinds of adenomyosis have stronger association with implantation failure and early miscarriage.

10.3 Adenomyosis Is Also Linked to Perinatal Complication

As mentioned above, recent clinical studies indicate an increase of implantation failure and early miscarriage in the presence of adenomyosis [16]. In addition to these findings, recent three retrospective studies indicate the relationship between adenomyosis and perinatal outcomes [17–19]. A nested case-control study evaluated specifically the risk of preterm birth [17], and two cohort studies by Japanese groups investigated the risk of poor pregnancy/perinatal outcomes in women with adenomyosis [18, 19]. Summary of these studies is demonstrated in Table 10.2. All the studies indicated a heightened risk of preterm delivery in adenomyosis patients [17–19]. Moreover, women with adenomyosis are at an increased risk of second trimester miscarriage, small-for-gestational age, preeclampsia, fetal malpresentation, placental malposition, and postpartum hemorrhage [18, 19]. To date, however, there are no large studies investigating the influence of adenomyosis on perinatal complications, and further accumulation of data is required to reveal this issue.

Table 10.2 Adenomyosis and risk of perinatal complications

Reference	Study description	Higher risk in pregnant women with adenomyosis
Juang et al. [17]	Nested case-control study Preterm delivery cases: 104 (those with adenomyosis: 16) Term delivery cases: 208 (those with adenomyosis: 19)	Preterm premature rupture of the membrane (adjusted OR 1.98, 95% CI 1.39–3.15) Preterm birth (adjusted OR 1.84, 95% CI 1.32–4.31)
Mochimaru et al. [18]	Retrospective cohort study Adenomyosis cases: 36 Non-adenomyosis cases:144	Small-for-gestational age (OR 4.3, 95% CI 1.8–10.3) Fetal malpresentation (OR 4.2, 95% CI 1.6–10.8) Preterm premature rupture of membranes (OR 5.5, 95% CI 1.7–17.7) Preterm birth (OR 5.0, 95% CI 2.2–11.4) Postpartum hemorrhage (OR 6.5, 95% CI 2.2–19.0) Cesarean delivery (OR 4.5, 95% CI 2.1–9.7)
Hashimoto et al. [19]	Retrospective cohort study Adenomyosis cases: 49 Non-adenomyosis cases: 245	Second trimester miscarriage (OR 11.2, 95% CI 2.2–71.2), preeclampsia (OR 21.0, 95% CI 4.8–124.5) Placental malposition (OR 4.9, 95% CI 1.4–16.3) Preterm birth (OR 3.1, 95% CI 1.2–7.2) Cesarean delivery (OR 4.0, 95% CI 1.9–8.6)

10.4 Possible Mechanisms of Adenomyosis-Related Infertility

It is speculated that the presence of adenomyosis leads to dysfunction of myometrium and endometrium, which must be the cause of adenomyosis-related infertility. However, its detailed mechanism remains unknown.

As for myometrial dysfunction caused by adenomyosis, disturbance of uterine peristalsis has been proposed. Uterine peristaltic activity is created from the JZ myometrium [20]. Normal architecture of the JZ myometrium is impaired in women with adenomyosis. Thus, the myometrium with hyperplastic adenomyosis lesion might cause dysfunctional uterine hyperperistalsis with increased intrauterine pressure [21]. During luteal phase during the menstrual cycle, uterine peristalsis is reduced perhaps due to embryo attachment and invasion to the endometrium [22]. Sperm transport into fallopian tubes, which is a fundamental reproductive function, is regulated by uterine peristalsis, and this function might be disturbed in the presence of adenomyosis. Uterine JZ hyperperistalsis in embryo transfer is linked to a decreased pregnancy rate [23]. Taken together, it is likely reasonable to suppose that disturbance of uterine peristalsis may affect fertility in patients with adenomyosis.

As endometrial dysfunction due to adenomyosis, altered endometrial receptivity has been proposed. The expression of leukemia inhibitory factor (LIF), an essential cytokine for embryo attachment onto the endometrium [24], is reduced

in the endometrium in women with adenomyosis compared to those without adenomyosis [25]. Expression of HOXA10, a homeobox transcriptional factor essential for embryo implantation [26], is also decreased in the endometrium of women with adenomyosis compared to fertile controls [27]. These findings suggest the impaired endometrial receptivity in women with adenomyosis. Altered endometrial steroid metabolism, abnormal inflammatory response, and excessive oxidative stress condition are reported in the endometrium in women with adenomyosis [13, 28]. The molecular and cellular environment required for successful implantation might be disrupted by adenomyosis-related molecular/cellular changes such as chronic inflammation, although future investigation is necessary to clarify these details.

10.5 Medical and Surgical Treatment for Adenomyosis-Associated Infertility

Several medical treatments for adenomyosis are reported, but those are mainly against menstruation-related symptoms such as dysmenorrhea and heavy menstrual bleeding. The use of progestins including a levonorgestrel-releasing intrauterine system, oral contraceptive pills, and danazol can temporarily improve these symptoms in the course of the treatment. However, there are very few reports showing therapeutic effects of these drugs for infertility. A study with small numbers of cases showed the treatment of an intrauterine device containing danazol resulted in successful conception of infertile patients [29]. In contrast, there are some reports showing infertile women achieving pregnancy after GnRH analog treatment for adenomyosis [30–33]. GnRH agonist can temporarily lead to regression of adenomyosis lesion, which may improve the implantation environment. Notably, a recent study showed that 2-month GnRH analog pretreatment improved rates of implantation, clinical pregnancy, and ongoing pregnancy in adenomyosis patients undergoing frozen embryo transfer [34]. Another study also showed that pretreatment with GnRH agonist before frozen embryo transfer resulted in a trend of high pregnancy rate in infertile patients with adenomyosis [35]. These studies indicate a possible benefit of GnRH analog pretreatment before frozen embryo transfer in infertile patients undergoing IVF/ICSI, although it remains unclear which types of adenomyosis can receive favors from GnRH analog.

Another option for adenomyosis patients with infertility may be cytoreductive surgery. Under the open or laparoscopic approach, adenomyosis lesions were dissected out as much as possible, and the remaining myometrium were used to reform the uterus. In order to resect the lesion, transverse, longitudinal, wedge-shaped, and transverse H-shaped incisions of the uterus were conducted according to the size and location of the lesion [36]. To restore the thickness of the reformed myometrium, closure techniques including double- and triple-flap methods were proposed [37, 38]. A combined medical treatment with cytoreductive surgery was applied in several studies [39–41]. A retrospective study performed by Kishi et al. showed that there was no apparent advantage of the surgery on fertility outcome

Table 10.3 Pregnancy outcome of adenomyosis patients after cytoreductive surgery in the previous retrospective studies

Reference	*N*	Follow-up length after surgery	Clinical pregnancy		IVF/ICSI pregnancy		Miscarriage	
Fujishita et al. [36]	11	23–69 months	6/11	(55%)	N/A		0/6	(0%)
Takeuchi et al. [39]	8	N/A	2/8	(25%)	N/A		0/2	(0%)
Wang et al. [40]	71	24 months	55/71	(78%)	N/A		6/55	(11%)
Wang et al. [43]	28	36 months	13/28	(46%)	N/A		4/13	(31%)
Al Jama [41]	18	4–30 months	8/18	(44%)	0/8	(0%)	2/8	(25%)
Osada et al. [38]	26	>24 months	16/26	(62%)	12/16	(75%)	2/16	(13%)
Huang et al. [45]	9	62–83 months	3/9	(33%)	6/9	(67%)	1/3	(33%)
Kishi et al. [42]	102	9–60 months	42/102	(41%)	27/42	(64%)	10/42	(24%)
Saremi et al. [46]	70	20–50 months	21/70	(30%)	14/21	(67%)	4/21	(19%)
Total	343	N/A	166/343	(48%)	59/96	(61%)	29/166	(17%)

Data above were cited by the previous retrospective studies in which pregnancy outcome of adenomyosis patients with cytoreductive surgery were evaluated. *N/A* not applicable

for patients over the age of 40 [42]. Another study performed by Wang et al. showed high delivery rate in the surgery group compared to GnRH analog group (33% vs. 8%) [43]. Laparoscopic surgery of adenomyosis resection might be proper for women with focal adenomyosis who failed infertility treatments including assisted reproductive technology [44]. Cytoreductive surgery in adenomyosis patients with infertility might have obvious benefits for not only reducing symptoms but also for improving the pregnancy outcome [41, 43]. Pregnancy outcome after cytoreductive surgery for adenomyosis is demonstrated in Table 10.3. This table covers the previous retrospective studies of women who wanted to conceive after cytoreductive surgery for adenomyosis [36–43, 45, 46]. Thirty-nine percent of the adenomyosis patients conceived without IVF/ICSI treatment after surgery, whereas 41% of them conceived through ICSI/IVF. In total cases of Table 10.3, 166 pregnancies and 29 miscarriages were displayed in 343 women with adenomyosis (clinical pregnancy rate 48%, miscarriage rate 17%). Generally speaking from these data, the pregnancy outcome after conservative cytoreductive surgery seems reasonably good, but further prospective studies are needed to elucidate the usefulness of adenomyosis cytoreductive surgery as a fertility treatment. In addition, the incidence of uterine rupture and placenta accrete in the pregnancy after the surgery remains unclear.

Conclusion

Adenomyosis is still a common gynecological disease with unclear pathogenesis and pathophysiology. Recent studies have demonstrated that the presence of adenomyosis may not only impair implantation due to myometrial and endometrial dysfunction but also damage later pregnancy outcome. Cytoreductive surgery for adenomyosis may be effective in women with adenomyosis with infertility. However, most of the current studies about adenomyosis are retrospective and insufficient, and better studies are required for understanding of the impact of adenomyosis on women suffering from subfertility.

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Surgical Treatments for Adenomyosis

11

Mari Kitade, Makoto Jinushi, Shinichiro Ikuma, Keisuke Murakami, Rie Ozaki, Ayako Masuda, Keiji Kuroda, and Jun Kumakiri

Abstract

The objective of this prospective study performed at Juntendo University School of Medicine was to evaluate a novel method of laparoscopic adenomyomectomy (LA). The subjects were 76 women with adenomyosis who wished to conserve fertility.

Two methods of LA and hysteroplasty were used: wedge resection (WR) and the double flap method (DF). WR was performed on 22 women with exteriorly growing focal adenomyosis close to the serosal membrane, and DF was performed on 54 women with adenomyosis growing interiorly close to the endometrium. WR was performed by making a V-shaped notch to remove the adenomyotic nodule and surrounding serosa with a electric cautery. The remaining muscle layer was sutured so that hysteroplasty could be performed. For the DF procedure, after a transverse incision, the adenomyotic nodule was removed, with the remaining serosal tissue serving as the upper and lower flaps, which were overlapped and sutured.

For the operative outcome in the WR and DF groups, the average surgical duration was 118.6 ± 43.3 min and 144.0 ± 44.5 min, and the estimated blood loss was 172.1 ± 175.2 mL and 245.3 ± 232.3 mL, respectively. The visual

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_11

analog scale of dysmenorrhea and hypermenorrhea was significantly decreased after surgery, and this trend continued for more than 3 years. Investigation by second-look laparoscopy showed that 3 out of 24 patients (12.5%) had de novo adhesion to the adnexa and 12 patients out of them (50%) had postoperative adhesions to the incision site of the uterus. All patients exhibited tubal patency with an indigo carmine solution used for chromotubation, but wound thinning was observed in only one patient by leakage of it. The postoperative pregnancy rate was 38.8% (12 out of 31 infertile patients), 73.3% (11 out of 15 pregnancies) of which resulted in live births. There were no severe complications during pregnancy and delivery.

In conclusion, LA was found to be safe and useful for minimally invasive surgery to conserve fertility.

Keywords

Adenomyomectomy · Laparoscopy · Conservative surgery · Focal adenomyosis · Laparoscopic adenomyomectomy

11.1 Introduction

Adenomyosis is a benign gynecological disease defined to invasion of ectopic endometrial glands and stroma into the uterine myometrium. Premenopausal women with adenomyosis typically suffer from dysmenorrhea, hypermenorrhea, chronic pelvic pain, and infertility.

Over two decades ago, the standard surgical treatment of adenomyosis is a hysterectomy. However with the changes in women's lifestyles, such as marrying later in life and having fewer children, adenomyosis is on the rise in unmarried and nulligravid women. A laparoscopic adenomyomectomy (LA) is minimally invasive and a conservative surgical option to preserve fertility for infertile women with disorder of implantation or previous miscarriage.

We demonstrated the details of perinatal complication due to adenomyosis experienced in our hospital in Fig. 11.1.

According to count, the majority of perinatal complications due to adenomyosis were premature birth, malpresentation, and PROM in our hospital. On the other hand, we should be careful of the uterine rupture during pregnancy as the postoperative complication [1].

11.1.1 Surgical Indication of LA

We show the option of the treatment for adenomyosis in Fig. 11.2.

LA is a palliative surgery for patients with focal adenomyosis who wish the conception, making it difficult to completely excise of adenomyosis; therefore, keeping the balance of radicality with fertility is difficult.

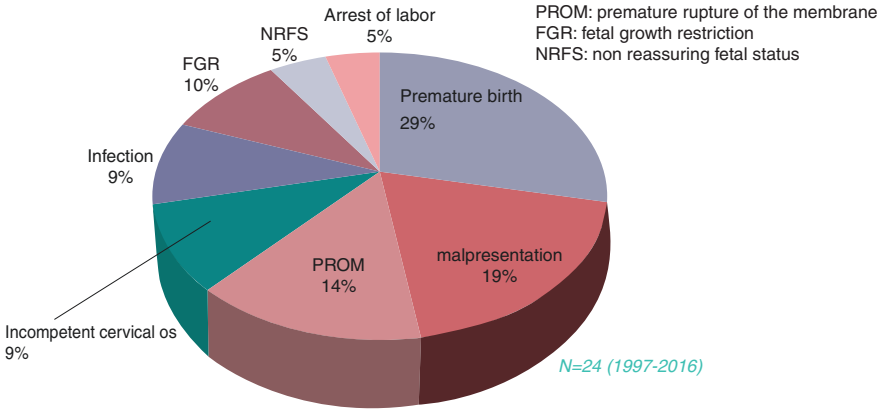

Fig. 11.1 The details of perinatal complication due to adenomyosis in our hospital

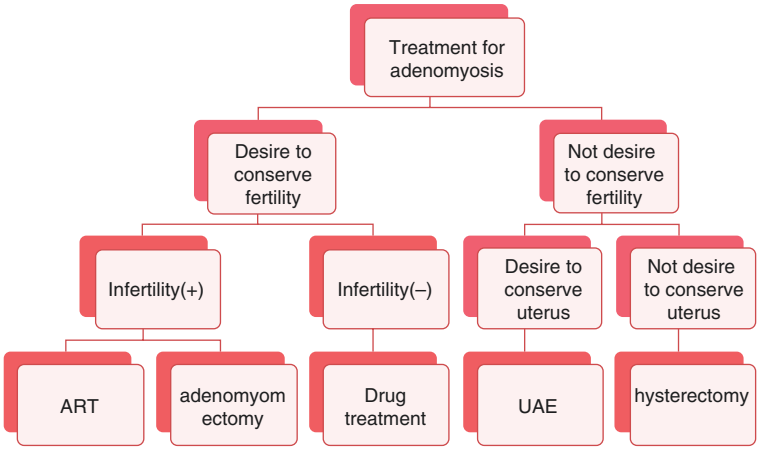

Fig. 11.2 The option of the treatment for adenomyosis. We usually apply the treatment for adenomyosis according to desire to conserve fertility or not, and infertility or not

Despite the surgical procedure of LA being highly difficult, there is also a high risk of recurrence and perinatal complications such as the postoperative premature birth or the uterine rupture. Thus, many surgeons often find it difficult to make a decision regarding the surgical indication. In our hospital, LA is often normally selected for infertile women whose symptoms due to adenomyosis cannot be controlled well, or uterine cavity is deformed, with a history of perinatal complication due to adenomyosis and increasing size of adenomyotic tumor rapidly in either surgical method of laparotomy or laparoscopy.

11.1.2 Two Types of LA Methods and Criteria for Selection

We demonstrate two types of laparoscopic LA for women with partial adenomyosis depending on development of adenomyotic lesion and aimed to evaluate the

efficacy of them. The focal type adenomyosis was classified into two types: exteriorly growing type where development of adenomyotic nodule occurred close to the serosal membrane and interiorly growing one where it appeared close to the endometrium.

The wedge resection method (WR) involves excision of the adenomyotic tissue together with the serosal membrane, and it is applicable to exteriorly growing type of adenomyosis. On the other hand, the double flap method (DF) is used for removing substantial tissue while preserving the normal muscle layer on the serosal site and is suitable for interiorly growing type adenomyosis (Fig. 11.3).

Because WR is the procedure used for removing adenomyotic tissue together with the surrounding serosal membrane and normal muscle layer, the extent of adenomyotic nodule excision is limited. On the other hand, the DF removes the adenomyotic tissue while the remaining serosal membrane and surrounding normal muscle layer serve as flaps to counteract the loss of myometrium. So we usually apply WR to exteriorly growing focal adenomyosis with a diameter of 5 cm or less and choose DF for interiorly growing one with a diameter under 8 cm.

Diffuse type of adenomyosis, which spans the entire uterus, was excluded as unsuitable for this procedure.

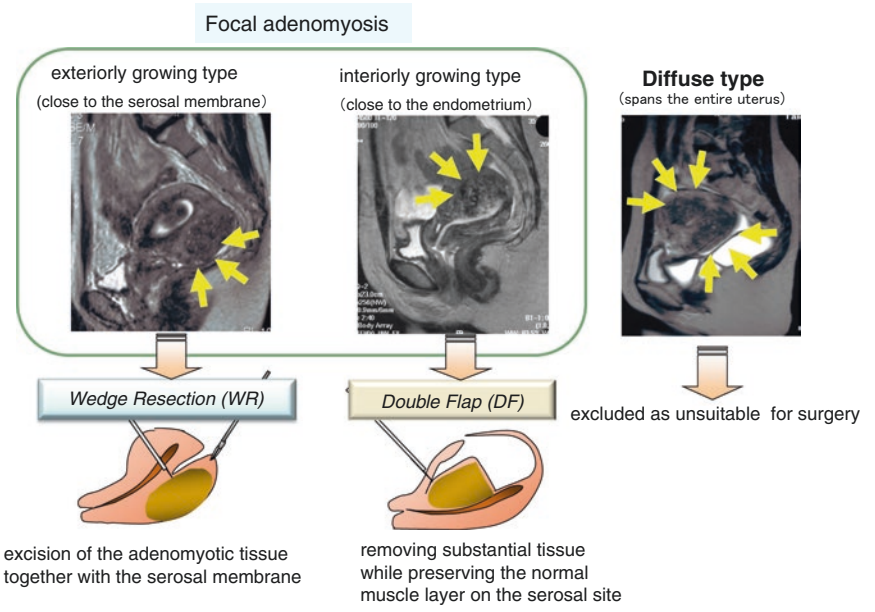

Fig. 11.3 Two types of LA methods and criteria for selection. WR is applicable to exteriorly growing focal adenomyosis with a diameter of 5 cm or less. In DF is suitable for interiorly growing one with a diameter under 8 cm. Diffuse type or larger one is not considered to perform laparoscopy

11.2 Surgical Procedure

Surgical preparation and commonality of two methods. The patients were placed in lithotomy, and the Uterine Manipulator (Ethicon, Tokyo, Japan) was replaced into the uterine cavity for mobilization of the uterus. After the pneumoperitoneum was established, a 11-mm scope was inserted periumbilically. Three additional puncture sites were made: two 5-mm sites 2 cm above the anterior superior iliac supine and a 12-mm site 3 cm above the umbilicus on the left anterior axial line. The operator stood on the patient's left side, controlling the 12-mm trocar with his right hand and manipulating the left lower 5-mm trocar with his left hand. The right lower 5-mm trocar was controlled by the assistant's right hand.

After observing the abdominal cavity, cystectomy was performed on the cases of adenomyosis complicated with endometrioma and lysis of adhesions, and the removal of deep endometriotic lesions around Douglas' pouch were performed for the complete cul-de-sac obliteration cases. Vasopressin 20 IU in 1 mL diluted 100 times with saline solution was infused into the adenomyotic tissue.

11.2.1 Wedge Resection (WR)

We apply wedge excision to adenomyosis 5 cm in diameter or less with outside spatial pattern. The adenomyotic nodule and sorounding serosa are removed by making a V-shaped notch with a electric cautery of monopolar needle or scissors. The remaining muscle layer was sutured so that hysteroplasty could be performed. Similar to LM (laparoscopic myomectomy), we begin to sew it up from the base of the muscularis which tore and sew it up in from two to four layers according to depth not to make dead space, but various invention is necessary for suture subsequently because it is hard to put myometrium unlike LM in the case of LA. We attach adhesion inhibitor on the uterus' wounded area after the saturation (Fig. 11.4).

11.2.2 Double Flap Method: (DF)

We add transverse incision in needle-formed monopolar from the top of adenomyosis to the limit reaching the endometrial cavity and resect a lesion of adenomyotic tissue as en bloc with the remaining serosal tissue serving as the upper and lower flaps, which were overlapped and sutured. Because normal muscularis remains in the serosa side in the case of inside spatial pattern, we leave this part and resect only an adenomyosis tissue. Left normal muscularis becomes the flap and can fill greatly defective normal muscularis. After excision of the adenomyotic tissue, any perforations to the endometrium were sewn up with 2/0 thread. The inner side of the lower serosal flap was sutured with 1/0 thread, and the upper fringe of the serosal flap was sutured to the muscle layer continuously. Likewise, the inner side of the upper serosal flap was sutured to the muscle layer, while overlapping with the lower serosal flap and the bottom fringe of the serosal flap, and lower serosal flap surface was continuously sutured to close the muscle layer incision [3].

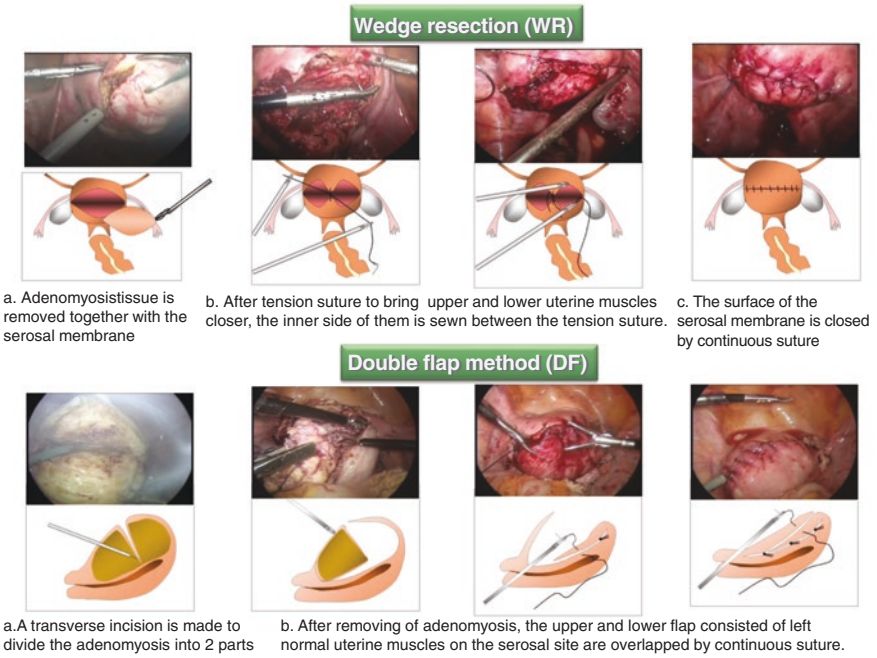

Fig. 11.4 Surgical techniques of two types LA. Wedge resection (WR): (**a**) adenomyosis is removed together with the serosal membrane. (**b**) Muscle layer was closed to each other by tension suture. (**c**) The inner side of muscle layer is sewn between the tension suture. (**d**) The surface of the serosal membrane is closed by continuous suture. Double flap method (DF): (**a**) a transverse incision is made to divide the adenomyotic region into two parts. (**b**) Adenomyosis is removed remaining the normal muscle layer on the serosal membrane side. (**c**) The upper and lower flaps consisting left normal muscularis are overlapped by continuous suture. Modified from [8]

11.3 Patient Characteristics

Seventy-six women who were diagnosed with adenomyosis underwent LA at our hospital during 2003 and 2013. In detail, WR was performed for 22 patients, and DF method was performed for 54 patients.

Each patient was sufficiently informed of all aspects of the trial before the surgery, and written consent was obtained. Additionally, this study was reported to and approved by the institutional review board.

Preoperatively, patients were diagnosed as having adenomyosis in a maximal junctional zone thickness >12 mm by MRI [2]. From past experience in laparoscopic myomectomy, the exclusion criteria are those with the size of over 80 mm in a low-intensity area or a uterine size equivalent to about 12 weeks of pregnancy.

The median age of the subjects was 36 years of age (range, 28–39 years). The details of past pregnancies were one case of spontaneous abortion, two cases of elective abortion, and one case of normal delivery. Symptoms included 14 cases of dysmenorrhea (100%), 8 cases of menorrhagia (57.1%), and 8 cases of infertility (57.1%). The details of infertility cases were five cases of primary infertility and three cases of secondary infertility. The median infertility period was 47 months (range 12–60 months).

The average of widest median diameter of adenomyosis measured by MRI was 47 mm (range 30–80 mm). The region of adenomyosis occurrence was the anterior wall for six cases and the posterior wall for eight cases.

59 cases were administered the GnRH agonist (leuprolide acetate1.88 mg, Takeda, Tokyo, Japan), and one case was administered dienogest, totaling ten cases with preoperative treatment.

Postoperative observations were made by MRI or second-look laparoscopy [3] to investigate wound healing and postoperative adhesion after adenomyomectomy.

SLL was performed for 24 patients who desired pregnancy in the future and the average of whose age was 35.4 ± 3.7.

11.4 Result

11.4.1 Operative Findings and Outcome

The details of complicated disease were as follows: endometrioma was 39.5% (30/76) and deep infiltlating endometriosis (DIE) was 43.4% (33/76). As the result of operative outcome in the WR group and the DF group, the average of surgical duration was 118.6 ± 43.3 (min) and 144.0 ± 44.5 (min), and the average of estimated blood loss was 172.1 ± 175.2, (mL) and 245.3 ± 232.3, respectively (Table 11.1).

The average of specimen in DF group was more heavy than the one in WR group comparing patients whose adenomyotic nodule was of the same size (Fig. 11.5).

No conversion to laparotomy or major complications occurred during surgery.

11.4.2 The Change of Symptoms

The postoperative improvement of symptoms indicated by changes in dysmenorrhea was evaluated using the visual analog scale (VAS). The visual analog scale of dysmenorrhea was significantly decreased from 9.3 (range, 9–10) before surgery to 3.5 (range, 1–6) after surgery, and this trend continued for over 3 years (Fig. 11.6).

All 45 patients with hypermenorrhea improved after surgery.

Table 11.1 Patient's background and operative findings

	WR (n = 22)	DF (n = 54)
Age (years)	35.8 ± 4.3	36.8 ± 3.8
Infertility (n)	9 (40.9%)	22 (40.7%)
Size of adenomyotic tumor (cm)	4.2 ± 0.9	5.0 ± 1.2
The level of CA125 (U/mL)	112.8 ± 120.4	209.9 ± 194.3
Administration of GnRHa (n)	18 (81.8%)	41 (75.9%)
Complication of endometrioma (n)	7 (31.8%)	23 (42.6%)
Complication of DIE (n)	11 (50.0%)	22 (40.7%)
r-ASRM	441 ± 48.1	40.1 ± 45.9
Surgical duration (min)	118.6 ± 43.3	144.0 ± 44.5
Estimated Blood loss (mL)	172.1 ± 175.2	245.3 ± 232.3

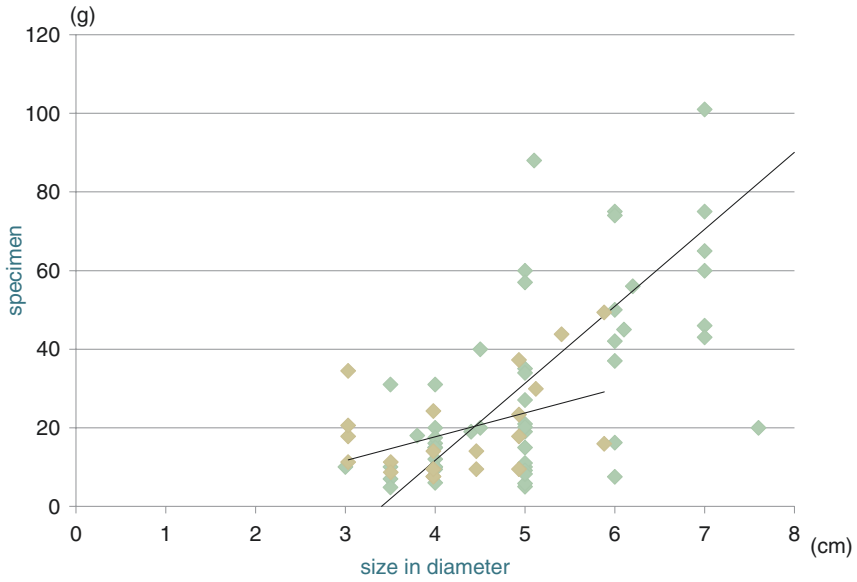

Fig. 11.5 The correlation between the size in diameter and specimen of adenomyosis. The average of specimen in DF group was more heavy than the one in WR group comparing patients whose adenomyotic nodule was of the same size

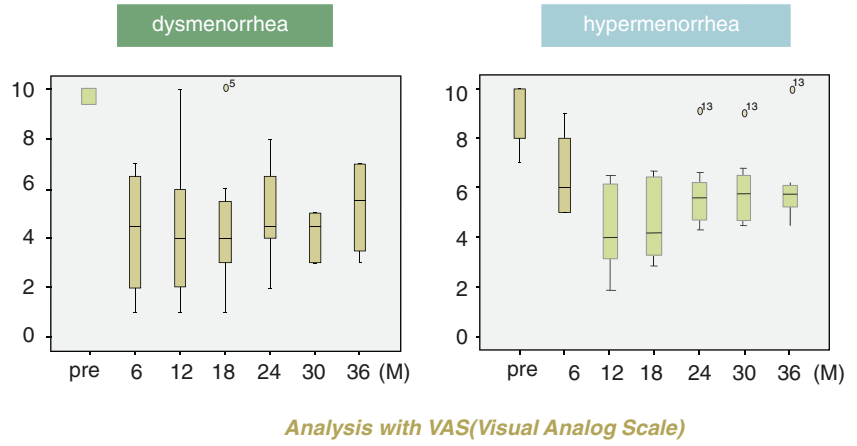

Fig. 11.6 Changes in dysmenorrhea and hypermenorrhea until 3 years postoperatively were evaluated using the VAS. Both decreased quickly after surgery and this trend continued for over 3 years

11.4.3 The Outcome of Second-Look Laparoscopy (SLL)

Three out of 24 patients (12.5%) had de novo adhesion to the adnexa and 12 out of them (50%) had postoperative adhesions to the incision site of the uterus.

All patients exhibited tubal patency, but wound thinning was only observed in one patient by leakage an indigo carmine used for chromotubation. The patient with

wound thinning underwent debridement and reconstructive surgery by laparoscopy.

Eight infertile patients (33.3%) conceived after SLL and the mean period until pregnancy (since performing SLL) was 15.6 months.

11.4.4 Postoperative Pregnancy Rate and Cumulative Pregnancy Rate

Twelve of the 31 infertile patients (38.8%) who underwent LA conceived and 85.7% of all these pregnancies were spontaneous pregnancies containing of timing method or IUI without ART. The mean period until pregnancy was 24.5 months, and three patients experienced repeated pregnancies.

The cumulative pregnancy rate was approximately 40% of patients who conceived 2 years after LA and approximately 60% of patients who got pregnant 3 years after surgery (Fig. 11.7).

11.4.5 Outcome of Pregnancy and Delivery

Although the count of postoperative pregnancy was 15 (from 12 pregnant women), only 73.3% of them (11/15) resulted in a live birth. The details of patients doing not well were two patients were stillborn and one experienced a miscarriage. There were no cases of uterine rupture or placenta accrete.

As a rule, planned cesarean sections are performed on post-LA patients; however, vaginal delivery was performed for two patients during the early period.

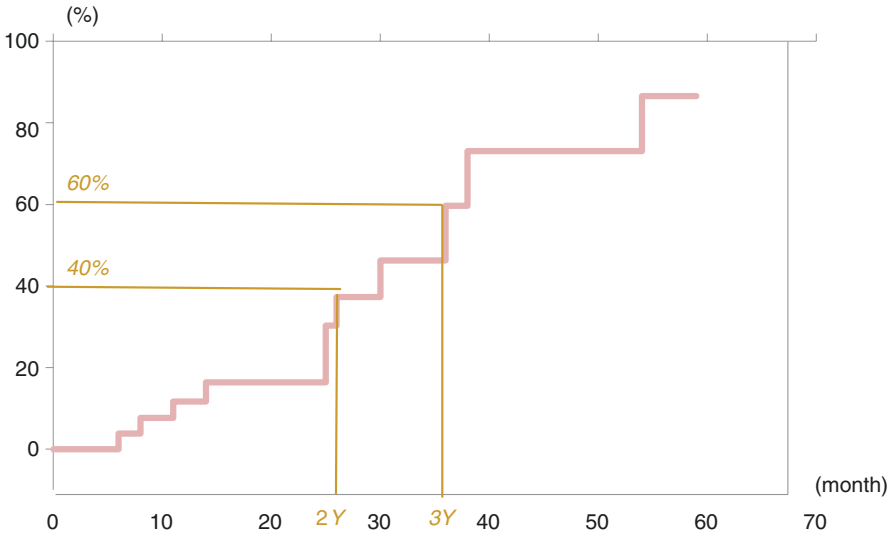

Fig. 11.7 Cumulative pregnancy rate after LA. At 2 years postoperatively, approximately 40% of patients had conceived and, at 3 years postoperatively approximately 60% of patients were pregnant

11.5 Discussion

Adenomyosis is a disorder often affecting woman in their late reproductive years who have already finished childbearing. Therefore hysterectomy has been a common surgical treatment. However, because of the recent trend of marrying later in life and having fewer children, the incidence of adenomyosis in unmarried and nulligravid women is increasing, and there is a higher demand for adenomyomectomy to conserve fertility and the uterus.

To be different from uterine fibroid, adenomyosis is a condition where the endometrial tissue has penetrated the uterine muscle layer and the boundary between the lesion and normal muscle layer is unclear. Therefore LA requires techniques for excising the adenomyotic tissue and reconstructing the remaining muscle layer after removing adenomyoma.

Adenomyomectomy is an operative procedure that requires time for the excision of adenomyotic tissue with unclear boundaries and the suturing process. Therefore controlling bleeding is an important factor. Preoperative administration of GnRH agonists to decrease uterine blood flow and vasopressin administration during the surgery is effective to control bleeding [4]. However, the boundary between adenomyotic tissue and the normal muscle layer may become even more unclear by administration of GnRHa; we usually use it only for cases of focal adenomyosis on the posterior uterine wall with suspicious existence of DIE.

We usually choose the surgical method according to the type classified by position of adenomyosis: exteriorly growing and interiorly growing one to conserve as much of the normal muscle layer as possible to avoid the postoperative uterine rupture during pregnancy. Besides it should be able to contribute to decrease the remnant of adenomyotic tissue.

We considered that WR removing adenomyotic tissue together with the surrounding serosal membrane is applicable to exteriorly growing type adenomyosis that the adenomyotic nodule occurred close to the serosal membrane but the extent of adenomyotic nodule excision is limited. On the other hand, DF is suitable for interiorly growing type adenomyosis that it appeared close to the endometrium while preserving the normal muscle layer on the serosal site as flaps to counteract the loss of myometrium.

The reports on LA are not so much; Wood [5] and Morita [6] reported for the wedge-shaped excision methods using laparoscopy, and Chung [7] were mentioned as robot-assisted one. Furthermore, laparoscopic DF was reported by Takeuchi [8] to modify the laparotomy techniques mentioned by Hyamus [9] and Osada [10]. After that, double or triple flap method for diffuse-type adenomyosis was reported by Huang [11].

LA requires time for adenomyoma removal and uterine reconstruction and is significantly more difficult to perform compared with laparoscopic myomectomy of similar size. However, we considered that with proficiency in the operative procedures, it is possible to operate on adenomyosis with a maximum size of 7–8 cm and uterine size equivalent to about 12 weeks of pregnancy.

As far as observing of above indication, our method for focal adenomyosis is effective and safe. There is no complication during surgery and related symptoms to adenomyosis like dysmenorrhea or hypermenorrhea have decreased considerably after surgery. Postoperative observations of the wound area by MRI and second-look laparoscopy have indicated that sufficient wound healing is possible even if the muscle layer is overlaid on the normal serosal membrane. In our cases, only one patient with wound thinning was observed by leakage an indigo carmine used for chromotubation at second look laparoscopy. She underwent debridement and reconstructive surgery by laparoscopy immediately.

Twelve of the 31 infertile patients (58.8%) who underwent LA conceived after surgery and 11 women (73.3%) out of them delivered normally in term. None of these cases experienced uterine rupture during pregnancy or delivery. But it is necessary to ensure that there is sufficient uterine wall strength to withstand pregnancy and labor after conservative surgery of uterus. This procedure should be attempted by surgeons with sufficient experience in laparoscopic myomectomy [12] or mastered suturing skill under laparoscopy.

Conclusion

LA was suggested to be safe and useful for minimally invasive surgery to conserving fertility and suffering from various related symptoms to adenomyosis. However unlike laparoscopic myomectomy, there are few reports of conservative LA and little data on indication and surgical performance. Further investigation on operative procedures and postoperative management of LA is necessary. Laparoscopic myomectomy is now widespread, and techniques for enucleation and suturing under laparoscopy are developing rapidly [13]. By applying laparoscopic myomectomy techniques, we believe we have established a safer and more effective technique for LA.

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Pregnancy Complications in Women with Adenomyosis

12

Hiroshi Tamura and Norihiro Sugino

Abstract

The risk of pregnancy complications in women with adenomyosis is debatable. However, only a limited number of studies on pregnancies in women with adenomyosis have been reported. Some control studies and a retrospective survey demonstrated that the risk of pregnancy complications, including miscarriage, preterm delivery, pregnancy-induced hypertension (PIH), fetal growth restriction (FGR), cervical incompetency, and uterine infection, was increased in pregnant women with adenomyosis. Although some studies have evaluated whether medication or surgical management can influence the pregnancy outcomes in women with adenomyosis, there is currently no evidence to suggest that medication or surgical intervention has any benefit in terms of the pregnancy complications in women with adenomyosis. The size and type of adenomyosis are important factors for pregnancy complications. We should be aware of the potential for pregnancy complications, especially in pregnant women with diffuse-type adenomyosis.

Keywords

Adenomyosis · Pregnancy complication · Miscarriage · Preterm delivery

12.1 Introduction

Uterine adenomyosis is defined as the presence of endometrial glands and stroma within the myometrium, and its reported prevalence in the literature shows extreme variation (14–66%) [1–4]. Based on past reports, the incidence of

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N. Sugino (ed.), *Uterine Fibroids and Adenomyosis*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-10-7167-6_12

pregnancy-associated adenomyosis appears to be 17% [5]. However, the frequency of adenomyosis-complicated pregnancy has been increasing in recent years due to improvements in imaging techniques and the growing number of women delaying their first pregnancy until their late thirties or early forties [6, 7]. Although poor pregnancy outcomes, including miscarriage and preterm delivery, have been recognized, only a limited number of studies have addressed the association between adenomyosis and pregnancy complications. Thus, the potential impact of adenomyosis on pregnancy outcomes remains unclear.

During pregnancy, the invasion of trophoblasts into the endometrium and the myometrial junctional zone causes decidualization and unique vascular changes [8]. The impaired decidualization of the myometrial spiral arteries is a predisposing factor for failed intravascular trophoblast invasion [9]. Defective deep placentation was reported to be associated with a spectrum of obstetric complications, including late miscarriage, preterm labor, fetal growth restriction, and preeclampsia [10]. Since the thickening and the disruption of the junctional zone appearance is strongly associated with uterine adenomyosis [8], adenomyosis might be related to placental insufficiency and poor placental perfusion.

Adenomyosis can be classified as focal and diffuse; focal lesions are localized in the anterior and posterior uterine walls, and diffuse lesions involve the entire uterus [11]. The type of adenomyosis is considered to be an important factor that affects pregnancy outcomes. In addition, it is unknown whether pretreatment for adenomyosis, including medication and conservative surgery before pregnancy, improves the outcomes of pregnancy.

This review aims to present and discuss the clinical and pathophysiological findings of adenomyosis-associated pregnancy complications in order to clarify the relationship between adenomyosis and pregnancy outcomes.

12.2 Adenomyosis and Fetal Loss

The impact of adenomyosis on miscarriage is controversial (Table 12.1); however, a systematic review and meta-analysis showed that women with adenomyosis had a significantly lower clinical pregnancy rate (relative risk [RR] 0.72, 95% confidence interval [CI] 0.55–0.95) and that their risk of miscarriage was increased twofold (RR 2.12, 95% CI 1.20–3.75) in comparison to women without adenomyosis [7, 12–19]. Recent reports demonstrated the high prevalence of second trimester miscarriage in pregnant women with adenomyosis [13, 18] (Table 12.1). In addition, a study based on an analysis of a Japanese survey reported that the incidence of cervical incompetency (5.3%, 14/262) was increased in pregnant women with adenomyosis [18]. It was significantly higher than the reported prevalence of cervical incompetency (1.2%, 2943/242,715) in Japanese women [20]. The close association between adenomyosis and miscarriage suggests that adenomyosis may be a risk factor for unexplained fetal loss.

Patients with adenomyosis also display high levels of inflammatory substances, such as prostaglandins, which have been suggested as a potential cause of uterine contraction [21]. Cyclooxygease-2 (COX-2), the rate-limiting enzyme that

Table 12.1 Studies addressing miscarriage in women with adenomyosis

Reference	Subjects	No. of subjects		Treatment	Miscarriage	
		Adenomyosis	Control		Adenomyosis	Control
Costello et al. 2011 [12]	Infertile women	37	164	IVF/ICSI	15.4% (2/13)	27.1% (16/59)
Mijatovic 2010 [15]	Infertile women	20	54	IVF/ICSI	36.4% (4/11)	46.7% (14/30)
Youm et al. 2011 [19]	Infertile women	136	302	IVF/ICSI	31.8% (21/66)	12.9% (29/224)
Martinez-Conejero et al. 2011 [14]	Infertile women	152	147	IVF/ICSI	32.8% (43/131)	16.3% (24/147)
Salim et al. 2012 [16]	Infertile women	19	256	IVF/ICSI	50% (2/4)	2.8% (3/108)
Tamura et al. 2017 [17]	Infertile women	535	–	ART or non-ART	29.8% (75/252)	Survey without control
Hashimoto 2017 [13]	Pregnant women	49	245	–	12.2% (6/49) (>12 weeks)	1.2% (3/245)
Tamura et al. 2017 [18]	Pregnant women	262	–	–	9.9% (26/262) (>12 weeks)	Survey without control

catalyzes the initial step in the formation of prostaglandins, has been shown to be overexpressed in adenomyosis [22]. Not only prostaglandins but also proinflammatory cytokines, including interleukin 1β (IL-1β), tumor necrosis factor-a (TNFa), and epidermal growth factor (EGF), are increased in the endometrium of women with adenomyosis [23, 24]. The chronic inflammation in pregnant women with adenomyosis may be a risk factor for fetal loss.

On the other hand, adenomyosis impairs the function of the myometrium. A recent report showed a significant increase in the myometrial stiffness estimated by shear wave elastography in patients with adenomyosis [25]. In fact, increased intrauterine pressure has been reported in pregnant women with adenomyosis [26, 27]. The uterine contractions caused by chronic inflammation and the high intrauterine pressure caused by the myometrial stiffness level may be possible factors that contribute to adenomyosis-related miscarriage and cervical incompetency.

12.3 Adenomyosis and Preterm Delivery

A nested case-control study by Juang et al. found a significant, nearly twofold increase in the risk of preterm delivery in women with adenomyosis [21]. The other study, a retrospective case-control study, indicated that there was an increased risk of preterm delivery (OR, 5.0; 95% CI 2.2–11.4) and preterm premature rupture of the membranes (PPROM) (OR, 5.5; 95% CI 1.7–17.7) [28] (Table 12.2). Recently, a high prevalence of preterm delivery was also reported in a case-control study of pregnant women with adenomyosis; the incidence of preterm delivery in the adenomyosis group (24.4%, 12/49) was higher than that in the control group (9.3%,

Table 12.2 Studies addressing pregnancy outcomes in women with adenomyosis

Author, year	Type of study	No. of pregnant women	Preterm delivery	PPROM	PIH	FGR (SGA)	Malpresentation	Others
Juang et al., 2007 [21]	Nested case-control	104 with preterm labor vs. 208 control (delivered after 37 weeks)	↑ OR 1.84 95% CI 1.32–4.31	↑ OR 1.98 95% CI 1.39–3.15				
Mochimaru et al., 2015 [28]	Retrospective cohort	36 with adenomyosis vs. 144 control (without adenomyosis)	↑ OR 5.0 95% CI 2.2–11.4 41.7% (15/36) vs. 12.5% (18/144) ($p < 0.001$)	↑ OR 5.5 95% CI 1.7–17.7 19.4% (7/36) vs. 4.2% (6/144) ($p = 0.01$)	→ OR 2.7 95% CI 0.4–17.2 5.6% (2/36) vs. 2.1% (3/144) ($p = 0.46$)	↑ OR 4.3 95% CI 1.8–10.3 33.3% (12/36) vs. 10.4% (15/144) ($p = 0.01$)	↑ OR 4.2 95% CI 1.6–10.8 27.8% (10/36) vs. 8.3% (12/144) ($p = 0.003$)	Postpartum hemorrhage ↑ OR 6.5 95% CI 2.2–19.0 25.0% (9/36) vs. 4.9% (7/144) ($p < 0.01$)
Hashimoto et al., 2017 [13]	Retrospective case-control	49 with adenomyosis vs. 245 control (without adenomyosis)	↑ OR 3.1 95% CI 1.2–7.2 24.4% (12/49) vs. 9.3% (23/245) ($p = 0.003$)		↑ OR 21.0 95% CI 4.8–124.5 18.3% (9/49) vs. 1.2% (3/245) ($p < 0.001$)	↑ OR 3.5 95% CI 1.2–9.0 20.9% (9/49) vs. 7.0% (17/245) ($p = 0.004$)	→ OR: NA 16.3% (8/49) vs. 8.9% (22/245) ($p = 0.120$)	Postpartum hemorrhage →
Tamura et al., 2017 [18]	Retrospective survey	262 pregnant cases without control	24.4% (64/262)	4.6% (12/262)	9.9% (26/262)	11.8% (31/262)		Cervical incompetency 5.3% (14/262) Uterine infection 7.3% (19/262)

PPROM preterm premature rupture of the membranes, *PIH* pregnancy-induced hypertension, *FGR (SGA)* fetal growth restriction (small for gestational age), *OR* odds ratio

23/245) (OR, 3.1; 95% CI 1.2–7.2) [13] and in a retrospective large-scale survey (24.4%, 64/262) [18] (Table 12.2).

Preterm delivery is often preceded by increased myometrial contractility, gradually elevated intrauterine pressure, and cervical effacement. Adenomyosis is associated with some biochemical pathways that lead to increased intrauterine pressure and result in preterm delivery. The presence of proinflammatory cytokines [23, 24], increased prostaglandin and COX-2 levels [21, 22], and increased myometrial stiffness have all been suggested as potential causes of increased intrauterine pressure [26, 27] and preterm delivery.

PPROM occurs in approximately 1–3% of all pregnancies and is associated with 30–40% of preterm deliveries [29, 30]. The high incidence of PPROM in pregnant women with adenomyosis was reported by Jung (OR 1.98, 95% CI 1.39–3.15) and Mochimaru (OR 5.5, 95% CI 1.7–17.7) (Table 12.2), and it is one of the important causes of preterm delivery [21, 28]. PPROM has been attributed to increased physical stress, which weakens the membranes. The high intrauterine pressure caused by increased prostaglandins and the degradation of the collagen of the membranes by inflammation [31] seem to be possible mechanisms of PPROM in pregnant women with adenomyosis.

12.4 Adenomyosis and Preeclampsia: Fetal Growth Restriction

Recent reports have demonstrated a significant relationship between the presence of adenomyosis and preeclampsia [13, 18] (Table 12.2). Preeclampsia is defined as new hypertension and substantial proteinuria at ≥20 weeks of gestation [32]. Although the cause of preeclampsia remains largely unknown, the leading hypotheses strongly rely on a disturbed placental function in early pregnancy [33]. The junctional zone is the inner 1/3 of the myometrium; it differs from the outer myometrium in its embryonic origin and functions [8, 34]. The disruption of the JZ prior to conception may have profound repercussions on deep placentation and subsequent pregnancy outcomes.

Adenomyosis is not a uniform disease; it represents a spectrum of lesions, ranging from the disruption of the junctional zone architecture with little or no endometrial invasion to overt diffuse adenomyosis and focal adenomyomas [8]. Since decidualization initiates some of the morphological changes in the spiral arteries of the junctional zone, the thickening and disruption of the junctional zone that appears in adenomyosis is strongly associated with impaired decidualization and defective deep placentation. Defective deep placentation was reported to be associated with a spectrum of obstetric complications, including late miscarriage, preterm labor, fetal growth restriction, and preeclampsia [10]. The deep defective placentation caused by failure in the remodeling of the spiral arteries in adenomyosis is considered to be an early defect that causes preeclampsia.

Pregnant women with adenomyosis have been reported to have an increased incidence of FGR [13, 18, 28]. A group of patients with adenomyosis was reported to

have a higher rate of FGR (33.3% vs. 10.4%; OR 4.3, 95% CI 1.8–10.8) in comparison to a control group [28]. The high incidence of FGR was also reported by Hashimoto (20.9% vs. 7.0%; OR 3.5, 95% CI 1.2–9.0) [13]. This evidence suggests that the presence of adenomyosis might be related to poor placental perfusion. The abnormal placentation in adenomyosis leads to reduced uteroplacental artery flow and irregular placental perfusion, resulting in fetal growth restriction. Yorifuji et al. [35] proposed another mechanism, in which the lesions of adenomyosis showed abundant blood flow while the placenta had diminished blood flow, suggesting that vascular steal by uterine adenomyosis was among the possible causes of FGR.

12.5 Adenomyosis and Uterine Infection

The functional changes that occur due to adenomyosis during pregnancy and after delivery include decidualization and hemorrhage within the lesion of adenomyosis. Since adenomyosis is characterized by chronic inflammatory disease, it could be responsible for severe infection and abscess formation within the adenomyotic foci [36, 37]. In fact, a case of a rapidly growing adenomyosis that was associated with preterm delivery and which involved postpartum abscess formation within the lesion was reported by Kim et al. [38]. Because the abscess formation was localized within the adenomyotic foci, antibiotic therapy was considered to be ineffective, and total abdominal hysterectomy was performed in these cases. A recent survey analyzing the relationship between adenomyosis and pregnancy complications showed an increase in the incidence of intrauterine infection (7.3%, 19 cases in 262 pregnancies) in pregnant women with adenomyosis [18] (Table 12.2). In two of the cases in the study, hysterectomy was necessary due to severe infection, including septic abortion, and postpartum abscess formation. Adenomyosis can be associated with inflammatory consequences, such as septic abortion, preterm delivery, and abscess formation in pregnant women.

12.6 Other Pregnancy Complications

The incidence of severe postpartum hemorrhage (defined as an estimated blood loss of ≥1000 mL in a vaginal delivery and ≥1500 mL in a cesarean section delivery) was reported to be high (25.0% vs. 4.9%; OR 6.5, 95% CI 2.2–19.0) (Table 12.2). It is possible that adenomyosis may disturb the postpartum uterine contractions.

Fetal malpresentation was significantly more common in patients with adenomyosis (27.8% vs. 8.3%; OR 4.2, 95% CI 1.6–10.8) than in a control group [28] (Table 12.2). The narrowing of the intrauterine cavity and decreased uterine extensibility is the presumed cause of the high incidence of fetal malpresentation in pregnant women with adenomyosis.

Only a limited number of studies have reported on the relationship between adenomyosis and the neonatal outcomes. The analysis of the neonatal outcomes revealed a significant difference in the median birth weight in an adenomyosis

group (2716 g) and a control group (2972 g) [13]. The median birth weight of an adenomyosis group was also reported to be low in comparison to controls (2463 g vs. 2946 g) [28]. There was no significant difference in the Apgar score at 5 min or the neonatal intensive care unit (NICU) admission rate between the adenomyosis and control groups [13, 28].

12.7 Pretreatment for Adenomyosis and Pregnancy Complications

Although substantial efforts have focused on improving the reproductive outcomes by pretreatment for adenomyosis, at present, there is no evidence to suggest the potential benefit of medication or surgical intervention in terms of the fertility prognosis [6]. Multiple treatment modalities including hormonal therapy with gonadotropin-releasing hormone (GnRH) agonists and conservative surgical procedures have been used to restore the fertility of women with adenomyosis; however, there is no agreement on the most appropriate therapeutic method for managing infertile patients with adenomyosis. A recent large-scale survey did not find any data suggesting that medication or surgery as a pretreatment for adenomyosis increased the pregnancy rate in infertile women [18]. No reports have evaluated whether medication or surgery, as pretreatments for adenomyosis, improve the pregnancy outcomes.

Some reports have shown successful delivery after when pregnant women with adenomyosis undergo long-term treatment with a GnRH agonist (3–6 months) before pregnancy [39, 40]. However, since available data on the relationship between GnRH agonist and pregnancy complications is still scant and limited to small-scale cases, the impact of GnRH agonist as a pretreatment for adenomyosis on pregnancy complications is still unclear.

Dienogest, a derivative of 19-norsteroid, is a novel progestin that is highly selective for progesterone receptors and which directly inhibited cellular proliferation and induced apoptosis in human adenomyotic stromal cells [41]. Further studies are required to evaluate whether dienogest improves the pregnancy outcomes in women with adenomyosis.

Successful pregnancy and deliveries after conservative surgery have been reported in women with adenomyosis [42–44]. The advantages of removing the affected area must be balanced against the disadvantages of leaving a possibly defective uterine wall. Thus, there is a recognized difficulty in establishing the optimum conservative surgical technique for adenomyosis, and several approaches have been proposed, including different operative options (open, laparoscopic), surgical techniques (adenomyomectomy, complete excision; partial adenomyomectomy, cytoreductive surgery), and modified surgical techniques (U-shaped suturing, overlapping flaps, triple-flap method, and transverse H incision) [44–47]. In addition, uterine rupture, which is a catastrophic obstetric complication, has been reported in pregnancies after conservative surgery [48, 49]. Since different facilities use different surgical techniques, it is difficult to analyze the association between the

reproductive outcomes and individual surgical techniques. A prudent argument is required to evaluate the involvement of each surgical technique in the complications of pregnancy and the risk of uterine rupture in women with adenomyosis.

12.8 The Type of Adenomyosis and Pregnancy Complications

Adenomyosis can be classified into two categories: focal adenomyosis, which is a restricted area of hypertrophic and distorted endometrium and myometrium, usually embedded within the myometrium; and diffuse adenomyosis, which is an extensive form of the disease, characterized by foci of the endometrial mucosa (glands and stroma) scattered throughout the uterine musculature [11]. In diffuse-type adenomyosis, the disruption and thickening of the junctional zone is observed in a large part of the uterus. Focal-type adenomyosis mimics a leiomyoma, showing a roughly spherical, intramural, space-occupying lesion; thus, the disruption and thickening of the junctional zone is limited to part of the junctional zone. It is possible that there are some differences in decidualization and deep placentation between diffuse-type and focal-type adenomyosis. These differences may influence the subsequent placental function and pregnancy complications. In addition to structural differences, functional differences between diffuse-type and focal-type adenomyosis have been proposed. The immune balance between regulatory T cells and helper T cells in the uteri of women with diffuse adenomyosis differed from those of women with focal-type adenomyosis [50].

Recently, Tamura et al. reported that the type of adenomyosis was associated with the types of pregnancy complications in a large-scale survey [18]. They found that the rate of PIH in cases involving diffuse-type adenomyosis (13.8%; 19 cases/138 pregnancies) was higher in comparison to cases involving focal-type adenomyosis (5.1%; 5 cases/99 pregnancies). The rate of intrauterine infection in cases of diffuse-type adenomyosis (10.9%; 15 cases/138 pregnancies) was higher than in those with focal-type adenomyosis (2.0%; 2 cases/99 pregnancies). Diffuse-type adenomyosis is associated with various findings, including the enlargement of the uterus involving the entire myometrium in the posterior and/or anterior wall and junctional zone thickening. Since the diffuse thickening of the junctional zone, as well as the whole myometrium, is a possible cause of the modification of the interface between the endometrium and myometrium, it is possible that impaired decidualization and defective deep placentation are more severe in cases of diffuse-type adenomyosis than in cases of focal-type adenomyosis.

12.9 Challenges and Future Directions

The association between adenomyosis and female fertility varies widely among individuals, because the conditions of the disease vary in size, type, localization, and severity. The presence of concomitant pathologic conditions, including leiomyoma (35–55%) and endometriosis (6–20%), might drastically influence the

fertility of women with adenomyosis [5, 26, 51, 52]. The chronic inflammation caused by endometriosis may be related to preterm delivery [53]. The presence of leiomyoma has been linked to an increased risk of spontaneous abortion, fetal malpresentation, placenta previa, preterm birth, cesarean section, and peripartum hemorrhage [54]. Since the prevalence of endometriosis in women with adenomyosis was reported to be high in the majority of the articles reporting on adenomyosis and fertility, the actual impact of adenomyosis on pregnancy outcomes is difficult to determine. Thus, women with endometriosis and leiomyoma should be excluded from studies which evaluate the impact of adenomyosis on pregnancy complications. A prospective and well-conducted randomized study is required to evaluate the true impact of adenomyosis on pregnancy outcomes.

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