

Molecular biomarkers for uterine leiomyosarcoma and endometrial stromal sarcoma

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Uterine leiomyosarcoma (u-LMS) and endometrial stromal sarcoma (ESS) are among the most frequent soft tissue sarcomas, which, in adults, lead to fatal lung metastases and patients have an extremely poor prognosis. Due to their rarity and heterogeneity, there are no suitable biomarkers for diagnosis and prognosis, although some biomarker candidates have appeared. In 2017, The Cancer Genome Atlas (TCGA) Research Network's work on u-LMS has confirmed mutations and deletions in *RB1*, *TP53* and *PTEN*. In addition, whole-exome sequencing of u-LMS has confirmed and demonstrated frequent alterations in *TP53*, *RB1*, *α-thalassemia/mental retardation syndrome X-linked (ATRX)* and *mediator complex subunit 12 (MED12)*. *MED12* is a useful biomarker to diagnose uterine-derived LMS and tumors arising from (LM) with a relatively favorable prognosis. *TP53* and *ATRX* mutations can be important mechanisms in the pathogenesis of u-LMS and are correlated with a poor prognosis. In an update based on the 2014 WHO classification, low-grade ESS is often associated with gene rearrangement bringing about the *JAZF 1-SUZ12* (formerly *JAZF1-JJAZ1*) fusion gene, whereas high-grade ESS is associated with the *YWHAE-NUTM* fusion gene. Low-grade ESS with *JAZF1* rearrangement may correlate with metastasis. However, high-grade ESS with metastasis with *YWHAE* rearrangement shows a relatively favorable prognosis. The genetic/molecular genetic aberrations in u-LMS and ESS are reviewed, focusing on molecular biomarkers for these primary and metastatic tumors.

KEYWORDS

endometrial stromal sarcoma, genetic aberrations, The Cancer Genome Atlas project, uterine leiomyosarcoma

1 | INTRODUCTION

Malignant soft tissue tumors (soft tissue sarcomas [STS]) are rare and diverse. Although they are rare in adults, accounting for $\leq 1\%$ of adult malignant tumors, they are known to account for 15% of pediatric malignant tumors.¹ These tumors originate in the bone and soft tissues (Clinically relevant molecular subtypes in Leiomyosarcoma muscle, fat and nerves) throughout the body, and ≥ 75 histological types have been identified. Soft tissue tumors in adults lead to fatal lung metastases in 30% of cases, and they have an extremely poor

prognosis (mean survival, 15 months). Due to the rarity and heterogeneity of these tumors, there are no suitable biomarkers and treatment methods, and the past few decades have not seen marked improvements in survival.²

Uterine leiomyosarcoma (u-LMS) and endometrial stromal sarcoma (ESS) are among the most frequent STS in adult women, accounting for 1%-2% of uterine malignant tumors, and their incidence tends to increase. LMS occurs frequently in 50-55-year-old women, while 15% of STS occur in women under the age of 40 years. ESS is classified into low-grade (LG-ESS) and high-grade

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(HG-ESS). LG-ESS frequently occurs before menopause. The prognoses of LMS and HG-ESS are extremely poor.³

With the recent developments in genomic analysis techniques, the pathogenesis of these tumors has been becoming clearer, and some biomarker candidates have appeared. In the present article, the development and pathological states of uterine sarcoma (u-LMS and ESS) are described based on findings from genomic analysis, and some molecular biomarker candidates for diagnosis and prognosis are discussed.

2 | UTERINE LEIOMYOSARCOMA

Soft tissue sarcomas have been traditionally classified by their location of occurrence. However, based on the development of genomic analysis in the last few years, a new concept has arisen that STS can be divided into 2 groups. One, which accounts for one-third of STS, is a relatively simple tumor and has a diploid karyotype with several chromosome abnormalities. The other, which accounts for two-thirds of STS, has a complicated karyotype, with instability of many genes and mutation of the *TP53* gene that encodes p53 in many cases;⁴ u-LMS is considered to belong to the latter group. Moreover, u-LMS demonstrates complicated tumor development and pathology that differs from LMS originating from other sites due to the involvement of estrogen.⁵

In 2017, The Cancer Genome Atlas (TCGA) Research Network performed a multiplatform molecular characterization of adult STS, including 53 soft tissue LMS and 27 u-LMS cases. They reported that sarcomas had low mutational burdens compared with other tumors in the TCGA projects. Moreover, in integrated and individual platform analyses, u-LMS and soft tissue LMS were generally more similar to each other than to other sarcomas. The analysis confirmed mutations and deletions in *RB1*, which encodes RB, *TP53* and *PTEN* (Figure 1).⁴ In addition, whole-exome sequencing of soft tissue LMS and u-LMS has confirmed and demonstrated frequent alterations in *TP53*, *RB1*, *α -thalassemia/mental retardation syndrome X-linked (ATRX)* and *mediator complex subunit 12 (MED12)*.^{6,7} Other shared features of LMS were elevated microRNA (miRNA)-143 and miRNA-145 expressions, low miRNA expressions of inflammatory response genes, and low leukocyte fraction on methylation analysis. In contrast, u-LMS and soft tissue LMS had significantly different methylation and miRNA expression signatures, with u-LMS showing a higher DNA damage response score and *hypomethylation of estrogen receptor 1 (ESR1)* target genes, while soft tissue LMS had a more prominent hypoxia inducible factor (HIF) 1 α signaling signature, suggesting that the use of different management approaches should be considered for u-LMS and soft tissue LMS due to the predicted differences in hormonal responsiveness and stress responses.⁴

2.1 | Whole molecular mechanisms of tumor development, metastasis and chemoresistance in leiomyosarcoma

To elucidate the molecular mechanisms of tumor development and metastasis, numerous experimental animal models have been created.

Hernando et al found a defect in the PI3K-AKT signal transduction pathway in many LMS. They succeeded in creating smooth muscle cell-specific *Pten* knockout animals (TagIn-Cre; *Pten*flox/flox). This mouse model showed smooth muscle cell hyperplasia and abdominal LMS. However, development of LMS was not observed in the uterus and in lung metastases.⁸ Xing et al introduced Cre recombinase into the anti-Mullerian hormone type II receptor (*Amhr 2*) locus in mice to conditionally inactivate p53 in the reproductive tract and reported that u-LMS developed in 50% of the mice within 13 months. Furthermore, when the tumor suppressor gene *BRCA1* was also inactivated, the frequency of tumor development increased to 82%.⁹ Strizzi et al reported that Cripto-1 (CR-1) protein, an active growth factor in the Wnt signaling pathway, is present in $\geq 70\%$ of u-LMS, and they created a CR-1 overexpression transgenic mouse model. Wnt signaling and c-Src and AKT signaling pathways were activated in these mice, and u-LMS were detected in approximately 20% of the mice. These findings suggested that crosstalk between the Wnt signaling pathway and the Src/AKT pathway may play a significant role in the development of u-LMS. Interestingly, p53 mutation was not involved in the development of u-LMS in these animals.¹⁰ Kawabe et al developed a uterine sarcoma tissue-derived orthotopic and metastatic mouse model using a green fluorescent protein stably expressed uterine sarcoma cell. They also identified the differential expression of genes related to cell proliferation and migration (*TNNT1*, *COL1A2* and *ZIC1*) between orthotopic tumors with high and low metastatic potential.¹¹ Animal models are indispensable tools for the study of u-LMS because a real clinical sample makes large-scale analysis of human samples challenging.

Doxorubicin is one of the key drugs in the treatment of u-LMS, although resistance is the major hurdle, with a response rate of only 19% due to drug resistance.¹² Overcoming resistance to chemotherapy and investigating molecular targeted therapies are challenges. Currently, *multi drug resistant 1 (MDR1)* is frequently associated with the overexpression of membrane-embedded drug efflux transporters, such as ATP-binding cassette transporters, known as P-glycoprotein 1 (P-gp) (also named ABCB1 ([TP-binding Cassette Sub-family B Member 1]), leading to the reduced accumulation of chemotherapeutic drugs and chemoresistance in LMS cells.¹³ Hung et al reported that the overexpression of frizzled 1 (*FZD1*) encoded by *FZD1* gene, which is a G-protein-coupled receptor and involved in the Wnt/ β -catenin pathway, was observed in LMS cells and contributed to doxorubicin-resistance via the activation of the protein kinase C δ /AP-1 signaling transduction pathway, resulting in enhanced ABCB1 expression.¹⁴ Lin et al reported on the important role of progesterone receptor membrane component 1 (*PGRMC1*) encoded by *PGRMC1*, which is an adapter protein mediating cholesterol synthesis, steroid signaling and cytochrome p450 activation. *PGRMC1* promoted cell proliferation and cell cycle progression to the S phase by mediating ERK activation, leading to doxorubicin-resistance in LMS cells.¹⁵ In the last decade, several anticancer drugs or multityrosine kinase inhibitors have demonstrated efficacy with respect to progression-free survival and overall survival, particularly the *tyrosine kinase inhibitor (TKI)* pazopanib in the treatment of u-LMS.¹⁶ However, the biological mechanisms or optimal predictive biomarkers for

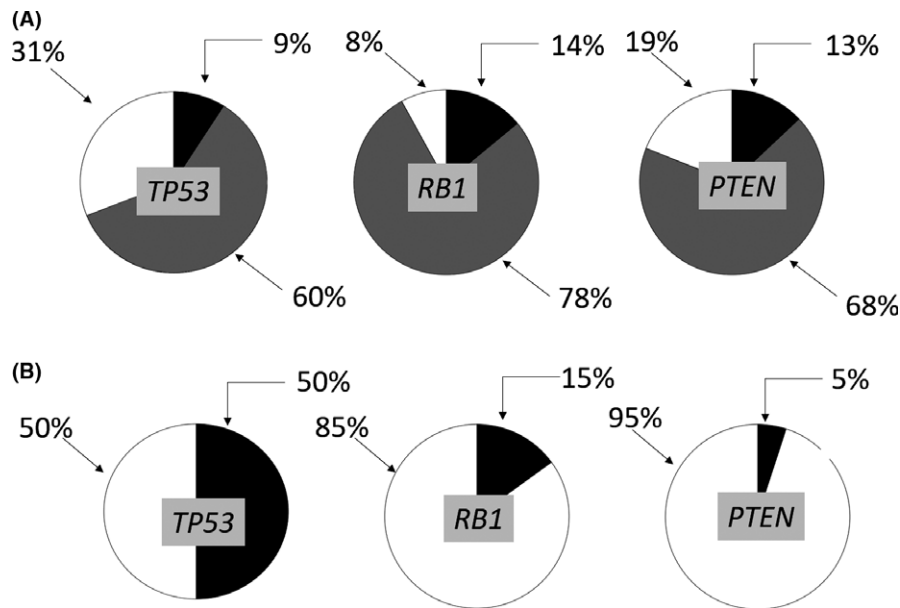


FIGURE 1 In the recurrent focal copy-number alterations, the ratios of deletions of the tumor suppressors (A) and mutations (B) of *TP53*, *RB1* and *PTEN* to each whole gene in leiomyosarcoma. The deletions of the tumor suppressors *TP53* (9% deep and 60% shallow deletions), *RB1* (14% deep, 78% shallow) and *PTEN* (13% deep, 68% shallow) (false discovery rate [FDR] is 0.25) are shown. Mutations of *TP53* in 50%, *RB1* in 15% and *PTEN* in 5% of samples are shown (FDR values computed by MuSic2 are $<1e-22$, $2e-15$ and 0.49, respectively). A, Black indicates deep, a striped pattern indicates shallow deletions and white indicates no alterations. B, Black indicates mutations, and white indicates no mutations. Data from The Cancer Genome Atlas Research Network that performed a multiplatform molecular characterization of adult soft tissue sarcoma⁴

these therapies are still being explored. The further development of in vitro or in vivo models using advanced genetic techniques will continue to increase our understanding of LMS biology.

2.2 | Molecular biomarker candidates for diagnosis and prognosis of primary uterine leiomyosarcoma

2.2.1 | Tumor suppressor gene

Molecular genetic studies have demonstrated that, in LMS (site of formation in the uterine or extrauterine tissues), frequent chromosomal losses are detected in the long arm of chromosome 10 (10q) and 13 (13q), the regions in which tumor suppressor *PTEN* and *RB1* genes are present. In particular, losses at 10q21.3 and 13q14.2-q14.3 were the most frequent, with frequencies of 75% at each of the loci. According to Yang et al,¹⁷ chromosomal gains were observed in 1q21, 5p14-pter, 8q, 12q13-15, 13q31, 17p11, 19p13 and 20q13.

Genetic deficiency of 10 q means inactivation of tumor suppressor gene *PTEN*, leading to activation of the PI3K/AKT pathway and the downstream mTOR pathway. Hernando et al reported abnormalities of the PI3K-AKT signaling pathway in many LMS. They genetically inactivated *Pten* in the smooth muscle cell lineage by crossbreeding *Pten* (loxP/loxP) mice with TagIn-cre mice. The mice developed widespread smooth muscle cell hyperplasia and abdominal LMS in the peritoneal cavity. In addition, mTOR inhibitor suppressed tumor growth.¹⁷ Thus, the PTEN-PI3K/AKT-mTOR pathway can play

important roles in not only the generation of LMS but also in the treatment and prediction of the prognosis of patients with LMS.^{18,19} However, this pathway has not been well investigated in u-LMS.

Loss at the 13q region leads to inactivation of the tumor suppressor *RB1* gene. The *RB1* gene is involved in the cell cycle, specifically at the G1-S phase checkpoint. Regulatory aberration at this region is known to induce cells to divide indefinitely. Dei-Tos et al²⁰ observed defects in RB-cyclin D1 signaling (*RB1*, *CDKN2A* which encodes p16, *CCND1* which encodes cyclin D1, and *CCND3* which encodes cyclin D3) in $\geq 90\%$ of u-LMS patients. It has also been reported that an abnormality in this pathway is an obvious factor related to poor prognosis.¹⁷

Many studies have shown that the frequencies of *TP53* mutation in uterine leiomyoma (u-LM), smooth muscle tumor of uncertain malignant potential (STUMP) and u-LMS are 0%, 6%-29% and 24%-30%, respectively. It has also been reported that the frequencies of *PTEN* mutation are 5%, 33% and 42%-58%, respectively.²¹ However, u-LMS has shown a lower frequency of *TP53* mutation, higher expression of *MDM2*, and a higher *TP53*/*MDM2* ratio than the other sarcomas.

2.2.2 | Genes related to the cell cycle

In general, it is difficult to distinguish between u-LM and u-LMS with protein expressions related to the cell cycle (p16, p21, p27, p53, Ki-67 and PHH3).²² In 2004, Quade et al investigated 4 normal uterine myometrium samples, 7 u-LM and 9 u-LMS using microarrays of

oligonucleotides representing approximately 7000 unique probe sets. They reported that, while there was a difference in *CDKN1A*, which encodes p21, which is involved in cell proliferation and the cell cycle, this difference was not significant. Moreover, they reported that there were no clear differences in cellular gene expressions between LMS that developed in the uterus vs other tissues.²³ Miyata et al reported that overexpression of cell cycle-related genes and frequent hypermethylation at the polycomb group target genes and protocadherin genes were observed in u-LMS in single nucleotide polymorphism arrays, gene expression array analysis, and analysis involving comprehensive detection of methylated DNA.²⁴ Furthermore, Aurora-A kinase, which is a serine/threonine kinase and is involved in the regulation of the M phase in the cell cycle and division, has recently been reported to be overexpressed in u-LMS. Matsumura et al demonstrate the important role of *acroganin*, which is also known as *PCDGF*, *progranulin* or *proepithelin*. Acroganin, an 88-kDa glycoprotein, is one of the pluripotent growth factors that mediate cell cycle progression and cell motility. It activates the extracellular regulated kinases and PI3K signal cascades, increases expressions of cyclins D and B, and is overexpressed in u-LMS.²⁵

2.2.3 | Genes related to oncogenesis of uterine leiomyosarcoma derived from uterine leiomyoma

There are currently many studies focused on the *mediator complex subunit 12 (MED12)* gene, which was discovered in genomic analysis of u-LM. Makinen et al observed a mutation in *MED12* that resides on the long arm of the X chromosome (Xq13.1.) in $\geq 70\%$ of u-LM and postulated that this mutation contributes greatly to the formation of u-LM. Mediator is a complex that is composed of approximately 30 subunits that acts as a bridge between transcription factors and RNA polymerase II in the nucleus. These molecules consequently augment or suppress gene expression in a cell-dependent manner. *MED12* is known to be involved in p53 and Wnt/ β -catenin pathways.²⁶ Interestingly, in vitro study of u-LM has shown that the u-LM cells with *MED12* mutation grow poorly in culture and cannot be maintained through passage.²⁷

In 2012, the same research group demonstrated that, albeit at low frequencies, *MED12* mutations are observed in u-LMS, as well as u-LM, while no mutations were found in other sarcomas or in gastrointestinal stromal tumors (GIST), suggesting that a subgroup of u-LMS may develop from an LM precursor. Furthermore, they report that such u-LMS do not have greater malignancy than those from the group without mutations.²⁸ In 2012, Pérot et al compared *MED12* protein expression between benign and malignant smooth muscle tumors. They postulated that *MED12* could be a tumor suppressor gene, and that *MED12* protein expression inhibits LMS oncogenesis.²⁹ Schwetye et al compared the frequencies of *MED12* gene mutations among normal myometrium adjacent to LM, pelvic LM and extrauterine LM. *MED12* mutations were detected in 54% of u-LM, 15% of cases in myometrium adjacent to LM, and 0% of extrauterine LM. Moreover, *MED12* mutations were also detected in 30% of u-LMS compared with 4% of extrauterine LMS, suggesting

that smooth muscle tumors in pelvic/retroperitoneal sites are subject to the same mutational changes as those of uterine myometrium.³⁰ Recently, Zhang et al showed the frequencies of *MED12*, *TP53* or *PTEN* mutations in LM, mitotically active LM, cellular LM, atypical LM, STUMP and u-LMS. STUMP and atypical LM were more similar to LMS, with high frequencies of *TP53* and *PTEN* mutations and low frequencies of *MED12* mutation. However, atypical LM shared more molecular alterations, including the selected microRNA, oncogenes and tumor suppressors that are highly relevant to uterine smooth muscle tumors with u-LMS, suggesting that atypical LM may be a precursor lesion of u-LMS or have similar genetic changes during its early stage (Table 1).²¹

Bertsch et al³¹ showed other genetic abnormalities in u-LM. The overexpression of *high-mobility group AT-hook 2 (HMGA 2)*, which is an oncogene, occurred in u-LM (40%) and u-LMS (25%) with no *MED12* mutation, suggesting that they may act differently in u-LM tumorigenesis. Because u-LM is related to familial hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome, heterozygous germ-line mutations in *fumarate hydratase (FH)*, which is a tumor suppressor and encodes fumarase of the tricarboxylic acid cycle, has been reported to be related to the development of u-LM.³² Recent studies have shown that a kind of u-LMS can be derived from the precursor lesion of u-LM based on the examination of 3 driver genes, *MED12*, *HMGA 2* and *FH*.³³

Based on several studies, *MED12* mutation is also observed in u-LMS, while it is more frequent in u-LM than in u-LMS. *MED12* mutations are rarely observed in other sarcomas or in GIST, as well as extrauterine LMS,^{29,30} suggesting that *MED12* can be a useful biomarker to diagnose uterine-derived LMS, although it is difficult to distinguish between u-LM and u-LMS only by examination of *MED12* mutation.

2.3 | Other biomarkers for primary uterine leiomyosarcoma

2.3.1 | Stathmin 1

Stathmin 1 encoded by *STMN1* is involved in the regulation of the cell cycle as the protein that destabilizes microtubules. It is also known as an activator of the PI3K-AKT-mTOR pathway. In 2017, the Gynecologic Oncology Group (GOG) and NRG showed that *STMN1* expression was significantly associated with shorter progression-free survival and overall survival for all analyzed uterine endometrial cancer cases in both GOG-177 and TCGA.³⁴ *STMN1* expression was observed in all u-LMS cases (100%), while it was observed in approximately 40% of benign u-LM cases.³⁵

2.3.2 | Insulin-like growth factor (IGF-II) mRNA binding protein 3 (IMP3)

IMP3 encoded by *IMP3* is an RNA-binding carcinoembryonic protein consisting of 580 amino acids that is observed only in advanced tumor tissues, but not in normal tissues.³⁶ Strong expression of

TABLE 1 Mutation analysis of *MED12*, *TP53* and *PTEN* and comparison with the literature for 6 types of uterine smooth muscle tumors

Mutations	LMS	STUMP	Atypical LM	Cellular LM	Mitotically active LM	LM	Number of references
<i>MED12</i>	15% (21/141)	10% (4/40)	12% (8/69)	12% (11/95)	48% (16/33)	67% (636/948)	13
<i>TP53</i>	28% (50/176)	17% (6/35)	10% (5/50)	0% (0/26)	0% (0/7)	0% (0/59)	7
<i>PTEN</i>	54% (76/142)	33% (6/18)	26% (11/42)	5% (1/22)	0% (0/7)	5% (1/20)	6

LM, leiomyoma; LMS, leiomyosarcoma.

IMP3 is observed in the cytoplasm in more than 50% of u-LMS cases, while it is not observed in typical u-LM.³⁶ Yasutake et al³⁷ reported a multivariate analysis that showed that advanced stage and IMP3 are independent predictors of a poor prognosis in u-LMS.

2.3.3 | Telomere-maintenance mechanism by alternative lengthening of telomeres

It has been reported that the correlation of alternative lengthening of telomeres (ALT) activation and loss of α -thalassaemia/mental retardation syndrome X-linked (*ATRX*) encoded by *ATRX* expression can suppress cell death in sarcoma.³⁸ Further studies using next-generation sequencing have shown that loss of function of p53 can be correlated with *ATRX* pathways involved in ALT. Although *ATRX* mutations were not correlated with *ATRX* protein expression, all *ATRX*-mutated LMS cases showed the ALT phenotype. *TP53* and *ATRX* mutations can be important mechanisms in the pathogenesis of u-LMS and are correlated with a poor prognosis.³⁹

2.3.4 | MicroRNA (miRNA)-181b

The Cancer Genome Atlas indicated that high miR-181b was more common in u-LMS and was an independent predictor of recurrence-free survival in a multivariate model, including tumor size. MiR-181b expression has been reported to promote proliferation and migration of vascular smooth muscle via the PI3K pathway.⁴

2.3.5 | KIT expression

Raspollini et al⁴⁰ showed a high frequency of positive immunostaining for KIT in u-LMS. However, imatinib is not theoretically applicable for u-LMS or ESS because of the lack of KIT hotspot mutations.⁴¹ Pazopanib inhibits VEGF, PDGFR, FGFR and c-KIT. After a recent successful placebo-controlled phase III trial, the PALLETTE study, it was approved by the FDA (April 2012) for use in STS, including LMS. On subgroup analysis, u-LMS patients had a partial response (11%), and median PFS was 3 months (95% CI 2.5–4.7 months), with median OS of 17.5 months (95% CI 11.1–19.6 months).⁴²

To date, several anticancer drugs, multityrosine kinase inhibitors or small molecule inhibitors, have demonstrated efficacy with respect to progression-free survival and overall survival. However, optimal predictive biomarkers for these therapies are still being explored. Some novel check point inhibitors, such as nivolumab, pembrolizumab and ipilimumab, have also been explored as potential

therapies, but their efficacy is still limited. In contrast, in LMS as a whole, aberrant PI3K-AKT-mTOR signaling may be crucial, given recurrent deletion/mutation of *PTEN*. Indeed, mTOR inhibitors such as everolimus and temsirolimus have shown some clinical efficacy in LMS, although the use of definitive predictive biomarkers is still challenging. Thus, further investigations are needed to evaluate the predictors for these therapies and to provide the optimal precision medicine.

2.4 | Molecular biomarker candidates for diagnosis and prognosis of metastases of uterine leiomyosarcoma

Recently, some researchers reported extremely interesting studies.^{11,43,44} They investigated the gene expression patterns of primary and metastatic lesions in patients and in vivo with distant metastatic u-LMS, and they identified genes that were overexpressed in each type of lesion. Several genes, which are listed along with their functions in Table 2, are overexpressed at primary lesion sites, and the following genes were overexpressed or down underexpressed in metastatic lesions.

Increasing evidence of intratumor genetic heterogeneity (ITH) is emerging, both within individual tumor biopsies and spatially separated between biopsies of the same tumor. Furthermore, sequential analysis of tumors has also provided evidence that ITH evolves during the course of the disease.⁴⁵ However, these genes may be useful in elucidating the mechanism of metastasis from u-LMS.

3 | ENDOMETRIAL STROMAL SARCOMA

Endometrial stromal sarcoma (ESS) is composed of cells similar to the endometrial stroma at the proliferative phase. Basically, it is diagnosed by histopathological criteria. The growing evidence provided by molecular genetic analysis has been increasingly used to diagnose ESS (Table 3) (Figure 2).

3.1 | Whole molecular mechanisms of tumor development, metastasis and chemoresistance in endometrial stromal sarcoma

Based on 2014 WHO classification, LG-ESS is often associated with gene rearrangement bringing about the *JAZF 1-SUZ12* (formerly *JAZF1-JJAZ1*) fusion gene, whereas HG-ESS is associated with the *YWHAE-NUTM* fusion gene. LG-ESS with *JAZF1* rearrangement may

TABLE 2 Gene expression patterns of primary and metastatic lesions in patients and in vivo with distant metastatic u-LMS

	Functions	References
Overexpressed gene at primary lesion site		
<i>Osteocrin (OSTN)</i>	Muscle metabolism	Davidson et al ⁴³
<i>Neurologin4X (NLGN4X)</i> and <i>Neurologin1 (NLGN1)</i>	Neural development, angiogenesis and tumor growth	Davidson et al ⁴³
<i>SLITRK4</i> encodes the Slitrk protein	An integral membrane protein similar to Trk tyrosine kinase receptors Slitrk is primarily involved in nervous system development.	Davidson et al ⁴³
<i>TSPAN7 (CD231)</i>	The expression of tetraspanins, a family of transmembrane adhesion molecules including integrins, claudin-1 and EGFR	Davidson et al ⁴³
Overexpressed genes in metastatic lesions		
<i>TSPAN10</i> (metastatic site)	The expression of tetraspanins, a family of transmembrane adhesion molecules, including integrins, claudin-1 and EGFR	Davidson et al ⁴³
<i>FOLR3</i> and <i>SHMT1</i>	Folate metabolism	Davidson et al ⁴³
<i>TDO2</i>	The expression of tryptophan conversion enzyme	Davidson et al ⁴³
<i>TNNT1</i>	The expression of skeletal muscle protein	Davidson et al ⁴³ Kawabe et al ¹¹
<i>Early growth response 2 (EGR2)</i>	Neural development and has been reported to be involved in the prognosis of Ewing's sarcoma,	Davidson et al ⁴³
<i>SGK1</i>	A factor involved in the activation of ion channels for the transport of K, Na and cellular signal transduction of serine/threonine kinases.	Davidson et al ⁴³
<i>Gas 6</i> and <i>Tyro 3</i>	<i>Gas 6</i> is a gene product expressed when cell growth arrest occurs. It is involved in various biological activities, such as the promotion of cell proliferation, chemotaxis or cell adhesion factors as a common ligand of <i>Tyro 3</i> receptor protein tyrosine kinase, <i>Axl</i> and <i>Mer</i> .	el Sayadi et al ⁴⁴
<i>COL1A2</i>	A genetic maker involved in aggressive malignancy	Kawabe et al ¹¹
Low expression gene in highly metastatic lesions		
<i>ZIC1</i>	A transcription factor gene that induces cell-cycle arrest/apoptosis and inhibits cell migration/invasion by blockade of PI3K/Akt and MAPK pathways	Kawabe et al ¹¹

u-LMS, uterine leiomyosarcoma.

correlate with metastasis. On the other hand, HG-ESS with metastasis with *YWHAE* rearrangement shows a relatively favorable prognosis.⁴⁶

Low-grade endometrial stromal sarcoma usually expresses ER/PR receptors and is an indolent tumor with a favorable prognosis. However, LG-ESS is characterized by late recurrences even in patients with stage I disease, suggesting the need for long-term follow-up.² In an in vitro study, Quan et al⁴⁷ found that the histone deacetylase (HDAC) inhibitor suberanilohydroxamic acid (SAHA) combined with inhibition of PI3K (LY294002, LY) and mammalian target of rapamycin (mTOR) led to the strongest growth inhibition and slowest growth recovery in an ESS cell line. Lee et al report that ESS with *YWHAE/NUTM* (formerly *YWHAE/FAM22A/B*) fusion which gives rise to a 14-3-3 oncoprotein displays higher-grade histology and more aggressive clinical course.⁴⁸

3.2 | Molecular biomarker candidates for diagnosis and prognosis for primaries of low-grade endometrial stromal sarcoma

In 1988, Dal Cin et al⁴⁹ first reported a chromosomal abnormality in LG-ESS. Based on the growing evidence, LG-ESS is classified into 3 categories based on molecular genetic abnormalities:

translocation t(7;17) (p15;q21); 6p21-rearrangements; and X;22 or 17-rearrangements.

Chromosomes 7 and 17 are recombined in the first genetic hallmark to be discovered in ESS; namely, the translocation t(7;17) (p15;q21). Two zinc finger genes, the *JAZF1* gene from chromosomal band 7p15 and *SUZ12* (formerly *JJAZ1*) from 17q11, are fused by this translocation.⁵⁰ Subsequent studies have shown that the fusion of *JAZF1* and *SUZ12* is recognized in almost all ESS cases and is more frequently expressed in LG-ESS than in HG-ESS.⁵¹ Micci et al⁵² show that the *PHD finger protein 1 (PHF1)* gene from chromosomal band 6p21 recombined with *JAZF1* as the partner gene of the *JAZF1* gene in LG-ESS. Further studies have shown that the *PHF1* gene from chromosomal band 6p21 recombined with *JAZF1*, *EPC1*, *MEAF6* or *BRD8*, which encode the proteins involved in regulation of protein acetylation and/or histone acetyltransferase activity.⁵³ All these fusions in ESS combined genes are involved in transcriptional regulation; that is, polycomb group complex-mediated aberration methylation/acetylation; hence, their presumed oncogenic effects may be involved in the pathogenesis of ESS.⁵²

Currently, ESS is considered to be a tumor with molecular genetic heterogeneity in gene rearrangement. Representative fusion

TABLE 3 Classification of ESS by molecular genetic examinations

Karyotype	Fusion transcript	Functions	Material and methods
LG-ESS			
t(7;17)(p15;q21)	JAZF1-SUZ12	JAZF1; zinc finger gene, one of the largest family of transcriptional repressor SUZ12; a member of the polycomb group protein family involved in transcriptional repression	FISH, PCR
t(6;7)(p21;p15)	JAZF1-PHF1	JAZF1; zinc finger gene, one of the largest family of transcriptional repressors PHF1; a member of the polycomb group protein family involved in transcriptional repression.	FISH
t(6;10)(p21;p11)	EPC1-PHF1	EPC1; part of the nucleosome acetyltransferase of histone H4 complex PHF1; a member of the polycomb group protein family involved in transcriptional repression.	FISH
t(1;6)(p34;p21)	MEAF6-PHF1	MEAF6; part of histone acetyltransferase multi-subunit complexes PHF1; a member of the polycomb group protein family involved in transcriptional repression	FISH
t(X;17)(p11;q21)	CXorf67-MBTD1	CXorf6; chromosome X open reading frame 67 MBTD1; a member of the polycomb group protein family involved in transcriptional repression	FISH, PCR
t(X;22)(p11;q13)	ZC3H7B-BCOR	ZC3H7B; it is involved in protein-nucleic acid interactions BCOR; it interacts with polycomb group proteins	FISH, PCR
HG-ESS			
t(10;17)(q21;p13)	YWHAЕ-NUTM	YWHAЕ; mediator of signal transduction by binding to phosphoserine-containing proteins	FISH, PCR

ESS, endometrial stromal sarcoma; HG-ESS, high-grade ESS; LG-ESS, low-grade ESS; PCR, polymerase chain reaction.

genes such as *JAZF1-SUZ12*, *PHF1-JAZF1*, *EPC1-PHF1*, *MEAF6-PHF1* and *ZC3H7-BCOR* are observed in more than 50% of ESS cases.^{54,55}

3.3 | Molecular biomarker candidates for diagnosis and prognosis for primaries of high-grade endometrial stromal sarcoma

In 2012, Lee et al⁴⁸ showed that a specific gene rearrangement, t(10;17)(q22;p13), brought about *YWHAЕ-NUTM* (previously known as *YWHAЕ-FAM22*) fusion, which is related to malignant potential. In an update based on 2014 WHO classification, LG-ESS is often associated with gene rearrangement bringing about the *JAZF 1-SUZ12* fusion gene, whereas HG-ESS is associated with the *YWHAЕ-NUTM* fusion gene, suggesting that LG-ESS and HG-ESS are independent units.⁴⁶ Clinically, HG-ESS shows more malignant potential than LG-ESS. Hoang et al (2017) show that ESS with *ZC3H7B-BCOR* fusion morphologically closely resembles mucinous LMS. They also suggest that tumors with *ZC3H7B-BCOR* fusion should be classified as ESS even if they are morphologically LMS.⁵⁶

3.4 | Other biomarkers for primary endometrial stromal sarcoma

3.4.1 | Cyclin D1 expression

More than 70% of HG-ESS cases with the *YWHAЕ-NUTM2A/B* fusion gene show diffusely moderate to high staining of cyclin D1.

In contrast, LG-ESS with the *JAZF1* fusion gene shows only 5% focal staining. Lee et al indicate that cyclin D1 is a sensitive and specific diagnostic immunomarker for *YWHAЕ-NUTM* ESS. When evaluating HG-ESS, cyclin D1 can be included in the immunohistochemical panel as an indicator of *YWHAЕ-NUTM* ESS.⁵⁷

3.4.2 | KIT expression

It has been reported that overexpression of KIT, PDGFRA or EGFR is observed in a few cases, while KIT expression is observed in HG-ESS with the *YWHAЕ-NUTM2A/B* fusion gene, which frequently spreads beyond the uterus. If the extruterine disease presents with a pelvic/abdominal mass, particularly in situations where its uterine origin is not definitive, an epithelioid GIST should be considered in the differential diagnosis because of the high frequency of KIT immunopositivity. Lee et al indicate that the high-grade round cell component of *YWHAЕ-NUTM2A/B* ESS consistently expresses KIT, but lacks *KIT* hotspot mutations, while GIST frequently contains activating gene mutations in *KIT*. Moreover, the expression of ANO1 (also known as *DOG1*), which is another widely used diagnostic immunomarker for GIST, is observed in approximately 90% of GIST, while ANO1 is not expressed by *YWHAЕ-NUTM2A/B* ESS, suggesting that the evaluation of only Kit expression may be a potential diagnostic pitfall in the evaluation of pelvic/abdominal masses in female patients. Thus, the additional diagnostic strategy including the immunoprofiling of another antibody such as ANO1 or cyclin D1 or a mutation screen of *KIT* is needed.⁵⁸ Likewise, expression of

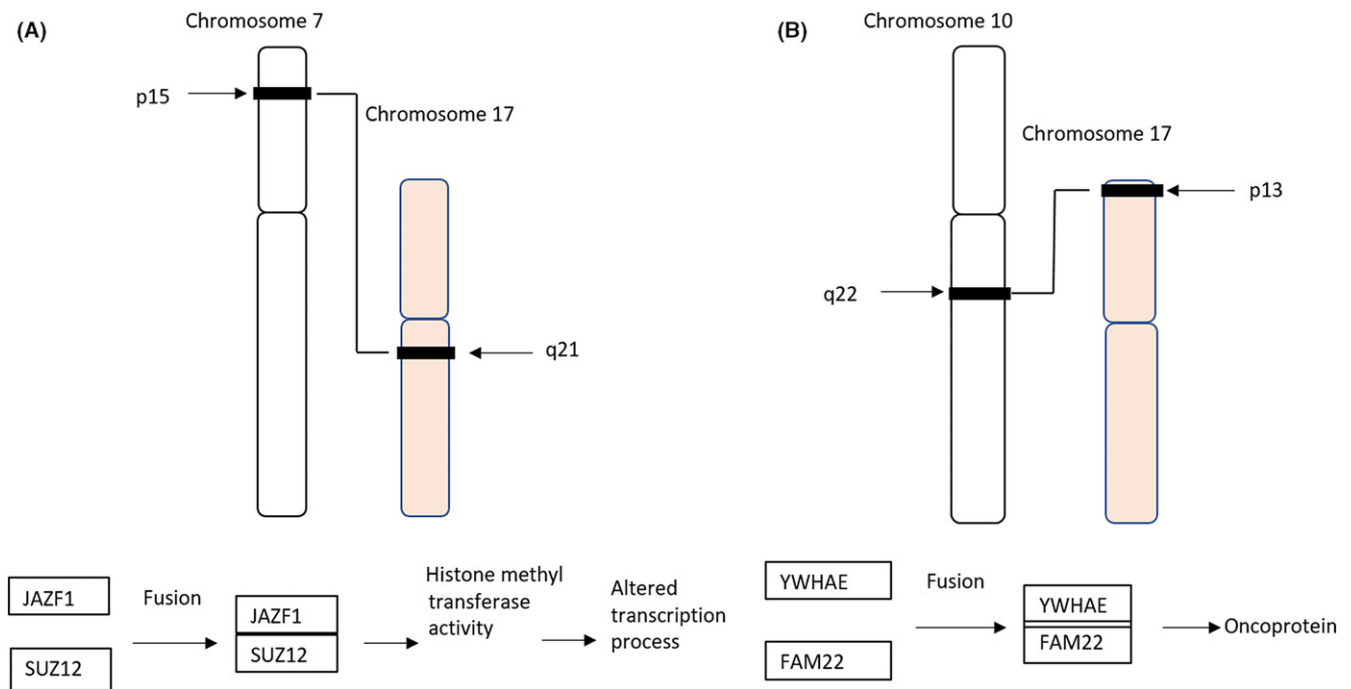


FIGURE 2 Schematic of chromosomal translocations present in low-grade endometrial stromal sarcoma (LG-ESS) (A) and high-grade endometrial stromal sarcoma (HG-ESS) (B). A, The most common translocation involves the short arm of chromosome 7 and long arm of chromosome 17(7;17) (p15;q21), leading to fusion of 2 zinc finger genes, *JAZF1* and *SUZ12*, and production of *JAZF1/SUZ12* gene fusion protein. B, Schematic of t(10;17) (q22;p13) chromosomal translocation present in HG-ESS results in the fusion of the *YWHAE* and *NUTM* genes, leading to production of *YWHAE/NUTM* gene fusion proteins, which contributes to oncoprotein

tyrosine kinase receptor was observed, but gene abnormalities were found in few cases.⁵⁹

3.4.3 | Murine double minute 2 amplification

Murine double minute 2 (*MDM2*) is an oncogene that can promote tumor occurrence and development by regulating the cell cycle and promoting cell proliferation. The evaluation of *MDM2* protein expression may not be helpful in the differential diagnosis because it is often observed in various neoplasms, including ESS or other sarcomas. In contrast, the assessment of *MDM2* gene amplification by FISH has become a routine ancillary tool in distinguishing well-differentiated and dedifferentiated liposarcoma from classic lipoma, while *MDM2* amplification is not common in ESS. Thus, examination of *MDM2* amplification in ESS, particularly with metastatic lesions with myxoid morphology, can be useful for differentiation from liposarcoma.⁶⁰

3.4.4 | Interferon-induced transmembrane protein 1

CD10 is a well-established marker for endometrial stromal differentiation, although it has limited specificity. IFITM1 has been demonstrated as a potential marker based on the bioinformatics approach to identify more specific markers of endometrial stromal differentiation by searching a public database of protein expression profiles, the Human Protein Atlas. Immunohistochemical analysis of IFITM1 has shown high sensitivity and specificity in LG-ESS and HG-ESS.⁶¹

3.5 | Molecular biomarkers for metastases of endometrial stromal sarcoma

In 2013, it was first reported that an LG-ESS case with a *PHF1-JAZF1* fusion gene in the metastatic lesion, as well as the primary lesion, had invasive progression and fatal lung metastasis.⁶² It has also been reported that an LG-ESS case with *JAZF1* rearrangement and *MDM2* amplification had lethal lung metastasis.⁶⁰ On the other hand, it has been reported that a case with the *JAZF1-SUZ12* fusion gene in the primary and metastatic lesion had metastatic but not invasive disease.⁶³

It has been reported that an HG-ESS case with metastasis with *YWHAE* rearrangement responded to anthracycline-based therapy and showed relatively long-term survival.⁶⁴ These facts indicate that further study to elucidate the relationship between the pattern of fusion genes and the prognosis or therapeutic effects in ESS is needed in the future.

4 | CONCLUSIONS

Further advances in genome analysis are expected to elucidate not only the pathogenic genes but also the molecular basis of various abnormal traits, such as metastasis, for uterine sarcoma, which is characterized by its rarity and diversity. The development of molecular imaging methods or molecular targeted drugs based on these findings is also expected in the future.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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