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Review Article

# Soft tissue sarcomas: From a morphological to a molecular biological approach

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**Recently developed molecular genetic techniques have led to the elucidation of tumor-specific genomic alterations and thereby the reclassification of tumor entities of soft tissue sarcoma. A solitary fibrous tumor-mimicking tumor with the *AHRR-NCOA2* gene has been isolated as angiofibroma of soft tissue. As for small round cell sarcomas, novel fusion genes such as *CIC-DUX4* and *BCOR-CCNB3* have been identified in these tumor groups. SMARCB1/INI1 deficient tumors with round cell morphology are also expected to be reclassified in three types, based on the combination of their morphology and genotype. The identification of the *MDM2* gene amplification in pleomorphic sarcomas has extended the entity of dedifferentiated liposarcoma (DDLS). Our recent molecular investigations elucidated candidates for novel therapeutic strategies. Activation of the Akt-mTOR pathway was correlated with poor prognosis or tumor grade in spindle cell sarcomas including malignant peripheral nerve sheath tumor. *In vitro* and *in vivo* studies of transcription factor Forkhead Box M1 (FOXM1) demonstrated the close correlation between aggressive biological behavior or chemosensitivity and FOXM1 expression in synovial sarcoma, so far. Finally, in regard to the investigation of cancer-testis antigens, myxoid/round cell liposarcoma and synovial sarcoma showed frequent and high expression of PRAME and NY-ESO-1.**

**Key words:** Akt/mTOR, cancer testis antigen, FOXM1, fusion gene, molecular target, small round cell sarcomas, SMARCB1/INI1, soft tissue sarcomas, spindle cell sarcomas

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Soft tissue sarcoma is a rare malignant tumor, with an incidence accounting for less than 1% of all malignant tumors annually.[1] There are more than 110 histo-types in all soft tissue tumors,[2] which occur at various anatomical sites in the body, including the extremities, head and neck, mediastinum, abdominal cavity, retroperitoneum, and pelvic cavity. As a result of these characteristics, a definitive histological diagnosis is often difficult for general surgical pathologists. In the soft tissue sarcomas registry at our institution, about two thirds of the sarcomas are histologically classified as liposarcoma, leiomyosarcoma, undifferentiated pleomorphic sarcoma (UPS) (previously known as malignant fibrous histiocytoma (MFH)), rhabdomyosarcoma, synovial sarcoma, myxofibrosarcoma and malignant peripheral nerve sheath tumors (MPNST) (Fig. 1). Moreover, 8% of the sarcomas do not conform to any of the established tumor entities and thus are categorized as unclassified sarcomas (Fig. 1).

The recent development of genome wide molecular analysis and its combined use with morphology has led to the establishment of novel tumor entities, especially in round cell or spindle cell tumors. It has also permitted reclassification of the previously established entities, according to molecular phenotype. Moreover, several investigators have used this technology to seek effective novel molecular targets in soft tissue sarcomas and to evaluate their therapeutic potential.

In this review article, we introduce our investigations concerning the molecular diagnosis and potential molecular therapeutic targets of soft tissue sarcomas. With respect to the molecular genetic diagnosis, we discuss novel fusion genes and their use in the reclassification of tumor entities, *SMARCB1/INI1* gene alterations, and *MDM2* gene amplifications. In our consideration of molecular therapeutic targets, we discuss investigations of the Akt/mTOR pathway, transcription factor Forkhead Box M1 (FOXM1) and cancer testis antigen.

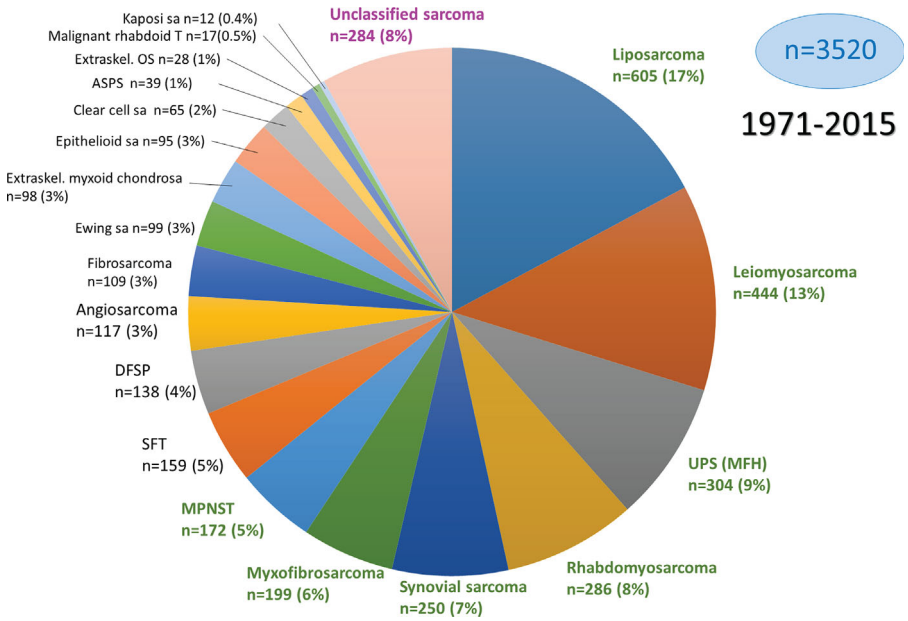


**Figure 1** Frequency of histological subtype in soft tissue sarcomas registered at the Department of Anatomic Pathology, Kyushu University between 1971 and 2015. UPS, undifferentiated pleomorphic sarcoma; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor; SFT, solitary fibrous tumor; DFSP, dermatofibrosarcoma protuberans; ASPS, alveolar soft part sarcoma; OS, osteosarcoma.

## MOLECULAR PATHOLOGICAL DIAGNOSIS

Soft tissue sarcomas are divided into two categories, translocation-associated sarcomas and non-translocation-associated sarcomas.[3] The latter carry complex chromosomal alterations. Translocation-associated sarcomas usually occur in younger patients, and disclose more uniform cellular morphology and fewer *p53* pathway alterations, compared with non-translocation-associated sarcomas.[3]

Certain soft tissue sarcomas exhibit specific reciprocal chromosome translocation and concordant fusion genes (Table 1). Perhaps the best know examples are, the detection of *SS18-SSX1* or *SSX2* in synovial sarcoma, *EWSR1-FLI1* or *EGR* in Ewing sarcoma and *PAX3* or *PAX7-FOXO1* in alveolar rhabdomyosarcoma, and detection of these genes have been applied for practical diagnosis in the last two decades. Generally, spindle sarcomas have tyrosine kinase genes as fusion partner genes, such as *PDGFB* in dermatofibrosarcoma protuberans (DFSP) and ALK in inflammatory myofibroblastic tumor (IMT) (Fig. 2). Tyrosine kinase inhibitors including imatinib and ALK inhibitors are practically applied for molecular target therapy in these sarcomas. On the other hands, round cell sarcomas such as Ewing sarcoma and myxoid/round cell liposarcoma frequently possess transcription factor genes including *FUS* and *EWSR1* (Fig. 2).

Recently, the development of molecular analytical methods such as next generation sequencing (NGS) has led to the identification of novel chimeric genes (Table 1). Some new tumor entities have also been established through the identification of their specific chimeric genes, such as *CIC-DUX4* or *BCOR-CCNB3* fusion-positive sarcoma.[4,5]

Moreover, some histologically different tumors have the same fusion gene. For instance, clear cell sarcoma, which is a high-grade malignant tumor, and angiomatoid fibrous histiocytoma, which is an intermediate-grade soft tissue tumor, share the same fusion gene, *EWSR1-CREB1/ATF1*[6] (Table 2). However, both tumors show distinctive clinicopathological features. Other types of tumors and soft tissue tumors, such as IMT and lymphoma also share the same fusion gene[7–20] (Table 2). Therefore, in focusing only on the molecular features without considering the histopathological findings, they may mistake the diagnosis.

### Solitary fibrous tumor (SFT) and angiofibroma of soft tissue (AFST)

In 2013, a novel specific fusion gene of SFT, *NAB2-STAT6,* was identified by NGS analysis.[21,22] The histology of SFT is characterized by hemangiopericytomatous staghorn-like branching vessels and so-called “patternless pattern” with variously oriented spindle-shaped tumor cells and collagenous stroma. The tumor cells are usually positive for CD34. The *NAB2-STAT6* fusion gene is also detected by STAT6-immunostaining.[23,24] This specific gene alteration is common with meningeal hemangiopericytoma, and thus both

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**Table 1** Chromosomal translocation and concordant fusion gene in soft tissue tumor

| Tumors | Chromosome | Fusion gene |
|---|---|---|
| Synovial sarcoma | t(X;18)(p11.2;q11.2) | *SS18-SSX1, SSX2* |
| Ewing sarcoma | t(11;22)(q24;q12) | *EWSR1-FLI1* |
| | t(21;22)(q22;q12) | *EWSR1-ERG* |
| | t(7;22)(p22;q12) | *EWSR1-ETV1* |
| | t(17;22)(q12;q12) | *EWSR1-E1AF* |
| Myxoid/round cell liposarcoma | t(12;16)(q13;p11) | *FUS-DDIT3* |
| | t(12;22)(q13;q12) | *EWSR1-DDIT3* |
| Alveolar rhabdomyosarcoma | t(2;13)(q35;q14) | *PAX3-FOXO1* |
| | t(1;13)(q36;q14) | *PAX7-FOXO1* |
| Clear cell sarcoma | t(12;22)(q13;q12) | *EWSR1-ATF1* |
| | t(2;22)(q32.3;q12) | *EWSR1-CREB1* |
| Extraskeletal myxoid chondrosa. | t(9;22)(q22;q12) | *EWSR1-NR4A3* |
| | t(9;17)(q22;q12) | *TAF15-NR4A3* |
| DSRCT | t(11;22)(p13;q12) | *EWSR1-WT1* |
| DFSP/Giant cell fibroblastoma | t(17;22)(q22;q13) | *COL1A1-PFGFB* |
| Infantile/congenital fibrosarcoma | t(12;15)(p13;q25) | *ETV6-NTRK3* |
| Alveolar soft part sarcoma | t(X;17)(p21;q25) | *ASPCR1-TFE3* |
| IMT | t(2;5)(p23;q35) | *TPM3/4-ALK* |
| | t(2;17)(p23;q23) | *CLTC-ALK* |
| | inv(2)(p23q13) | *RANBP2-ALK* |
| Angiomatoid fibrous histiocytoma | t(2;22)(q32.3;q12) | *EWSR1-CREB1* |
| | t(12;22)(q13;q12) | *EWSR1-ATF1* |
| Low grade fibromyxoid sa./ | t(7;16)(q34;p11) | *FUS-CREB3L2* |
| Sclerosing epithelioid fibrosa. | t(11;16)(p11;p11) | *FUS-CREB3L1* |
| Hemosiderotic fibrolipomatous t. | t(1;10)(p22;q24) | *MGEA5-TGFBR3* |
| Myxoinflammatory fibroblastic sa. | | |
| Mesenchymal chondrosarcoma | del(8)(q13.3q21.1) | *HEY1-NCOA2* |
| Epithelioid hemangioendothelioma | t(1;3)(p36.3;q25) | *WWTR1-CAMTA1* |
| Soft tissue myoepithelioma | t(1;22)(q23;q12) | *EWSR1-PBX1* |
| GCT of tendon sheath | t(1;2)(p13;q37) | *COL6A3-CSF1* |
| Solitary fibrous tumor | inv(12)(q13q13) | *NAB2-STAT6* |
| Nodular fasciitis | t(17;22)(p13;q13) | *MYH9-USP6* |
| Pseudomyogenic hemangioendothelioma | t(7;19)(q22;q13) | *SERPINE1-FOSB* |
| Soft tissue angiofibroma | t(5;8)(q15;q13) | *AHRR-NCOA2* |
| CIC-DUX4 sarcoma | t(4;19)(q35;q13) | *CIC-DUX4* |
| | t(10;19)(q26;q13) | *CIC-DUX4* |
| BCOR-CCNB3 sarcoma | inv(X)(p11.4;p11.22) | *BCOR-CCNB3* |
| Phosphaturic mesenchymal t. | t(2;8)(q35;p11) | *FN1-FGFR1* |

DSRCT, Desmoplastic small round cell tumor; DFSP, Dermatofibrosarcoma protuberans; IMT, Inflammatory myofibroblastic tumor; GCT, Giant cell tumor

tumors, SFT and hemangiopericytoma are demonstrated to be molecularly and genetically identical tumor.[25,26]

The prior classification of SFT-associated tumor entities, hemangiopericytoma, giant cell angiofibromas and lipomatous hemangiopericytoma, have been reappraised as cellular, giant cell and lipomatous variants of SFT, respectively. The reappraisal was based on the fact that these previous SFT-associated tumors also have the *NAB2-STAT6* fusion gene and STAT6 nuclear immunoexpression.[27] Classical SFT is now regarded as the fibrous variant of SFT.

Angiofibroma of soft tissue AFST, a new tumor entity not described in the World Health Organization (WHO) classification of 2013, is most often seen in the lower extremities of middle-aged women.[28] Histologically, typical cases show numerous slit-like small vessels and haphazard arrangement of oval-shaped tumor cells in fibromyxoid stroma. However, some cases have prominent hemangiopericyomatous vessels, in which SFT is important for the differential diagnosis. Immunohistochemically, half of all cases are positive for EMA. A specific fusion gene, *AHRR-NCOA2* is also detected by RT-PCR.[29] AFST is regarded as an entirely benign tumor with no metastasis, but in 14% of the cases it shows local recurrence.[28]

We retrieved 13 cases of AFST from among previously diagnosed SFT and unclassified fibrous tumors and analyzed them by histological, immunohistochemical and FISH or RT-PCR methods.[30] Our detailed histological review revealed novel histological variations, such as a multinodular structure and dense fibrous stroma. Moreover, nuclear atypia and pleomorphism were observed in some cases, and these findings could lead to a misdiagnosis of

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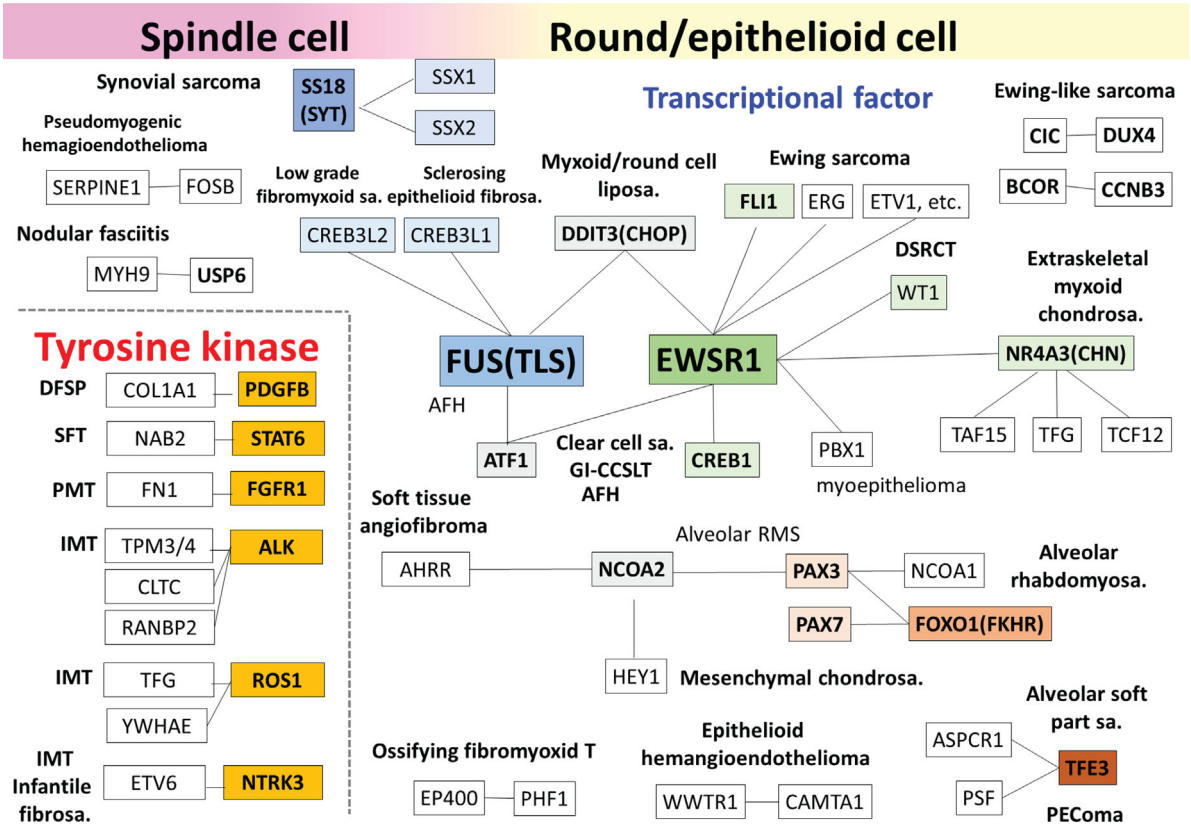


**Figure 2** Fusion genes in soft tissue tumors. PMT, phasphaturic mesenchymal tumor; IMT, inflammatory myofibroblastic tumor; GI-CCSLT, gastrointestinal clear cell sarcoma-like tumor; AFH, angiomatoid fibrous histiocytoma; RMS, rhabdomyosarcoma; DSRCT, desmoplastic small round cell tumor.

**Table 2** Tumors sharing the same fusion gene

| Fusion gene | Tumor type |
|---|---|
| *FUS-ERG* | Ewing sarcoma |
| | t(16;21) acute myeloid leukemia[7] |
| *TMP3/4-ALK* | Inflammatory myofibroblastic tumor[8] |
| | Anaplastic large cell lymphoma[8] |
| *CLTC-ALK* | Inflammatory myofibroblastic tumor |
| | Diffuse large B cell lymphoma[9] |
| | Extramedullary plasmacytoma[10] |
| *RANBP2-ALK* | Inflammatory myofibroblastic tumor |
| | Diffuse large B cell lymphoma[11] |
| | Myeloid leukemia[12] |
| *EWSR1-CREB1/ATF1* | Angiomatoid fibrous histiocytoma |
| | Clear cell sarcoma |
| | Gastrointestinal clear cell sarcoma-like tumor[13] |
| | Primary pulmonary myxoid sarcoma[14] |
| *ETV6-NTRK3* | Infantile fibrosarcoma |
| | Inflammatory myofibroblastic tumor[15] |
| | Congenital mesoblastic nephroma, cellular type[16] |
| | Acute myeloid leukemia[17] |
| | Secretory carcinoma (breast)[18] |
| | Mammary analogue secretory carcinoma (salivary gland)[19] |
| *ASPACR1-TFE3* | Alveolar soft part sarcoma |
| | Renal cell carcinoma[20] |

malignancy. Although no specific immunohistochemical marker has been established in AFST, we found frequent expression of CD163 and estrogen receptor in this entity, and both may be supportive diagnostic markers for AFST.[30] In addition, our results suggested that RT-PCR and FISH methods should be combined for the molecular diagnosis of AFST.

### Small round cell sarcomas with *CIC-DUX4* or *BCOR-CCNB3* fusion genes

Small round cell sarcomas (SRCSs) without known fusion transcripts, such as *EWSR1-FLI1/ERG* in Ewing sarcoma, *SS18-SSX* in poorly differentiated synovial sarcoma, or *PAX3/7-FOXO1* in alveolar rhabdomyosarcoma, have been practically diagnosed as atypical Ewing sarcoma or undifferentiated SRCS, not further specified. Recently, novel fusion genes such as *CIC-DUX4*[31] or *BCOR-CCNB3*[32] were identified from these SRCSs.

*CIC-DUX4* sarcomas arise in the soft tissue of children or young adults as well as in elderly patients.[4,33] *BCOR-CCNB3* sarcomas involve various soft tissues and bone.[4,34,35]

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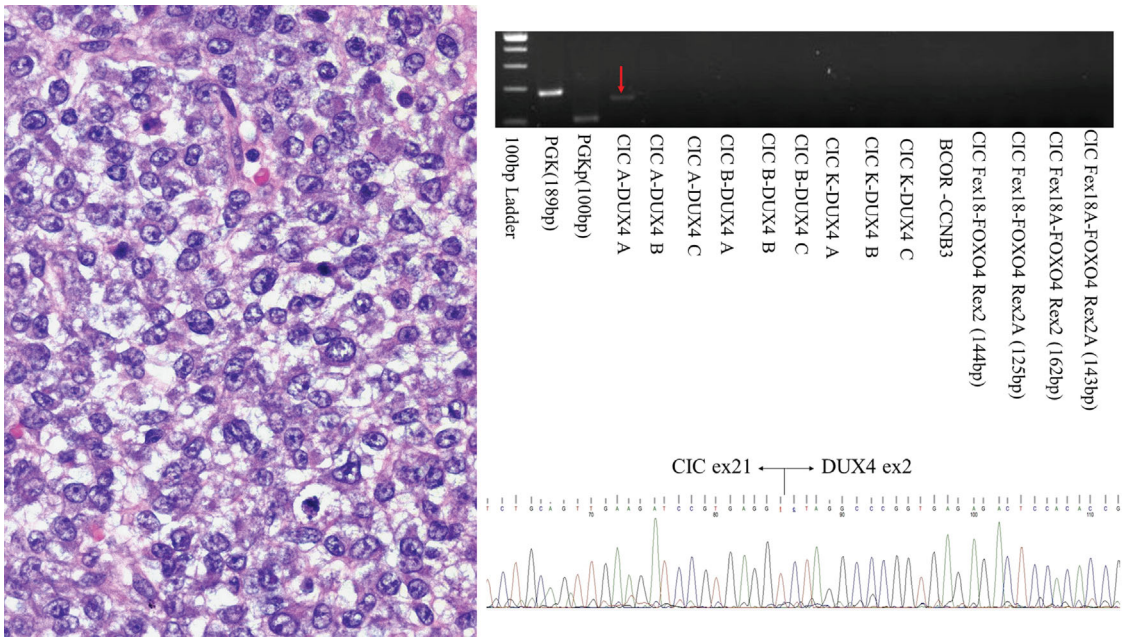


**Figure 3** *DIC-DUX4*-positive sarcoma. Histologically, the tumor is composed of round to oval cells with prominent nucleoli and scanty cytoplasms, arranged in sheets or nests. The fusion transcript was detected between *CIC* gene exon-21 and *DUX4* gene exon-2 by RT-PCR.

As compared with classical Ewing sarcoma, *CIC-DUX4* sarcoma and *BCOR-CCNB3* sarcoma have been reported to show poor[33] and better[34,35] prognosis, respectively.

We retrieved 9 nine cases of *CIC-DUX4*/*CIC* gene rearrangement-positive (Fig. 3) and 7 seven cases of *BCOR-CCNB3* fusion-positive sarcomas after systematic review of 164 unclassified small round cell tumors in our institution using CCNB3 immunohistochemistry, *CIC* gene rearrangement by FISH, detection of *CIX-DUX4* or *BCOR-CCNB3* fusion transcripts by RT-PCR and direct sequencing.[4] We showed that these sarcomas consisted of not only small round-shaped cells, but also an area of short spindle tumor cells on a myxoid background in the case of *BCOR-CCNB3* sarcomas, and areas of epithelioid tumor cells in the case of *CIC-DUX4* sarcomas. Therefore, MPNST, synovial sarcoma and myxofibrosarcoma are important differential diagnoses for *BCOR-CCNB3* sarcoma, and carcinomas, malignant melanoma and epithelioid sarcoma should be differentiated from *CIC-DUX4* sarcoma. These unusual components in both sarcomas showed relatively low proliferative activity, compared with small round cell components. As for an immunohistochemical marker, CCNB3 was confirmed to be a useful and specific marker in *BCOR-CCNB3* sarcoma in our study. On the other hand, expression of TLE1 or CD56 was also frequently observed in our *BCOR-CCNB3* sarcoma cases. Recently, Siegel *et al.* reported that DUX4 immunohistochemistry is sensitive and specific marker for *CIC-DUX4* sarcoma.[36]

## Inflammatory myofibroblastic tumor (IMT)

Inflammatory myofibroblastic tumor was previously called inflammatory pseudotumor (IPT), and indeed, differential diagnosis between IMT and IPT-like lesions is sometimes challenging.[37] About half of the cases of IMT have *ALK* gene rearrangement,[38] but the genetic alterations of the remaining half of cases have been unclear.

According to our recent investigation, 2 out of 36 cases of IMT (5.6%) showed immunohistochemical expression of ROS1, and lacked *ALK* rearrangement.[15] In addition, one case had a *TFG-ROS1* fusion gene transcript, which was detected by RT-PCR.[15]

ETV6 gene rearrangement was also detected in 2 of 36 cases. One case had an *ETV6-NTRK3* fusion gene transcript, which is also identified in infantile fibrosarcoma. Another case was positive for *ETV6* split by FISH, but *ETV6-NTRK3* was not detected. In this particular case, *ETV6* may have been fused with an unknown partner gene other than *NTRK3*.

As demonstrated in the previous investigations, including our own reports, IMT and lung adenocarcinoma share common RTK fusion genes, such as ALK, ROS1, NTRK, and RET.[15,38] Therefore, tyrosine kinase receptor inhibitors, including crizotinib, may be a promising therapeutic strategy in both types of tumors.

Some populations of IMTs have *ETV6-NTRK3* fusion in common with infantile fibrosarcoma.[15] However, the two

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entities have different clinicopathological features, including different age, anatomic sites and histology.

### SMARCB1/INI1-deficient tumors, including malignant rhabdoid tumor (MRT) and epithelioid sarcoma (ES)

Malignant rhabdoid tumor usually arises in a deep axial location in infants and children. This tumor shows very aggressive biological behavior and dismal survival rate. Microscopically, the tumor is composed of a diffuse proliferation of rounded or polygonal tumor cells with eccentric nuclei and prominent nucleoli. Glassy eosinophilic cytoplasms containing hyaline-like inclusion bodies are characteristic.[39] Immunohistochemically, tumor cells are positive for epithelial markers such as cytokeratin and EMA.

Distal-type conventional epithelioid sarcoma (DES) arises in a superficial location in the distal extremities of young adults. The metastatic rate is 40–50% and the 5-year survival rate is 60–80% (WHO). The tumor shows a characteristic pseudogranulomatous pattern with central necrosis on the low power view. The tumor is mainly composed of epithelioid cells, and aggregates of rhabdoid cells are often prominent.[40]

Proximal-type epithelioid sarcoma (PES) predominantly arise in the deep soft tissue of the pelvoperineum, genitals and inguinal tracts. This tumor shows a more aggressive clinical course than DES. The tumor is composed of large epithelioid cells with eosinophilic cytoplasms, vesicular nuclei and prominent nucleoli. Rhabdoid feature is prominent throughout the tumor.[40] Therefore, some investigators have proposed that PES is an adult counterpart of MRT.

The *SMARCB1 /INI1* gene is located in the long arm of chromosome 22. This protein is one of the core subunits of the SWI/SNF ATP-dependent chromatin remodeling complex,[41] and is ubiquitously expressed in the nuclei of all normal cells.[42] In a 1990 report, frequent deletion of 22q was demonstrated in an atypical teratoid/rhabdoid tumor (AT/RT), and the presence of a tumor suppression gene was suspected.[43] In 1998, *SMARCB1/INI1* gene alterations were identified in this region.[44] Subsequently, several investigators demonstrated that not only MRT cases, but the vast majority of DES and PES cases show a complete loss of immunohistochemical SMARCB1/INI1 expression in tumor cells.[45–48] In our series of 54 cases of ES, 76% of PES and 93% of DES revealed loss of SMARCB1/INI1 expression.[49] Other studies demonstrated approximately the same rate of SMARCB1/INI1-deficiency.[45–48] According to our previous investigations of SMARCB1/INI1-deficient tumors, 89% of MRT cases showed *SMARCB1/INI1* gene alterations causing loss of the protein,[50] whereas only 26% of PES cases demonstrated this gene alteration.[49] In DES, no gene alterations related with loss of the SMARCB1/INI1 protein were detected.[49] Therefore, analysis of the *SMARCB1/INI1* gene alteration status may be helpful for distinguishing MRT from ES, especially PES.

In ES, homozygous deletion is predominant and mutations are rare among the *SMARCB1/INI1* gene alterations.[45,49,51,52] However, their relevance tends to vary according to the methods of analysis. Dr. Papp *et al.*[51] demonstrated that no promoter hypermethylation was detected and proposed the possibility of microRNA (miR) regulation of the SMARCB1/INI1 protein. We compared miR expression profiles in SMARCB1/INI1 protein-deficient tumors between MRT with *SMARCB1/INI1* gene alterations and ES without gene alterations. The comparison revealed that miR193a-5p was significantly overexpressed in SMARCB1 deficient tumors without gene alterations.[53] Therefore, miR193a-5p may be correlated with the posttranscriptional regulation of SMARCB1/INI1 protein expression.

We reviewed 52 cases of pediatric SMARCB1/INI1-deficient tumors, including 33 MRT, 11 AT/RT and 8 cases of extra-CNS unclassified sarcoma. We were able to reclassify these tumors into three types, the conventional-type, AT/R-type and small cell-type.[54] The AT/R-type showed the same histological features as CNS AT/RT, and two cases were retrieved from the extra-CNS unclassified sarcomas. The small cell-type mimics malignant lymphoma, and six cases were identified from the unclassified sarcoma. The microRNA expression profiles of the small cell-type unclassified sarcomas were similar to those of conventional MRT. These data support the possibility that this small cell-type is one subtype of MRT.

Aberrant SMARCB1/INI1 expression patterns, such as complete loss, mosaic expression and reduced expression, have been reported to occur in various tumors. The mosaic expression pattern has been recognized in ossifying fibromyxoid tumor,[55,56] gastrointestinal stromal tumor[57] and familial schwannomatosis,[58] and the reduced expression pattern has been reported in synovial sarcoma.[59,60] Kadoch and Crabtree[61] demonstrated that the SS18-SSX fusion protein competes for assembly with wildtype SS18, forming an altered complex that leads to the loss of SMARCB1/INI1 protein expression.

### *MDM2* amplification in well/dedifferentiated liposarcoma and allied tumors

*MDM2* and *CDK4* amplification are characteristic molecular genetic features in well differentiated liposarcoma (WDLS) and dedifferentiated liposarcoma (DDLS). 90% of DDLS cases occur de novo, and 10% develop in recurrence of WDLS.

Microscopically, classical DDLS consists of a WDLS component and undifferentiated high-grade component, and

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transition between the two components is abrupt. The concept of DDLS extends to the tumor composed of a WDLS component and low-grade component, because *MDM2* gene amplification or protein expression has also been demonstrated in the low-grade component.[62,63]

*MDM2* amplification can be detected by FISH analysis, and protein expression is also detectable immunohistochemically. Nuclear expression of MDM2 is observed in the high-grade undifferentiated sarcoma component, which is histologically similar to undifferentiated pleomorphic sarcoma.

The concept of MFH, which was formerly considered the most common soft tissue sarcoma, has also been changed.[64] That is, in the 2002 WHO classification, MFH was renamed UPS.[65]

In the 2013 WHO classification, tumors having pleomorphic, round cell, spindle cell and epithelioid subsets and showing no identifiable line of differentiation were all categorized as undifferentiated/unclassified sarcoma.[66] Among them, tumors with prominent nuclear pleomorphism appeared to correspond with the tumors previously called MFH.

Based on the 2013 WHO classification, the undifferentiated/unclassified sarcoma, including so-called MFH and other unclassified round/spindle/epithelioid cell sarcomas, are the second most common tumors in our series, accounting for 16.7% of all soft tissue sarcomas in the 3520 sarcoma cases we collected.

Recently, the question of whether peripheral so-called MFH/UPS with *MDM2* gene amplification or expression are actually DDLPS attracted attention. A French group reported in 2014 that peripheral MFH/UPS without any lipomatous component but with *MDM2* amplification are molecular-genetically DDLS.[67] Recently, such tumors have tended to be diagnosed as DDLS, despite the absence of a lipomatous component.

The retroperitoneum is a common anatomical site of involvement of soft tissue leiomyosarcoma. It is also known that a certain population of DDLS shows myogenic differentiation in the dedifferentiated component. Therefore, previously diagnosed retroperitoneal leiomyosarcoma-like tumors may share a lineage with DDLS. In our series, four of 20 cases of previously diagnosed retroperitoneal leiomyosarcoma were immunohistochemically positive for MDM2, and *MDM2* gene amplification was detected in these four cases by FISH analysis.[68] These leiomyosarcoma-like tumors lack any lipomatous component, even after extensive sampling. Therefore, it may be appropriate to reclassify these tumors as DDLS with smooth muscle differentiation. On the other hand, 42% of 36 conventional DDLS cases were positive for at least two myogenic markers, including smooth muscle actin, desmin, calponin and h-caldesmon. Based on these findings, we considered that evaluation of *MDM2* gene amplification is necessary to distinguish retroperitoneum leiomyosarcoma from DDLS.

## A MOLECULAR-BIOLOGIC APPROACH TO IDENTIFYING THERAPEUTIC MOLECULAR TARGETS

### Akt-mTOR pathway

The Akt/mTOR pathway is located in the region downstream of several growth factor receptors, and it has been reported to be activated and correlated with cell proliferation, survival and protein synthesis in malignant tumors.[69,70] Recently, mTOR inhibitors have been shown to be useful for molecular target therapy in certain kinds of malignant tumors. A recent large randomized phase III trial evaluated the effects of an mTOR inhibitor against 711 metastatic sarcomas, including tumors with several types of histological phenotype.[71] Although ridaforolimus, an mTOR inhibitor, led to a significant improvement in progression free survival, there was no significant improvement in overall survival.[71] We considered that a detailed evaluation of the effect of mTOR inhibitors in each histological phenotype would be needed in order to examine its therapeutic potential.

First, we performed an immunohistochemical study to evaluate the activation profiles of Akt/mTOR pathway proteins using 135 MPNST samples, and found that phosphorylated-AKT (p-AKT), p-mTOR, p-S6RP, p-p70S6K and p4EP1 expressions were positive in more than 50% of primary MPNSTs.[72] In MPNST associated with neurofibromatosis type 1, these phosphorylated protein expressions were mainly observed in the malignant component, whereas they were absent in the benign neurofibroma component. By Western blot, the expression levels of p-Akt, p-mTOR, p-p70S6K in tumor tissue were higher than those in normal tissue. An in vitro study demonstrated that an mTOR inhibitor, everolimus, inactivated S6RP expression and suppressed proliferation, motility and invasion, without paradoxical activation of the Akt/ MAPK pathway in six MPNST cell lines. In clinical samples, the cases with Akt/mTOR/S6 pathway activation showed significantly worse prognosis. These results indicated that mTOR inhibitors may be candidates for novel anticancer drugs for MPNST.[72]

We also demonstrated that the Akt/mTOR pathway was activated and associated with high grade histology or worse prognosis in patients with other spindle cell sarcomas such as leomyosarcoma,[73] synovial sarcoma,[74] SFT[75] and myxofibrosarcoma.[76]

It is well known that there is cross-talk between the Akt/mTOR and MAPK pathways.[77,78] Several authors have reported that dual inhibition of both pathways enhanced the antitumor effect.[77,79] We clarified the status of these pathways in DDLS.[80] Immunohistochemically, positive staining

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for phosphorylated mTOR and MEK was significantly more frequent in the dedifferentiated component than the well-differentiated component. An *in vitro* study demonstrated that mTOR and MEK inhibitors dose-dependently decreased the expression of downstream phosphorylated S6RP and phosphorylated ERK, respectively. However, after mTOR inhibition, the phosphorylated ERK expression of one cell line was up-regulated, which indicated that there was cross-talk between the Akt/mTOR and MAPK pathways in DDLS. The combined administration of the two inhibitors enhanced the anti-proliferative activity against two cell lines, especially FU-DDLS-1, compared with administration of a single inhibitor. These findings suggest that these pathways could be a therapeutic target for patients with DDLS.[80]

HSP90, a member of the HSP family, refolds and activates certain denatured proteins known as "client proteins" under stress conditions. One of the important client proteins is Akt.[81] We analyzed the HSP90 expression, the Akt/mTOR pathway activation and the correlation between HSP90 expression and its pathway activation[82] in UPS.[83] Immunohistochemically, HSP90 expression was correlated with p-Akt, p-mTOR and p-S6RP. The univariate and multivariate analysis indicated that the positivities for p-Akt, p-mTOR, p-S6RP and HSP90 were poor prognostic factors in UPS. *In vitro* study showed that an HSP90 inhibitor led to decreased viability and invasiveness of UPS cells and inactivated the Akt/mTOR pathway without enhancing the MAPK pathway. Therefore, we considered that HSP90 inhibition is a potential treatment option for UPS.[82]

## Transcription factor FOXM1

FOXM1 is a transcription factor overexpressed in a wide variety of carcinomas. FOXM1 is associated with many functions of tumor cells, such as DNA repair, cellular proliferation, angiogenesis, cancer drug resistance, cellular senescence and invasion.[84]

We performed a cDNA microarray analysis of synovial sarcoma using clinical samples.[85] The genes correlated with *FOXM1* were retrieved after the clustering of cDNA levels. Functional analysis revealed that many of the retrieved genes were associated with cell cycle and mitosis. FOXM1 expression was observed in the tumor cell nuclei of synovial sarcoma by immunohistochemical staining. The cases with high FOXM1 expression showed significantly worse prognosis, compared with those with low FOXM1 expression. Based on the results from these clinical samples, the effect of FOXM1 knock-down was examined in synovial sarcoma cell lines. *FOXM1* knock-down inhibited the cell proliferation and increased doxorubicin-sensitivity. Moreover, cellular proliferation was decreased in a concentration dependent manner by thiostrepton, a FOXM1 inhibitor.

In addition, the combination of doxorubicin and thiostrepton inhibited cellular proliferation more strongly. Thus, FOXM1 was proposed as a novel prognostic factor or therapeutic target in synovial sarcoma.[85] The same results were also obtained in clinical samples and cell lines of leiomyosarcoma.[86] According to our previous investigations, FOXM1 expression was also correlated with poor prognosis and alveolar histology in rhabdomyosarcoma.[87] *In vitro* study demonstrated that *FOXM1* inhibition caused decreases in VEGF expression, invasiveness and migration activity in rhabdomyosarcoma cell lines. In the patients with angiosarcoma, FOXM1 expression also had significant correlation with adverse prognosis, and in angiosarcoma cell lines, a correlation between *FOXM1* inhibition and decreased proliferative activities or increased sensitivity for docetaxel was observed.[88] Based on these results, FOXM1 was thought to be a potential therapeutic target of various soft tissue sarcomas.

## Cancer testis antigen

Recently, immunotherapy has been under development as a novel therapeutic strategy against malignant tumors. Cancer testis antigens (CTAs) are considered promising immunotherapy targets because of their confined expression in normal tissue.[89] Several investigators demonstrated frequent expression of NY-ESO-1, one of CTAs, in synovial sarcoma (SS)[90] and myxoid/round cell liposarcoma (MRLS).[91,92]

We found that PRAME was also highly expressed in MRLS, using cDNA microarray.[93] PRAME is also one of the CTAs, and it is also highly expressed in various kinds of cancers and is a candidate target of immunotherapy. Next, therefore, we examined PRAME and NY-ESO-1 expression in each of the histological subtypes of liposarcomas.[93] Immunohistochemical study showed that both PRAME and NY-ESO-1 were expressed in the vast majority of MRLS (Fig. 4a,b), and their expression levels were higher in MRLS than other liposarcoma types. High expression of PRAME or NY-ESO-1 was correlated with larger tumor size, presence of necrosis, a more than 5% of round cell component, higher histological grade, higher clinical stage and worse prognosis. The results of immunostaining and Western blotting or quantitative RT-PCR were well correlated. According to these findings, we concluded that PRAME and NY-ESO-1 could be not only useful prognostic markers but also immunotherapeutic targets for MRLS.[93]

We also conducted similar investigations in SS.[94] NY-ESO-1, PRAME, and MAGEA4 were frequently expressed in SS specimens (Fig. 4c,d) and the triple-positive SS patient group showed the worst prognosis. Therefore, these results indicate that the potential utility of NY-ESO-1,

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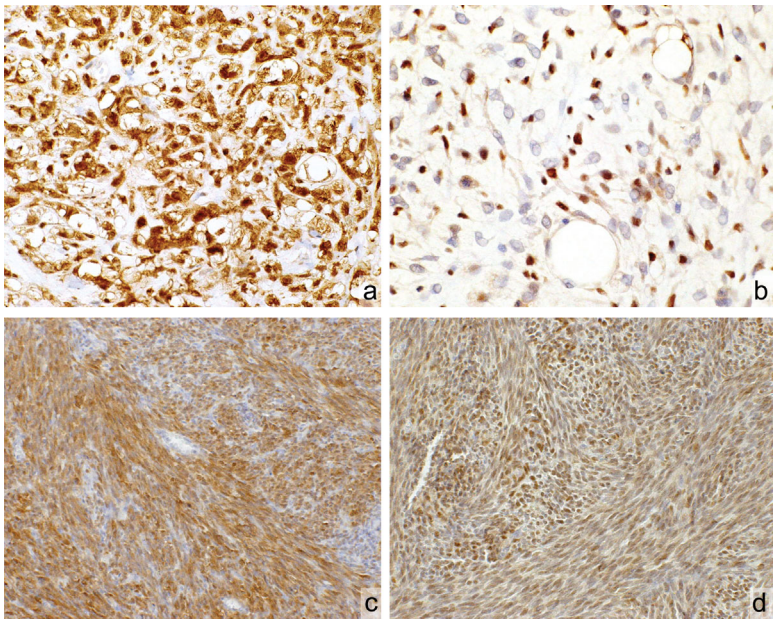


**Figure 4** NY-ESO-1 **(a)** and PRAME **(b)** expression in myxoid/round cell liposarcoma. NY-ESO-1 **(c)** and PRAME **(d)** expression in biphasic **(c)** and monophasic fibrous **(d)** synovial sarcoma.

PREME, and MEGE4 as immunotherapy targets and ancillary biomarkers in SS.[94]

We also compared the expression profiles of CTAs between uterine leiomyosarcoma (ULMS) and non-uterine soft tissue leiomyosarcoma (NULMS).[95] ULMS showed significant frequent expression of MAGEA1, MAGEA3, MAGEA4 and GAGE7, compared with NULMS, suggesting possible utility of these CTAs in ULMS as an immunotherapy target.[95]

## CONCLUSION

Molecular genetic investigation of soft tissue sarcomas may reveal further novel tumor entities according to their specific genotypes. However, it is important to evaluate both histopathological findings and molecular features together, because certain tumors with different morphologies or biological behaviors may share the same genotype. This line of research may also lead to development of novel molecular target therapies in soft tissue sarcomas, a rare and complex cancer.

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## DISCLOSURE STATEMENT

None declared.

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