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Review Article

Molecular classification of soft tissue sarcomas and its clinical applications

Shilpa Jain[1], Ruliang Xu[1], Victor G. Prieto[2], Peng Lee[1,3,4,5]

Department of [1]Pathology, [3]Urology and [4]Cancer Institute, New York University School of Medicine, [5]New York Harbor Healthcare System, New York, NY and [2]Department of Pathology, UT, M. D. Anderson Cancer Center, Houston, TX

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Abstract: Sarcomas are a heterogeneous group of tumors that are traditionally classified according to the morphology and type of tissue that they resemble, such as rhabdomyosarcoma, which resembles skeletal muscle. However, the cell of origin is unclear in numerous sarcomas. Molecular genetics analyses have not only assisted in understanding the molecular mechanism in sarcoma pathogenesis but also demonstrated new relationships within different types of sarcomas leading to a more proper classification of sarcomas. Molecular classification based on the genetic alteration divides sarcomas into two main categories: (i) *sarcomas with specific genetic alterations*; which can further be subclassified based on a) reciprocal translocations resulting in oncogenic fusion transcripts (e.g. EWSR1-FLI1 in Ewing sarcoma) and b) specific oncogenic mutations (e.g. KIT and PDGFRA mutations in gastrointestinal stromal tumors) and (ii) *sarcomas displaying multiple, complex karyotypic abnormalities with no specific pattern*, including leiomyosarcoma, and pleomorphic liposarcoma. These specific genetic alterations are an important adjunct to standard morphological and immunohistochemical diagnoses, and in some cases have a prognostic value, e. g., Ewing family tumors, synovial sarcoma, and alveolar rhabdomyosarcoma. In addition, these studies may also serve as markers to detect minimal residual disease and can aid in staging or monitor the efficacy of therapy. Furthermore, sarcoma-specific fusion genes and other emerging molecular events may also represent potential targets for novel therapeutic approaches such as Gleevec for dermatofibrosarcoma protuberans. Therefore, increased understanding of the molecular biology of sarcomas is leading towards development of newer and more effective treatment regimens. The review focuses on recent advances in molecular genetic alterations having an impact on diagnostics, prognostication and clinical management of selected sarcomas.

Keywords: Sarcomas, soft tissue tumors, molecular classification, specific genetic translocation, molecular genetics,

INTRODUCTION

Sarcomas are relatively rare malignant tumors arising in connective tissues, including fat, muscle, blood vessels, deep skin tissues, nerves, bones and cartilage, comprising less than 10% of all malignancies. Sarcomas are widely believed to develop as a result of genetic mutations in mesenchymal progenitor/ stem cells, but the precise cellular origin of most of these tumors remains unknown. Based upon molecular cytogenetic analysis, sarcomas can be divided into two main categories (**Table 1**): (i) sarcomas with specific genetic alterations, which can further be subclassified based on a) reciprocal translocations resulting in oncogenic fusion transcripts e.g. EWSR1-FLI1 in Ewing sarcoma, FUS-DDT3 in myxoid liposarcoma, and SS18-SSX in synovial sarcoma, and b) specific oncogenic mutations e.g. KIT and PDGFRA mutations in gastrointestinal stromal tumors and (ii) sarcomas displaying multiple complex karyotypic abnormalities with no specific pattern, including malignant fibrous histiocytoma (MFH / undifferentiated sarcoma), leiomyosarcoma, and pleomorphic liposarcoma. Fusion genes are most readily detected by fluorescence in situ hybridization and reverse transcription-PCR technologies. These specific translocations or characteristic genetic alterations aid in the diagnosis of sarcomas type and, in some cases, impart prognostic or predictive information influencing clinical management. The genetic abnor-

malities associated with sarcomas also represent potential targets for novel therapeutic approaches, which are desperately required to improve outcome in certain categories of sarcomas. Here we review sarcomas that have associated molecular genetics and its clinical application to diagnosis, prognosis or clinical management.

SARCOMAS WITH SPECIFIC GENETIC ALTERATIONS

Sarcomas with reciprocal translocation encoding specific fusion genes

The unique genetic events associated with sarcomas may give rise to at least three types of oncogenic mediators: aberrant transcription factors; constitutively active receptor tyrosine kinases (RTKs) and constitutively active growth factors. This leads to further subdivision of this category.

Fusion genes involving TET genes

Nearly half of the fusion proteins believed to participate in sarcoma initiation and development contain a portion of TET gene family products, named after the initials of TLS/FUS, EWSR1, and TAFII68. TET-family proteins contain a characteristic 87-aminoacid-RNA recognition motif that is thought to be implicated in protein-RNA binding and to participate in transcription and RNA metabolism. [1]

ET family members may be interchangeable in the development of a subgroup of sarcomas. Thus, both EWSR1-DDIT3 and FUS-DDIT3 are found in myxoid liposarcoma with an indistinguishable phenotype; EWSR1-ERG as well as some FUS-ERG fusions are associated with Ewing Sarcoma Family Tumors, and both EWSR1 and TAFII68 fused to NR4A3 are found in myxoid chondrosarcoma [1].

Ewing sarcoma/Primitive peripheral neuroectodermal tumor (PNET)

Ewing sarcoma is a small blue cell tumor that primarily affects long bones or the vertebral area in young adults and children. Histologically, it is characterized by sheets of small round cells that may form Homer-Wright rosettes. Along with PNET, Ewing sarcomas are referred to as Ewing Sarcomas Family Tumors (ESFT), in which lesions with evidence of neuroendocrine differentiation are sometimes subclassified as primitive neuroectodermal tumor (PNET) [2]. However, the distinction between Ewing sarcoma and PNET is no longer considered to be critical for clinical management as their prognosis and therapy is similar.

ESFTs are the prototype of tumors with fusion genes involving the TET gene family. They characteristically harbor a recurring t(11;22)(q24;q12) that juxtaposes the FLI1 and EWSR1 genes encoding a chimeric RNA and protein. About 10% of Ewing family tumors have an alternative translocation involving the EWSR1 gene, implying that disruption of EWSR1 is the critical molecular event underlying tumorigenesis. Except for FUS–ERG, all fusion transcripts identified so far in ESFTs consist of EWSR1 and an ET-family transcription factor, including the FLI1, ERG, ETV1, ETV4, and FEV genes[3]. Approximately 85–90% of EFSTs are associated with the EWSR1–FLI1 fusion gene, 9–14% with EWR1–ERG, and the remaining 1–5% with other rare variants[3].

Karyotype is an analytic method for the initial workup of a suspected Ewing family tumor because the characteristic t(11;22) is evident in approximately 90% of the tumors. However, in a case of strong suspicion of Ewing sarcoma/PNET with apparent normal karyotype, fluorescence in situ hybridization (FISH) should be considered as an additional test to try to detect the genetic defects. FISH, using a break-apart probe targeting the EWSR1 gene, is an excellent method to identify or exclude an EWSR1 gene rearrangement. FISH may be carried out on metaphase cells (requiring fresh tissue) or on interphase cells (feasible on a wide variety of sample types including fine needle aspirates, touch preparations, smears, or paraffin-embedded tissue) [4]. One advantage unique to reverse transcription polymerase chain reaction (RT-PCR) is its ability to define which of two prognostically relevant transcript structures is present: type 1 fusion transcripts (EWSR1-FLI1) are not transcribed as actively and carry a good prognosis compared with the alternative fusions [5]. Another advantage of RT-PCR is to detect minimal residual disease after therapy [6]. Immunohistochemical analysis of CD99 and FLI1 reveal overexpression in most Ewing/PNET tumors harboring a t(11;22) [7].

Desmoplastic small round cell tumor (DSRCT)

DSRCT exhibits nests and cords of primitive small cells enclosed by an abundant collagenous (desmoplastic) stroma arising most commonly in the abdomen of adolescents and young adults. DSRCT often resembles other small round cell tumors but it characteristically displays a multilineage differentiation immunophenotype with expression of epithelial, mesenchymal (fibrous and/or myogenic), neural, and WT1 markers [8-10]. These tumors usually harbor a recurring t(11;22) involving the EWSR1 gene on chromosome 22 and the WT1 (Wilms tumor 1) gene on the short arm of chromosome 11 (11p13). In contrast, the t(11;22) described above in association with Ewing sarcoma involves the FLI1 gene in the long arm of chromosome 11 (11q24). The appearance of the derivative chromosome 11, as analyzed by karyotype, can therefore distinguish these alternative gene partners. Another method to distinguish the two alternative gene partners for EWSR1 is cDNA amplification across the breakpoint of each fusion transcript. The 5' portion of the EWSR1 gene apparently controls the expression of the fused EWSR1-WT1 chimeric protein, whereas the 3' end of WT1 functions as a transcription factor that up-regulates oncogenic factors, such as platelet-derived growth factor (PDGF). The fibrosis/desmoplasia that is typical of this subtype of sarcoma is probably a consequence of PDGF-related recruitment of fibroblasts, and thus PDGF inhibitors are being explored as novel treatment strategies.

Clear cell sarcoma (CCS)

CCS, also known as melanoma of soft parts, is a rare but distinct neoplasm, which has a predilection for occurring in the tendons and aponeuroses of the distal extremities in adolescents and young adults.

CCS is characterized by a nested or fascicular growth of pale-staining or clear cells displaying phenotypic features shared with malignant melanoma, including the presence of melanin, ultrastructural evidence of melanosomes, and immunohistochemical expression of S-100 protein and melanoma-associated markers such as gp100 (with HMB-45), Melan-A, and microphthalmia transcription factor (MITF). The specific recurrent chromosomal translocation t(12;22)(q13;q12) or a resultant fusion of the EWSR1 gene on 22q12 and the ATF1 gene on 12q13 are detected in most cases with CCS. ATF1 is a member of the CREB transcription factor family with bZip, via which ATF-1 binds to CRE following activation by cAMP-dependent protein kinase A (PKA). Upon fusion with EWSR1, the PKA site of ATF-1 was replaced by the N-terminus of EWSR1, resulting in a cAMP-independent and constitutively active transactivator that is driven by the EWSR1 promoter. EWSR1–ATF1 binds to the promoter region and induces the expression of the MITF gene, a master regulator of melanocyte differentiation. Upregulation of MITF expression is found in CCS, endowing tumor cells with melanocytic features. Recently, another variant fusion gene, EWSR1-CREB1, probably resulting from a yet elucidated chromosomal translocation t(2;22)(q34;q12), has been found in a subset of CCS that arise exclusively in the gastrointestinal tract [11]. The gene product afforded by this translocation, the EWSR-ATF1 fusion transcript, can be detected by RT-PCR, and thus allowing diagnosis of clear cell sarcoma by molecular means.

Angiomatoid fibrous histiocytoma (AFH)

Angiomatoid fibrous histiocytoma (AFH) is a distinct soft tissue tumor of intermediate malignancy with a partial myoid phenotype. Despite not having obvious melanocytic differentiation, it shares a similar fusion gene, EWSR1–ATF with CCS.

Extraskeletal Myxoid Chondrosarcoma

Cytogenetic studies have demonstrated the presence of a translocation, t(9;22)(q22;q12), in approximately 70% of extraskeletal myxoid chondrosarcomas [12]. This translocation results in the fusion of the EWS gene on chromosome 22 with a novel orphan nuclear receptor gene, designated CHN (also known as TEC or NOR1), on chromosome 9 [13]. Additional fusion partners to CHN were subsequently identified (TAF2N, TCF12, and TFG) [14, 15]. Skeletal myxoid chondrosarcomas analyzed by RT-PCR lack this translocation, suggesting that extraskeletal myxoid chondrosarcomas and skeletal myxoid chondrosarcomas represent two distinct entities [16]. cDNA microarray analyses of extraskeletal myxoid chondrosarcomas samples exhibited uniform expression of CHI3L1, which encodes for a secreted glycoprotein (YKL-40, cartilage glycoprotein-39) that has been

implicated in various pathological conditions of extracellular matrix degradation as well as in cancer [14].

Myxoid/Round Cell Liposarcoma

Myxoid/round cell liposarcoma comprises 50% of all liposarcoma arising usually in the thigh. Myxoid liposarcoma is composed of univacuolar and multivacuolar lipoblasts embedded in a richly myxoid ground substance. Characteristically, there is an acute angle (plexiform) branching capillary vasculature. Round cell, also called hypercellular liposarcoma is characterized by a relative increase in the cellularity of the tumor so that individual tumor cells lie in direct continuity with each other without intervening matrix. Myxoid/round cell liposarcoma is treated by wide surgical excision with or without radiation therapy. Approximately 30% of patients develop distant metastases. Most myxoid/round cell liposarcomas demonstrate a specific chromosomal translocation t(12;16)(q13;p11) that results in the rearrangement of the CHOP and FUS genes. A minority demonstrates variants of this translocation that typically also involve the 12q13 breakpoint.

Low-grade fibromyxoid sarcoma (LGFMS)

LGFMS, also known as Evans tumor, usually occurs in deep soft tissue, and is characterized by a very bland histological appearance. The translocation t(7;16)(q33;p11) so far seems to be specific for LGFMS resulting in the FUS-CREB3L2 fusion gene. Interestingly, most of the break points are located within exon 6, intron 6, or exon 7 of the FUS gene and within exon 5 of the CREB3L2 gene; those of other translocation-associated sarcomas are usually positioned within introns. As FUS and CREB3L2 gene segments having break points within exons may be directly fused without interposed introns in many LGFMSs, DNA-based PCR for detecting the FUS-CREB3L2 fusion gene also seems to be applicable even when using formalin-fixed, paraffin-embedded (FFPE) tumor tissues, despite the limited length of extracted nucleic acids (e.g. DNA). FISH is another tool for detecting tumor-associated fusion genes using FFPE tumor specimens. FISH provides several advantages in comparison to RT-PCR-based or DNA-based PCR method: the possibility of contamination can be ruled out when using FISH on a histologic section, and one examination can detect any variants of FUS rearrangement including FUS-CREB2L2 with variable breakpoints and FUS-CREB3L1.[17]

Fusion genes involving the genes encoding receptor tyrosine kinases (RTKs) genes

Congenital Mesoblastic Nephromas (CMN)

CMN are uncommon renal tumors diagnosed generally within the first 3 months of life. CMN are characterized by a variably cellular proliferation of bland spindle cells arranged in interlacing bundles, and their clinical behavior is generally benign. Recently, CMN were shown to contain a novel t(12;15)(p13;q25) translocation, resulting in ETV6-NTRK3 gene fusion. ETV6 is a member of the ETs family of transcription factors containing a basic helix-loop-helix (bHLH) dimerization domain, which was originally found at the breakpoint in translocations in leukemia and myeloproliferative syndromes. NTRK3 is the cell surface receptor for neurotropin 3 expressed primarily in the central nervous system. The ETV6–NTRK3 fusion protein forms a homodimer or heterodimer with wild-type NTRK3, which displays receptor tyrosine kinase (RTK) activity and undergoes autophosphorylation at tyrosine domain [18].

Cytogenetic t(12;15) translocations may not identified in several cases (CMN4, CFS1, and CFS5), although RT-PCR and FISH analyses reveal ETV6-region rearrangements in those same cases. The detection of ETV6-NTRK3 fusion transcripts in most CMN using RT-PCR methods with archival, FFPE tissues therefore is a useful diagnostic adjunct.

Congenital Fibrosarcoma (CFS)

CFS is an uncommon pediatric soft tissue sarcoma with low-grade behavior, principally arising in the extremities, generally in the first year of life. CFSs have broad histological overlap with CMNs, and their clinical course is relatively benign, especially in comparison with the aggressive clinical behavior of histologically similar fibrosarcomas in adult patients. CFSs contain the same t(12;15)(p13;q25) translocation described recently in CMN. This translocation is associated with an ETV6-NTRK3 fusion gene, in which the ETV6 HLH domain is coupled with the NTRK3 tyrosine kinase residues [19].

Table 1. Sarcomas with specific alterations

Sarcomas with fusion genes					
Fusion genes involving TET genes					
	Gene (N-C)	Chromosomal location	Clinical Significance	Proposed function of gene product	Detection Method
Ewings/PNET	*EWSR1- FLI1* *EWSR1- ERG* *EWSR1-ETV1* *EWSR1-ETV4* *EWSR1- FEV* *FUS-ERG* *FUS-FEV* *EWSR1-ZSG*	t(11;22)(q24;q12) t(21;22)(q22;q12) t(7;22)(p22;q12) t(17;22)(q12;q12) t(2;22)(q33;q12) t(16;21)(p11;q22) t(2;16) inv (22)	Diagnostic EWSR1 associated with Good prognosis	Overexpression of Oncogene e.g. *MYC, ID2, CCND1, IGF1*	IHC (FLI1) Karyotype FISH (EWSR1 break apart probe), RT-PCR
Desmoplastic Small Round Cell Tumor	*EWSR1- WT1* *EWSR1-ERG*	t(11;22)(p13;q12) t(21;22)(q22;q12)	Diagnosis, therapeutic (PDGF inhibitors)	up-regulates oncogenic factors e.g. *PDGF, IL2Rβ, BAIALP3, TALLA1,MLF1*	IHC (WT1) FISH (EWSR1 break-apart probe) Karyotype
Clear cell sarcoma(CCS)	*EWSR1-ATF1* *EWSR1-CREB1*	t(12;22)(q13;q12) t(2;22)(q33;q12)	Diagnosis	Up-regulation of *ARNT2,ATM, GPP34, MITF* gene	FISH (EWSR1 break apart probe), PCR
Angiomatoid Fibrous Histiocytoma	*FUS-ATF1* *EWSR1-ATF1* *EWSR1-CREB1*	t(12;16)(q13;p11) t(12;22)(q13;q12) t(2;22)(q33;q12)	Diagnosis		FISH
Extraskeletal myxoid chondrosarcoma	*EWSR1-NR4A3* *TAF2N-NR4A3* *TCF12-NR4A3* *TFG-NR4A3*	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;15)(q22;q21) t(9;22)(q22;q15)	Diagnosis		FISH, RT-PCR (NR3A3-EWS fusion)
Myxoid/ round cell liposarcoma	*FUS-DDIT3 (CHOP)* *EWSR1-DDIT3 (CHOP)*	t(12;16)(q13;p11) t(12;22)(q13;q12)	Diagnosis, Potential therapeutic	Overexpression of MDM2, CDK4,*MET.PDGFα*	FISH (FUS break-apart probe)
Low Grade Fibromyxoid Sarcoma / HSCT	*FUS-CREB3L2* *FUS-CREB3L1*	t(7;16)(q33;p11) t(11;16)(p11;p11	Diagnosis		FISH (FUS break-apart probe), RT-PCR
Fusion genes involving RTK genes					
Congenital mesoblastic nephroma	*ETV6-NTRK3*	t(12;15)(p13;q25)	Diagnosis		FISH, RT-PCR
Congenital fibrosarcoma	*ETV6-NTRK3*	t(12;15)(p13;q25)	Diagnosis		FISH, RT-PCR
Inflammatory myofibroblastic tumor	*TPM3-ALK* *TPM4-ALK* *CLTC-ALK* *RANBP2-ALK*	t(1;2)(q25;q23) t(2;19)(q23;q13) t(2;17)(q23;q23) t(2;2)(p23;q13)	Diagnosis		IHC (ALK protein) FISH, RT-PCR
Fusion genes involving chromatin remodeling genes					
Synovial sarcoma	*SS18-SSX1* *SS18-SSX2* *SS18-SSX4* *SS18L1-SSX1* *TLE1 gene*	t(X;18)(p11;q11) t(X;18)(p11;q11) t(X;18)(p11;q13) t(x;20)(p11;q13)	Diagnosis, Better prognosis of SS18-SSX2		FISH (SYT probe), RT-PCR, IHC (TLE1 protein)

(Continued **Table 1**)

Endometrial stromal sarcoma	*JAZF1-SUZ12* *JAZF1-PHF1* *EPC1-PHF1*	t(7;17)(p15;q21) t(6;7)(p21;p15) t(6;10)(p21;p11)	Diagnosis		RT-PCR
Fusion genes involving growth factors genes					
Dermatofibrosarcoma protuberans	*COL1A1-PDGFB*	t(17;22)(q22;q13)	Diagnosis, Therapeutic (Gleevec responsive)	Up-regulate the expression of *PDGFR*	FISH, RT-PCR
Giant Cell Fibroblastoma	*COL1A1-PDGFB*	t(17;22)(q22;q13)	Diagnosis		FISH, RT-PCR
Other type of fusion genes					
Alveolar Rhabdomyosarcoma	*PAX3-FOXO1A* *PAX7-FOXO1A* *PAX3-MLLT7* *PAX3-NCOAI*	t(2;13)(q35;q14) t(1;13)(q36;q14) T(2;X)(p35;q13) T(2;2)(q35;q23)	Diagnostic Better Prognosis with PAX7-FOXO1A		FISH (*FOXO1A* Break-apart probe), Karyotype, RT-PCR
Alveolar soft part sarcoma	*ASPSL-TFE3*	t(X;17)(p11;q25)	Diagnosis		IHC (TFE3), RT-PCR
Aneurysmal bone cyst	*CDH11-USP6* *THRAP3-USP6* *CNBP-USP6* *OMD-USP6* *COL1A1-USP6*	t(16;17) t(1;17) t(3;17) t(9;17) t(17;17)	Diagnosis		FISH, RT-PCR
Tenosynovial giant cell tumor	*CSF1-COL6A3*	t(1;2)	Diagnosis		
Hemangiopericytoma		t(12;19)	Diagnosis		
Pericytoma	*ACTB-GLI1*	t(7;12)	Diagnosis		
Sarcomas with specific oncogenic mutation					
Gastrointestinal Stromal Tumors	KIT or PGDFRA	Occult 4q12	Diagnosis, *C*-Kit Gleevac responsive	Activation Tyrosine kinase Receptor	IHC (C-Kit), PCR
Rhabdoid tumor	SMARCB1	del 22q11.22	Diagnosis	LOH	IHC (loss of INI1)
Atypical lipomatous tumor/ Well-differentiated liposarcoma	giant marker and in*Micatio*3		Cyclin dependent kinaser	FISH (*MDM2, CDK4 amplification)*	
Fibromatosis	APC inactivation	Trisomies 8 and 20 Deletion of 5q	Diagnosis		IHC(β catenin)
Sarcoma with variable complex genetic alteration (discussed in text)					

Inflammatory Myofibroblastic Tumor (IMT)

IMTs are mesenchymal solid tumors that occur preferentially in children and young adults. They present as myofibroblastic cell proliferations accompanied by plasmocytes and lymphocytes. Recent cytogenetic studies showed abnormalities of chromosomal band 2p23 resulting in a rearrangement of the ALK gene, thus suggesting a neoplastic rather than a reactive inflammatory process. ALK is a tyrosine kinase oncogene initially found to be rearranged in anaplastic large-cell lymphomas (ALCL). NPM-ALK fusions, found in 80% of ALCL, have not been identified in IMT and TMP3-ALK fusions are rare in ALCL [20]. Interestingly, the breakpoints within ALK, and also within some of the ALK fusion gene partners, such as TPM3 or CLTC, are similar in IMT and ALCL. The consistent involvement of ALK, together with the diversity of partner genes, underlines both the central role of ALK constitutive activation in IMT development, as well as the importance of homodimerization mechanisms of the chimeric fusion proteins in this activation. Immunohistochemical analyses performed on PPFE sections have shown positive ALK expression with cytoplasmic localization in half of the IMT cases containing the molecular ALK rearrangement. Therefore, detection of a rearrangement of ALK by FISH is a useful, complementary tool for IMT diagnosis.

Fusion genes involving chromatin-remodeling genes

Synovial Sarcoma (SS)

SS, often arises deep in the soft tissue near a joint in the extremity of a young adult patient. Most synovial sarcomas are characterized by t(X;18)(p11.2;q11.2), resulting in a fusion between the SS18(SYT) gene on chromosome 18 and one of the SSX genes on the X chromosome, creating SS18-SSX1, SS18-SSX2 or SS18-SSX4 chimeric genes [21-27]. There are two common histologic and genetic variants of synovial sarcoma: (i) a monophasic variant comprised of vimentin-expressing spindle cells, usually carrying the SS18-SSX2 translocation and (ii) a biphasic variant comprised of a mixture of vimentin-expressing spindle cells and keratin-expressing glandular epithelial cells harboring the SS18-SSX1 or SS18-SSX2 translocation [28]. The biphasic variant may resemble adenocarcinoma but more typically resembles carcinosarcoma and, at least in early stage patients, carries a worse prognosis.

Karyotype is helpful when it is positive, but negative results could either reflect failure of the tumor cells to divide sufficiently in culture or other mechanisms of false negative results as alluded to above. The two translocations are indistinguishable using traditional cytogenetics. FISH using an SS18 break-apart probe is helpful for demonstrating t(X;18), but it cannot distinguish which partner gene is involved for prognostic purposes. RNA from either frozen or paraffin-embedded tissue is suitable for RT-PCR to detect and distinguish the two common translocation variants [29]. From a mechanistic standpoint, the translocation creates a chimeric gene encoding a fusion protein that redirects the transcription factor function of SS18. Relevant downstream targets include cyclin D1 (CCND1) that enhances cell cycle progression. TLE1 encodes a transcriptional co-repressor that is overexpressed in synovial sarcomas. Recently, gene and tissue microarray studies have identified TLE1 as an excellent bio-marker for distinguishing the synovial sarcoma from other soft tissue malignancies on immunohistochemistry [30].

Endometrial Stromal Sarcoma (ESS)

ESS are rare uterine neoplasms including benign stromal nodules, low-grade ESS, and undifferentiated endometrial sarcomas (UES), the latter representing the most aggressive form. ESS are low-grade, well-differentiated neoplasms composed of cells resembling those of the proliferative endometrial stroma and a rich network of thin-walled arteriolar type vessels. The distinction between well-differentiated smooth muscle neoplasms, such as cellular leiomyoma, and low-grade ESS can be problematic when stromal sarcomas show prominent smooth muscle or fibroblastic differentiation. Recently, a gene fusion on chromosome 7 that includes two zinc-finger genes (JAZF1 and JJAZ1) has been discovered in these tumors. The molecular analysis of the non-random translocation t(7;17) disclosed the fusion gene JAZF1/JJAZ1 (juxtaposed with another zinc finger gene 1/joined to JAZF1) in most examples of low-grade ESS. Although the JAZF1/JJAZ1 fusion t(7;17) is also common in benign endometrial stromal nodules, it is helpful when differentiating ESS from UES.

Fusion gene involving growth factors

Dermatofibrosarcoma Protuberans (DFSP)

DFSP is a rare skin tumor of low-grade malignant behavior that shows frequent local recurrence. Either the reciprocal chromosomal translocation t(17;22) (q11;q13.1) or a supernumerary ring chromosome derived from t(17;22) may be found in this type of tumor, resulting in the fusion of the COL1A1 gene on chromosome 17 with the PDGFB gene on chromosome 22 [31]. Since the point of fusion is highly specific for PDGFB but spread over almost the entire locus for COL1A1, the role of the COL1A1 gene may be simply to up-regulate the expression of PDGFR, which acts as an auto- or paracrine growth factor. Imatinib mesylate (gleevec®) is an inhibitor of tyrosine kinases, among them PDGFR, showing a dramatic response in adult in treatment. The use of other PDGFR inhibitors such as sunitinib and sorafenib is being tested in patients with metastatic DFSP [32].

Giant Cell Fibroblastoma (GCF)

GCF represents the juvenile form of dermatofibrosarcoma protuberans (DFSP). Although histologically different these two diseases share a number of similarities: clinical localization and course, CD34 positivity, and most importantly, genetic background. The same fusion gene COL1A1 with PDGFB is also found in giant-cell fibroblastoma [31].

Other types of fusion genes

Alveolar Rhabdomyosarcoma (ARMS)

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and young adults, often presenting as a muscle mass in the extremities, paranasal sinus, or retroperitoneum. The main histological subtypes are alveolar (ARMS) (20%), embryonal (ERMS) (60%), and pleomorphic (PRMS); for these subtypes survival rates vary from <25% to >95%, respectively [33]. In alveolar rhabdomyosarcoma, expression profiling has identified further subsets of patients having good, intermediate, and poor outcomes [34]. Histologically, there is striated muscle differentiation with concomitant expression of vimentin, muscle-specific actin, desmin, myogenin, and MyoD1. PAX3 and PAX7 are transcription factors that initiate myogenesis in muscle stem cells, and the aberrant fusion of their DNA binding domain with the transactivation domain of FOXO1A creates a potent transcription factor that stimulates myogenesis and resists apoptosis. Prognosis of ARMS is poor compared with embryonal rhabdomyosarcoma, justifying the effort to distinguish these two tumor types.

In alveolar rhabdomyosarcomas, 70% of ARMS harbor the translocation t(2;13)(q35:q14), which fuses the 5' end of PAX3 with the 3' end of the FOXO1A gene. An additional 10% of ARMS are associated with fusion of PAX7 to the FOXO1A gene. The remaining 20% of ARMS do not have these fusion genes detectable by routine RT-PCR and comprise cases with a very low expression of a fusion gene, a rare variant fusion, or are true fusion negative cases. FISH using a break-apart probe identifies rearrangement of the FOXO1A gene [35, 36]. Karyotype analysis typically reveals either t(2;13) (q35;q14) PAX3-FOXO1A or t(1;13)(p36;q14) PAX7-FOXO1A. The latter is less common but is associated with a better prognosis. ARMS tend to have a poor prognosis overall, especially when presenting with disseminated disease, so genetic testing may be not relevant in stage IV patients. Testing is most useful when the histologic features are not classic (e.g. mixed alveolar and embryonal patterns).

Alveolar Soft-Part Sarcoma (ASPS)

ASPS is a rare, malignant soft-tissue tumor that mostly occurs in adolescents and young adults and is usually located in the extremities. Lung metastases are often found at presentation, despite the relatively slow growth of the tumor. The prognosis associated with ASPS is poor. Cytogenetic studies have revealed an unbalanced, recurrent der(17)t(X;17)(p11;q25) translocation in ASPS that leads to the fusion of alveolar soft-part sarcoma chromosome region, candidate 1 (ASPSCR1; also known as ASPL) on the long arm of chromosome 17 to the transcription factor for immunoglobulin heavy-chain enhancer 3 (TFE3; located at Xp11). While the telomeric region of chromosome 17 is lost, the fused short arm of chromosome X is frequently duplicated. The consistent detection of the ASPL-TFE3 fusion in ASPS using FISHor RT-PCR identifies it as a marker of potential clinical utility to add to the growing list of translocation-based molecular diagnostic markers in

sarcomas.

Sarcomas with specific oncogenic mutations

Gastrointestinal Stromal Tumors (GIST)

GIST is the most common mesenchymal tumor in the gastrointestinal tract. Activating mutations of the c-kit gene, which encodes a tyrosine kinas receptor for stem cell / platelet-derived growth factor receptor, were found in 75–80% of cases, and those of PDGFRA were found among the rest (5–10%). GISTs without detectable c-kit/PDGFRA mutation are extremely rare. The c-kit protein comprises a long extracellular domain, a transmembrane segment, and an intracellular part. In all cases, mutated receptors transmit growth signals in a ligand-independent manner, inducing dysregulated cell proliferation. Mutations generally occur in the DNA encoding the intracellular part exon 11, but can also occur in exons 9 and rarely 13 and 1. Mutations in c-kit and PDGFRA are mutually exclusive. Detection of either overexpression or mutation of KIT/ PDGFRA has become a standard diagnostic tool for GISTs. Targeting the activating tyrosine kinase with medications such as imatinib mesylate (Glivec/Gleevec®), has been used successfully to treat patients with metastatic GISTs showing the above described mutations. D816V point mutations in c-kit exon 17 are responsible for resistance to targeted therapy drugs like imatinib mesylate [37].

Malignant Rhabdoid Tumors (MRT)

MRT are highly aggressive pediatric tumors that develop mainly in the kidney but also at virtually any extra-renal anatomic site, particularly affecting infants. SMARCB1/hSN5/INI1 is a component of the SWI/SNF complex and represses the expression of cyclin D1, which promotes cell cycle progression acting as a tumor suppressor. Loss-of-function mutations of the *INI1* gene accompanied by a loss of the wild-type allele are frequently found in this type of tumor. Loss of INI-1 staining by immunohistochemistry correlates well with mutations or deletion of the INI-1/hSNF5/SMARCB1/BAF47 locus and is a well-established diagnostic marker for MRT [38]. Recently, frequent loss of INI1 protein expression was reported in epithelioid sarcomas, which are highly malignant adult sarcomas of unknown origin, although the frequency of genetic deletions was low [39].

SARCOMAS WITH VARIABLE COMPLEX GENETIC ALTERATIONS LACKING A SPECIFIC PATTERN

Tumors in this group account for about 50% of soft tissue sarcomas and characterized by pleomorphic/spindle cell morphology, including Pleomorphic Malignant Fibrous Histiocytoma / undifferentiated (PMFH), pleomorphic liposarcoma (PLPS), leiomyosarcoma (LMS), pleomorphic rhabdomyosarcoma (PRMS) and malignant peripheral nerve sheath tumor (MPNST). Chromosomal breakpoints in these tumors are widely scattered, with no predilection of any of the recurrent breakpoints and losses to any of the morphological subtypes. However, high grade dedifferentiated liposarcoma in addition have distinctive genomic abnormalities (giant chromosome, ring chromosomes, amplification of 12q13-q21), similar to well-differentiated liposarcoma.

Pleomorphic Malignant Fibrous Histiocytoma/ Undiffrentiated sarcoma (PMFH)

PMFH has been considered to be the most frequent soft tissue sarcomas in adults. The cellular origin of PMFH has long been unknown, although different theories claimed that the tumor originates from histiocytes, fibroblasts, or primitive mesenchymal cells.

High-grade PMFH exhibits multiple complex chromosomal abnormalities with no specific aberrations, they often present cytogenetic signs of gene amplification, that is, homogeneously staining regions (hsr), double minute chromosomes (dmin), as well as add (19p) [20]. These hsr composed of amplified DNA sequences from 12q13→q15 are commonly detected. Moreover, amplicons of the human homologue of MDM2, cyclin-dependent kinase 4 (CDK4), sarcoma amplified sequence (SAS), and high-mobility group protein IC (HMGIC) genes, which are mapped to this region, have been detected in PMFH. Both the MDM2 and CDK4 genes play a major role in permitting override of the G1-S cell cycle checkpoint in cell proliferation. Since the HMG proteins can bind to DNA and are involved in the organization of chromatin during DNA transcription, they have been referred to as architectural transcription factors. Ezrin (villin2), a protein that serves as an intermediate between the plasma membrane and the actin cytoskeleton, is considered

as a marker of cancer progression and a potential target for cancer therapy. It plays a key role in cell morphology, adhesion, migration, and organization. Ezrin is over-expressed in many neoplasms including PMFH, and this overexpression was found to correlate with increased metastatic potential and reduced survival [40].

Pleomorphic Liposarcoma (PLPS)

PLPS is the least common variant of liposarcoma, accounting for less than 5%. It usually occurs in elderly people (median age 55–65 years) predominantly in deep soft tissues (75% of cases) of the lower extremities, especially the thigh. PLSP characteristically shows high chromosome counts and complex structural arrangements. Deletion of 13q14.2-q14.3, targeting the RB1 pathway, is observed in about 60% of PLPS [41]. Despite their morphologic similarity, PLPS and high-grade dedifferentiated LPS show distinct chromosomal imbalances. Gains of 5p13-p15, 1p21, 1q21-q22, and 7q22 are more frequently observed in PLPS whereas high -level amplifications within chromosomal subregion 12q13-q21 are observed in dedifferentiated LPS. MAD2, a gene involved in the RB1 signaling pathway over-expressed by PLPS (and dedifferentiated LPS), might constitute a therapeutic target [42].

Leiomyosarcoma (LMS)

LMS accounts for about 8–10% of adult soft tissue sarcomas. These malignant neoplasms show varying degrees of smooth muscle differentiation and can develop anywhere in the body. LMS usually show complex karyotypic alterations that differ generally from one tumor to another. Many LMSs show chromosomal imbalances or aberrations in the form of gains (chromosomes 1, 5, 6, 8, 15, 16, 17, 19, 20, 22, X), losses (chromosomes 1p, 2, 3, 4, 6q, 8, 9, 10p, 11p, 12q, 11q, 13, 16, 17p, 18 19, 22q), and amplifications (chromosomes 1, 5, 8, 12, 13, 17, 19, 20). Some gains and losses of chromosomal material are more frequently observed and tend to correlate with poor outcome, large tumor size, and metastatic dissemination (e.g. loss of 1p12-pter, 2p, 13q14-q21 (targeting the Rb pathway), 10q (targeting PTEN) , and 16q; gains of 17p, 8q, and 5p14 pter). Activation of the PI3K–AKT pathway through different mechanisms (e.g., activation of IGFR, inactivation of PTEN (a negative regulator of the PI3K–AKT) also plays a crucial role in the development and maintenance of LMS. This activation leads to the concomitant activation of downstream effectors such as mTOR and its targets (ß-catenin, pS6, p4E-BP1, etc.), as well as to MDM2 stabilization. Recent clinical trials showed that analogs of rapamycin, such as the mTOR inhibitor everolimus (RAD001), have some efficacy in patients with LMS [43]. It has also been realized that the more differentiated retroperitoneal LMSs tend to behave more aggressively and that this was mainly dependent upon amplification/overexpression of myocardin. Myocardin is a transcriptional cofactor of SRF that regulates smooth muscle differentiation. Inactivation of the myocardin pathway results in a significant reduction of smooth muscle differentiation, cell proliferation, and cell migration and was associated with less differentiated histology. These data suggest that myocardin might constitute a promising therapeutic target.

Pleomorphic Rhabdomyosarcoma (PRMS)

PRMS usually occurs in the extremities, especially the thigh, often in middle-aged men. It is an aggressive tumor, prone to recurrence and metastasis to the lungs. It shows nonspecific complex karyotypes. Numerical and unbalanced structural abnormalities are common with gains (chromosomes 1, 5, 8, 14, 18, 20, and 22) and loss (chromosomes 2, 5, 6, 10, 11, 13, 14, 15, 16, 17, 18, 19 and Y) of chromosomes, of which losses of chromosomes 2, 13, 14, 15, 16, and 19 are the most frequent. PRMS does not contain the t(2;13) or t(1;13) characteristic of alveolar rhabdomyosarcoma.

Malignant peripheral Nerve Sheath Tumor (MPNST)

MPNST occurs mostly in middle- to advanced-aged adults, without sex predilection. Fifty percent to 70% develop in a preexisting neurofibroma, especially in plexiform neurofibroma associated with NF1 and sporadic MPNSTs are less frequent. However there are no significant prognostic differences between NF1-associated and sporadic MPNSTs. Cytogenetically, MPNSTs display complex karyotypes and clonal chromosomal aberrations and loss of chromosome seem to be more frequent than gains. CGH studies demonstrate losses in chromosomes

17, 19p and 22q in NF1-associated neurofibromas, suggesting inactivation of tumor suppressor genes in the development, however, in MPNST gains are more frequent than losses, probably relating to proto-oncogene activation during MPNST progression. Losses of chromosomes (1p12-13, 1p21, 1p36, 3p21-pter, 9p13 -21, 9p22-24, 10, 10p11-15, 11p, 11q21-25, 13q14, 15p, 16/16q24, 17/17p, 17q11-12, 17q21-25, 22, 22p, 22q13, and 22q11-12) were the most frequent abnormalities observed. Gains mainly involved the regions 7p21-q36, 7p22, 7q, 8, 8q11-23, 1q25-44, and 5q13-35. Gain of 7p15-p21 was also found associated with poor prognosis and shortened survival [44]. cDNA microarray analysis has found that six genes (keratin 18, survivin, tenascin C, adenosine deaminase, collagen type VIa3, and collagen type VIIa1) were significantly upregulated in MPNST, whereas one gene, insulin-like growth factor binding protein 6, was downregulated in MPNST. MPNST is a highly aggressive sarcoma for which no effective treatment currently exists. PDGF-BB was identified as the most effective factor that induces MPNST cell invasion and proliferation. Expression of PDGF-BB receptor (PDGFR-β) mRNA was detected more frequently and its protein was expressed at higher levels in MPNST tissues than benign peripheral nerve sheath tumors (schwannomas and neurofibromas). The effectiveness of imatinib mesylate *in vitro* suggests that targeting PDGFR-β may result in the establishment of novel treatments for MPNST [45]. Survivin and tenascin C expression was validated on RT-PCR. Immunohistochemistry confirmed upregulation of survivin in MPNST at the protein level.

In summary, genetic analysis of soft tissue sarcomas has proven to be invaluable for diagnostic and clinical management. In the future, clinical decisions will increasingly be based on a combination of histologic criteria and the molecular identification of genetic abnormalities that are indicative of biologic properties. Furthermore, elucidation of the functions of sarcoma-specific fusion proteins will continue to improve understanding of the oncogenic process and will lead to the identification of new therapeutic targets for the treatment of sarcomas.

Please address correspondence to: Victor Prieto, MD, PhD, Department of Pathology, UT, MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, Phone: (713) 792-3187, E-mail: vprieto@mdanderson.org; or Peng Lee, MD, PhD, Departmentof Pathology and Urology, New York University School of Medicine, 423 e.23RD Street, New York, NY 10010, Phone: (212)9513-418, E-mail: peng.lee@nyumc.org

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