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Molecular Pathology of Soft Tissue Tumours: New Insights and Applications

Donald M Salter[1*], and Herng-Sheng Lee[2]

[1]*Centre for Molecular Medicine, MRC IGMM, University of Edinburgh, Western General Hospital, United Kingdom*
[2]*Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, Republic of China*

Soft tissue tumours are a heterogeneous group of neoplastic lesions that arise from mesenchymal cells. Classification of these tumours has historically been based on morphology with ancillary histochemical and immunohistochemical techniques. Improved cytogenetic studies and advances in molecular genetics have led to increased understanding of the pathology and classification of these tumours. Genetically, soft tissue tumours can be divided into four categories. These include tumours with reciprocal translocations and otherwise simple karyotypes, tumours with characteristic amplifications, tumours with specific driver mutations, and those with complex karyotypes associated with chromosomal instability. Knowledge of these genetic abnormalities and application of molecular diagnostic techniques such fluorescence in situ hybridisation and mutation screening in histopathological practice is currently driving a revolution in how these tumours are diagnosed and managed.

Key words: soft tissue tumour, cytogenetic, fluorescence in situ hybridisation (FISH), mutation

INTRODUCTION

Soft tissue tumours are most common in the extremities but may be seen in any part of the body. The majority of soft tissue tumours are benign and present as painless superficial lumps. Malignant tumours, soft tissue sarcomas, are rare and account for only 1% of all cancers. Like their benign counterparts sarcomas of the extremities or trunk typically present as lumps, although they are usually larger size and located deep to the superficial fascia.

As the incidence of different types of soft tissue tumour varies with age, site and gender, the diagnosis of soft tissue tumours is based on a multidisciplinary approach in which clinical information, radiological and histological features all contribute. Embryonal and alveolar rhabdomyosarcomas are common in children whilst pleomorphic rhabdomyosarcomas are predominantly seen in adults. Most forms of liposarcomas are extremely rare in children being recognised as almost exclusively an adult cancer. Synovial sarcomas show a peak incidence in young adults. Cutaneous angiosarcomas are most frequent in elderly patients and located in the head and neck region. Many retroperitoneal soft tissue tumours are liposarcomas. Thus, knowledge of the patient's age and sex, the size and site of tumour as well as family history and past medical history are important for diagnosis. Magnetic resonance imaging (MRI) is, in general, the preferred imaging procedure although computer tomography and ultrasound are also used in the diagnostic pathway.

Classification and Grading

The histological diagnosis of soft tissue tumours has historically been dependent on morphological description complemented by a restricted number of special histochemical stains, electron microscopy and, in some cases cytogenetics. Over the last few decades the evolution of immunohistochemistry and molecular genetic techniques has had a significant impact on diagnosis and classification of these tumours. The World Health Organization (WHO) classification of soft tissue tumours[1] reflects the significance of the application of these ancillary techniques on the diagnosis of soft tissue sarcoma in everyday practice. The WHO classification of soft tissue tumours recommends separation of soft tissue tumours into 4 categories: benign, intermediate locally

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*Corresponding author: Professor Donald M Salter, Centre for Molecular Medicine, MRC IGMM, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, United Kingdom. Tel: +44-31-242-7125; Fax: +44-31-242-7169; E-mail: Donald.Salter@ed.ac.uk

aggressive tumours, intermediate rarely metastasising and malignant. Benign lesions tend not to recur and are cured by complete excision. Intermediate, locally aggressive tumours are locally infiltrative and destructive. They require complete excision with good clearance to avoid recurrences. Intermediate rarely metastasising tumours are also locally aggressive but show a low, less than 2% risk of metastasis. Malignant tumours are locally destructive, recur and have a significant potential to metastasise. Histological grading systems help predict clinical outcome and the French or FNCLCC (French Fédération Nationale des Centres de Lutte Contre le Cancer)[2] is currently preferred in the UK. This system assesses three parameters: tumour differentiation, mitotic count and tumour necrosis to generate three histological grades by adding scores in each of the different parameters. Grading is useful as it selects patients at risk of developing metastasis; helps predict the clinical course and the need for adjuvant therapy. Grading does not have prognostic value in all sarcomas and is not recommended for use in some entities including alveolar soft part sarcoma, clear cell sarcoma and epithelioid sarcoma.

Aetiology

The aetiology of most soft tissue tumours is unknown. The majority arise spontaneously but genetic or environmental contributing factors are recognised. Patients with inherited syndromes such as neurofibromatosis and Li-Fraumeni syndrome show increased susceptibility for development of benign and malignant soft tissue tumours. In type 1 neurofibromatosis type 1 or von Recklinghausen disease a mutation of the tumour suppressor gene neurofibromin 1[3] on chromosome 17q11.2 results in an increased incidence of neurofibromas some of which may progress to malignant peripheral nerve sheath tumours. Germline mutations of the p53 tumor suppressor gene are present in patients with the Li-Fraumeni syndrome[4] and are associated with an increased risk of rhabdomyosarcoma and osteosarcoma. Irradiation is a recognised environmental risk factor with patients with a range of conditions treated by radiation showing increased incidence of angiosarcomas, osteosarcomas and undifferentiated sarcomas developing in the irradiated field. The risk of secondary sarcoma developing appears to correlate directly with the radiation dose.

Molecular Genetics of Sarcomas

Over the last few decades, as a result of improved cytogenetic studies and advances in molecular genetics, the ability to accurately diagnose and classify different subtypes of sarcoma has improved. This has led to an increase in the understanding of the molecular abnormalities that give rise to benign and malignant bone and soft tissue sarcomas and which are beginning to indicate new pathways for targeted therapy. Genetically, sarcomas can be roughly divided into four categories. These include tumours with reciprocal translocations and otherwise simple karyotypes, tumours with characteristic amplifications, tumours with specific driver mutations, and those with complex karyotypes associated with chromosomal instability.

Translocation Sarcomas

A reciprocal chromosomal translocation is seen in approximately 20% of sarcomas and includes 15 different tumour types.[5] Reciprocal chromosomal translocations are structural alterations of chromosomes in which pieces of two chromosomes are swapped creating chimeric or derivative chromosomes. A reciprocal chromosomal translocation introduces breaks within two cellular genes and results in the joining of portions of the two genes to produce a novel chimeric or fusion gene. Transcription of the fusion gene and translation of the fusion mRNA results in formation of a fusion oncoprotein that may influence a range of cellular processes such as proliferation, apoptosis, differentiation, spread and migration either by acting as a transcription factor or by leading to unregulated autocrine or paracrine stimulation of an involved receptor protein.

Many of the oncogenic fusion genes associated with sarcomas belong to the TET gene family including EWSR1 and FUS. Both of these genes encode for proteins that contain a characteristic RNA recognition motif and are thought to be involved in RNA binding and transcriptional activation. Typical translocations result in a fusion protein which acts as a powerful transcription factor containing the transactivating domain of the TET family gene with the DNA binding domain of a fusion partner.[6] Translocations involving the EWSR1 gene located at band 12 on the long arm of chromosome 22 (22q12) are relatively frequent occurrences in soft tissue tumours and are seen a range of histologically diverse benign and malignant soft tissue tumours including Ewing's sarcoma, desmoplastic small round cell tumour, extraskeletal myxoid chondrosarcoma, clear cell sarcoma, myoepithelial tumour of bone and soft tissue and angiomatoid fibrous histiocytoma. Interestingly EWSR1

rearrangements are also described in non-soft tissue tumours such as cutaneous hidradenoma and salivary gland mucoepidermoid carcinoma.[7]

Translocations involving EWSR1 frequently involve members of the erythroblastosis virus-transforming sequence (avian ETS) transcription factor family which includes FLI1, ERG, ETV1, ETV4, and FEV. EWSRI gene fusion with different partners may give rise to morphologically similar and dissimilar tumour types. In Ewings sarcoma a t(11;22)(q24;q12) translocation resulting in a fusion between EWSR1 and FLI1 is the most commonly translocation identified but translocations and fusions of EWSR1 with at least 8 other genes including ERG, NFATC2, FEV, ETV1, E1AF and SP3 have been described.[8] Although, in general, morphologically different soft tissue tumours that contain a EWSRI translocation have different fusion partners this is not always the case. Both angiomatoid fibrous histiocytoma and clear cell sarcoma have a t(12;22)(q13;q12) translocation fusing EWSR1 with ATF1 whereas a t(21;22)(q22;q12) translocation involving EWSR1 and ERG is seen in both a subset of Ewings sarcoma and desmoplastic small round cell tumour

The FUS gene translocations appear to be are more restricted being seen in only myxoid liposarcomas and low-grade fibromyxoid sarcomas (LGFMS). Most myxoid/ round cell liposarcomas demonstrate a t(12;16)(q13;p11) translocation that results in the rearrangement of and fusion between FUS, located at 16p11, and the CHOP / DDIT3 gene, a member of the CCAAT/enhancer-binding protein family involved in adipocyte differentiation, but a subset shows a translocation involving EWSR1 rather than FUS. The normal function of DDIT3 is to promote growth arrest but when involved in a translocation antiproliferative activity appears to be lost. In LGFMS there is a t(7;16)(q33;p11) translocation leading to a FUS-CREB3L2 (cAMP responsive element binding protein 3-like 2) fusion gene.[9] CREB3L2 is a bZIP transmembrane transcription factor and in a subset of cases of LGFMS a t(11;16) translocation results in a fusion gene between FUS and CREB3L1 another bZIP transmembrane transcription factor.[10]

Synovial sarcomas are characterized by a t(X;18)(p11;q11) translocation that results 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.[11] The SS18(SYT)-SSX fusion protein brings together the transcriptional activating domain of SS18 and the transcriptional repressor domains of SSX. The SS18 gene encodes a 387-amino acid protein that is ubiquitously expressed. It is localized in the nucleus and thought to be a transcriptional activator whereas members of the SSX gene probably function, in association with other proteins, to repress target genes.

The majority of alveolar rhabdomyosarcomas are characterised by a translocation t(2;13)(q35:q14), which fuses the 5' end of PAX3 with the 3' end of the FOXO1A gene.[12] A minority are associated with fusion of PAX7 to the FOXO1A gene.[13] The fusion protein combines the the paired and the homeo DNAbinding domains of the PAX gene with the transactivation domain from the FOXO1 gene producing a potent transcription factor. In addition fusion genes may be overexpressed by increased copy number of the gene in the case of PAX3-FOXO, or as a result of gene amplification in the case of PAX7-FOXO1.[14]

In dermatofibrosarcoma protuberans (DFSP) and giant cell fibroblastoma[15] a reciprocal chromosomal trans-location t(17;22) (q11;q13.) or, in some instances, a supernumerary ring chromosome derived from t(17;22) results in the fusion of the COL1A1 gene on chromosome 17 with the platelet-derived growth factor beta chain (PDGFB) gene on chromosome 22. The translocation puts the PDGFBgene under control of the promoter for collagen type 1A1 thus driving overproduction of PDGFB and excessive autocrine or paracrine signalling.[16]

Tumours with specific cytogenetic amplifications

Amplifications of specific parts of the genome resulting in formation of ring chromosomes and giant marker chromosomes is seen in some bone and soft tissue tumours. In atypical lipomatous tumors / well-differentiated liposarcoma and dedifferentiated liposarcomas arsing from these lower grade tumours show amplification of regions of choromosome 12.[17] These regions contain genes for MDM2, an antagonist of p53 tumor-suppressor activity, and CDK4 a protein involved in cell cycle progression both of which are thought to be important in tumour formation.

Tumors with Specific Mutations,

Specific transforming mutations or deletions may be seen in some soft tissue tumours and have also been identified in bone tumours. These include GISTs (gastrointestinal stromal tumours)[18], desmoid type fibromatosis[19] and epithelioid sarcoma[20] in soft tissue and fibrous dysplasia[21] in bone. GISTs are one of the most common soft tissue tumours of the gastrointestinal tract and are characterised by activating mutations in either the KIT gene,

most commonly, or less commonly the PDGFRA gene. KIT encodes for a tyrosine receptor kinase, ckit/CD117. The most common site of KIT mutations is in the 5' end of exon 11, which codes for the juxtamembrane domain of CD117. The mutations may be in-frame deletions, point mutations or deletions preceded by substitutions. Activating mutations act to promote CD117 dimerization in the absence of its physiological ligand scf (stem cell factor) inducing constitutive activation. The less common mutations that are seen in the PDGFRA gene result in uncontrolled signalling through alpha-type platelet-derived growth factor receptor. Interestingly KIT and PDGFR mutations appear mutually exclusive in GISTs. A small subset of GIST cases are driven by $BRAF^{V600E}$ mutations.[18]

Desmoid type fibromatosis are characterised by dysregulated beta catenin activity. This arises through two separate mechanisms. Sporadic desmoid type fibromatosis are frequently associated with somatic mutations of codons 41 and 45 of exon 3 of CTNNB1, the gene that codes for beta catenin.[19] Tumours that occur in the background of familial adenomatous polyposis, in contrast, contain inactivating germline mutations in the APC gene.[21,22] In sporadic desmoids type fibromatosis mutations of CTNNB1 result in stabilisation and activation of the beta catenin protein. In patients with APC gene mutations there is ineffective degradation and inactivation of the activated beta catenin.

Specific loss of the INI1 gene product appears to be important in epithelioid sarcomas and malignant rhabdoid tumours.[20,23] INI1 is located on chromosome 22q11.2 and belongs to the SWI/SNF chromatin remodelling complex. In malignant rhabdoid tumours loss of INI is commonly the result of partial or complete loss of the SMARCB1/INI1 gene expression as result of point mutations leading to frameshifts, premature stop codons or deletions.[24] In epithelioid sarcoma loss of INI gene expression appears to occur through gene silencing by promoter methylation.[25,26]

Activating GNAS1 point mutations are seen in fibrous dysplasia, a low-grade, bone-forming neoplasm and intramuscular myxomas.[27,28] The most common mutations are at codon 201 in GNAS1[20,29] and appear to arise as post-zygotic genetic events with mutated cells consequently being distributed throughout the body. As such fibrous dyplasia may affect one or multiple bones or be associated with benign intramuscular myxomas or endocrine tumours as part of McCune–Albright Syndrome and Mazabraud Syndrome respectively.

Tumours With Complex Karyotypes

As a group these account for around 50% of soft tissue sarcomas. They are typically high grade tumours, are often poorly differentiated and include the majority of leiomyosarcomas, angiosarcomas, pleomorphic spindle cell and undifferentiated sarcomas (previously termed malignant fibrous histiocytoma). These tumours have a complex karyotype including numerous chromosomal gains, losses and amplifications that are not consistent within and between tumour types. High grade undifferentiated sarcomas may show complex cytogenetic rearrangements involving as much as 30–35% of the genome, Nevertheless certain oncogenic signalling pathways such as p53, Rb and PI3k/Akt appear to be involved in tumour growth and spread. Indeed, the p53 tumor-suppressor pathway is one of the most frequently disrupted in sarcoma.[30]

Although most high-grade karyotypically complex sarcomas present de novo, a small number progress from lower grade lesions and show the typical genetic abnormalities in addition to those arising as a result of the genetic instability. Thus, some high grade liposarcomas and histologically undifferentiated pleomorphic sarcomas, especially in the retroperitoneum arise from well differentiated liposarcomas. Similarly benign cartilage tumours of bone, enchondromas, can progress to low and ultimately high grade chondrosarcomas.

Application of Molecular Pathology in Diagnosis and Management of Soft Tissue Sarcoma.

Subtypes of soft tissue tumour may be morphologically distinct and readily diagnosed by an experienced soft tissue pathologist. However knowledge of the molecular genetics of soft tissue tumours has led to advances in the use of immunohistochemistry and molecular diagnostics that allow improved diagnosis and classification. Furthermore knowledge of the specific genetic abnormalities and how these influence tumour cell behaviour may provide prognostic information and are indicating molecules and pathways which are potentially targetable by novel therapies.

Roles in Diagnostic Pathology

Accurate histological diagnosis of soft tissue tumours is recognised as being one of the most difficult areas in surgical tumour pathology. This is because of the relative rarity of these tumours and the morphological overlap between different entities including between benign and malignant soft tissue tumours. Morphological overlap also exists between mesenchymal and epithelial tumours

further complicating the diagnostic process. As such immunohistochemistry and molecular diagnostics are now a necessary tool for the pathologist practising in this area especially when faced with a lesion with unusual morphology, immunohistochemistry or clinical presentation.

Immunohistochemistry for molecules associated with lineage differentiation of mesenchymal cells such as smooth muscle actin, desmin, h-caldesmon, S100, CD99, CD34 and C31 have historically been very useful for diagnosis and subclassification of soft tissue tumours. When used in a panel with antibodies against CD45, cytokeratin and melanoma specific markers the diagnosis and classification of many soft tissue tumours can be readily made. More recently antibodies to INI, MUC-4, CDK4, p16 and MDM2 have been shown to have diagnostic utility. INI staining is absent in epithelioid sarcomas reflecting the genetic abnormality.[26] Gene expression profiling has identified differential upregulation of the mucin 4 (MUC4) gene in LGFMS and MUC4, a transmembrane glycoprotein is expressed in LGFMS but not morphologically similar soft tissue tumours.[31] The relationship of this altered gene and protein expression to the translocation is however not clear. As the genes for CDK4, p16 and MDM2 are frequently amplified in well-differentiated and dedifferentiated liposarcomas an immunohistochemical panel with antibodies against these molecules is useful for distinction from other adipocytic tumors.[32]

Molecular diagnostics are particularly useful for the diagnosis of soft tissue tumours with reciprocal translocations or specific transforming mutations. In the case of translocation sarcomas fluorescence in-situ hybridisation (FISH) on paraffin-embedded slides is generally used to detect these translocations.[33] With appropriately designed two colour probes a normal cell will show a two fusion pattern whereas a cell with a translocation involving that gene will show one fusion and separation of the other probes. Commercially available probes are available for the genes including EWSR1, FOXO1, FUS and SS18. FISH identifies the presence of a translocation but not a specific gene-fusion partner. If this information is required RT-PCR would be carried out on either fresh frozen or paraffin embedded tissue.

Prognosis and Treatment

As in other fields of cancer management molecular diagnostics have the potential to provide additional information with regards to patient prognosis and treatment options. In alveolar rhabdomyosarcoma patients with tumours harbouring a PAX7-FOXO1A fusion appear to have a better prognosis than those with a PAX3-FOXO1A translocation.[34] Initial associations with the type of EWSR1-FLI1 fusion product in Ewings sarcoma and prognosis have not been subsequently supported. Whether the nature of the SS18 fusion in synovial sarcoma is associated with prognosis has also recently been debated.[35] Specific mutations in CTNNB1 correlate with local recurrence in sporadic desmoid tumors.[36] In untreated GISTs exon 11 mutations are associated with poor outcomes.[37]

Treatment aimed at the aberrant signalling that occurs as a result of activating mutations of KIT/CD117 or PDGFRA in GISTs is a prime example of how knowledge of the molecular abnormality in a soft tissue tumour can influence patient management. Depending on the nature of the mutation in KIT and PDGFRA, tumours have distinct responses to imatinib mesylate.[38] GISTs with KIT exon 9 mutations, PDGFRA exon 18 mutations, and wild-type KIT are resistant to imatinib. Overexpression of PDGFB in DFSP following formation of the COL1A1-PDGFB fusion protein is also targetable by small-molecule inhibitors of PDGFR, such as imatinib mesylate.[39] Similarly over expression of the receptor tyrosine kinase ALK in a subset of cases of inflammatory myofibroblastic tumour with an ALK rearrangement may be targeted with specific inhibitors of ALK activity.[40]

CONCLUSION

There has been a large increase in the knowledge of the genetic and molecular abnormalities that are present in soft tissue and bone tumours. These are aiding the surgical pathologist to improve diagnosis and classification of this heterogeneous group of neoplasm. Application of molecular diagnostics to the assessment of soft tissue tumours will provide additional information that will be important for assessing prognosis and management in the clinical setting.

DISCLOSURE

The author declares that this study has no conflict of interest.

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