# Paediatric soft tissue tumours: from histology to molecular diagnosis

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## Abstract

Paediatric sarcomas comprise a diverse group of relatively uncommon neoplasms, with morphological features that range from primitive round cell tumours to fasciculated spindle cell cancers. These histologies frequently overlap and cause diagnostic confusion or uncertainty, so that ancillary testing has become the standard of practice. In previous years, immunohistochemistry and electron microscopy were commonly used, but the genetic revolution has led to the discovery of unique molecular markers that can be detected using routinely fixed and embedded tissues. Detection of characteristic genetic features of paediatric sarcomas has become more readily available and offers advantages over other ancillary tests, so that general pathologists should be familiar with molecular markers and the procedures used to detect them. This brief review outlines the more common types of paediatric sarcomas and describes the morphological and genetic features that facilitate their diagnosis.

**Keywords** diagnosis; fluorescence in-situ hybridization; genetic testing; immunohistochemistry; morphology; paediatric; reverse transcriptase-polymerase chain reaction; sarcoma

## Introduction

As a result of their frequent undifferentiated or morphologically bizarre nature, diagnosis of paediatric soft tissue sarcomas has traditionally been a challenging enterprise. However, the early 1980s witnessed the initial discoveries of characteristic genetic findings that continue unabated to this day. Although standard histopathology remains the keystone of pathological diagnosis, genetic techniques yield powerful tools that in many cases simplify what once exposed surgical pathologists to the sharp horns of diagnostic dilemmas. Chief among these tools for genetic diagnosis of paediatric sarcomas are standard cytogenetics, reverse transcriptase-polymerase chain reaction (RT-PCR), and fluorescence in-situ hybridization (FISH), each having advantages and disadvantages that should be weighed with particular diagnoses and individual cases.[1] These tests detect gene fusions and deletions that typically occur with paediatric sarcomas (Table 1).[2]

Coincident with the discovery of genetic tools came the description of characteristic protein expression that engendered diagnostic immunohistochemistry. In the early descriptions of ancillary immunohistochemical stains, reports focused on gene expression that reflected the phenotype of normal cells analogous to neoplastic entities. For example, diagnostic research on rhabdomyosarcoma focused on markers of skeletal muscle differentiation. However, ancillary diagnosis of neoplasms has often been stymied by anomalous protein expression noted on individual cases or unexpected non-specificity seen with study of large series. In more recent times, studies have focused on expression of proteins that can be targeted by biological agents, and hence immunohistochemistry has become useful for therapy as well as for diagnosis.[3–5]

Electron microscopy has also played an important role in the characterization of paediatric sarcomas, and some have considered it more useful than immunohistochemistry. Its usage has waned since the advent of molecular diagnosis, but it continues to be useful in difficult lesions that cannot be characterized by other methods. In some tumours, such as malignant peripheral nerve sheath tumour (MPNST), it still remains the most effective method of ancillary diagnosis.[6]

The following review will list paediatric soft tissue sarcomas that may be encountered in surgical pathology practice, and it will recount the characteristic morphology, protein expression and genetic features that allow a diagnosis. Readers should be aware that this remains an evolving field. Some questions have not been fully answered, and individual tumours may still lack characteristic genetic findings and possess divergent features. As a result, the diagnosis of 'undifferentiated sarcoma, not further classified' remains in our lexicon.

## Rhabdomyosarcoma

As the name implies, the abortive formation of skeletal muscle characterizes rhabdomyosarcoma. However, this lesion is not restricted to skeletal muscle primary sites, as a substantial number occur in areas normally devoid of muscle, such as vagina, urinary bladder and gallbladder. Rhabdomyosarcomas comprise the single most frequent sarcoma that arises in the paediatric age group but are distinctly rare in adulthood. Several histologically distinctive subtypes exist: embryonal rhabdomyosarcoma, alveolar rhabdomyosarcoma and pleomorphic rhabdomyosarcoma. Variants have been included with these groups, such as the botryoid and spindle cell variants of embryonal rhabdomyosarcoma, and the solid and mixed variants of alveolar rhabdomyosarcoma. Newer subtypes, such as the sclerosing rhabdomyosarcoma, do not fit comfortably into any of these groupings, and other characterizations, such as anaplasia, occur within all groups.

In spite of great initial enthusiasm, molecule testing has not replaced histopathology for the diagnosis of rhabdomyosarcoma. Primarily this is because of the discovery of powerful diagnostic stains such as myogenin. Only alveolar rhabdomyosarcomas possess proven specific genetic markers, associated with translocations of chromosomes 1 or 2 to chromosome 13. Approximately 30–50% of alveolar rhabdomyosarcomas contain a *PAX3–FOXO1* (formerly *PAX3–FKHR*), and approximately 20% contain a *PAX7–FOXO1*. A substantial number of alveolar rhabdomyosarcomas do not appear to contain a fusion.[7] Gene expression arrays indicate that fusion-negative alveolar rhabdomyosarcomas constitute a heterogeneous group that overlaps with embryonal

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**Recurrent chromosomal translocations in paediatric malignant soft tissue tumours**

| Tumors | Translocations | Fusion genes |
|---|---|---|
| Alveolar rhabdomyosarcoma | t(1;13)(p36;q14) | *PAX7—FOXO1* |
| | t(2;13)(q35;q14) | *PAX3—FOXO1 (formerly PAX3/FKHR)* |
| Ewing sarcoma/primitive neuroectodermal tumour | t(11;22)(q24;q12) | *EWS—FLI-1* |
| | t(21;22)(q22;q12) | *EWS—ERG* |
| | t(7;22)(p22;q12) | *EWS—ETV1* |
| | t(2;22)(q33;q12) | *EWS—FEV* |
| | t(17;22)(q21;q12) | *EWS—E1AF* |
| | t(16;21)(p11;q22) | *FUS—ERG* |
| Desmoplastic small round cell tumour | t(11;22)(p13;q12) | *EWS—WT1* |
| Synovial sarcoma | t(X;18)(p11.2;q11.2) | *SYT—SSX1* |
| | | *SYT—SSX2* |
| | | *SYT—SSX4* |
| Clear cell sarcoma of soft tissue | t(12;22)(q13;q12) | *EWS—ATF-1* |
| | t(2;22)(q33;q12) | *EWS—CREB1* |
| Alveolar soft part sarcoma | t(X;17)(p11;q25) | *ASPL—TFE3* |
| Infantile fibrosarcoma | t(12;15)(p13;q25) | *ETV6—NTRK3* |
| Dermatofibrosarcoma protuberans | t(17;22)(q21;q13) | *COL1A1—PDGFB* |
| Low-grade fibromyxoid sarcoma/hyalinizing spindle cell tumour with giant rosettes | t(7;16)(q33;p11) | *FUS—CREB3L2* |
| | t(11,16) (p11;p11) | *FUS—CREB3L1* |
| Myxoid liposarcoma | t(12;16)(q13;p11) | *FUS—CHOP* |
| | t(12;22)(q13;q12) | *EWS—CHOP* |

**Table 1**

rhabdomyosarcoma, whereas *PAX3—FOXO1* tumours comprise a molecularly homogeneous entity with a uniformly poor prognosis.[8] *PAX7—FOXO1*-positive tumours exhibit gene amplification rather than overexpression; this subset may have a prognosis superior to other alveolar genetic subtypes.[9]

Embryonal rhabdomyosarcomas constitute a heterogeneous group, perhaps because of the molecular plasticity of the primitive myoblast. Their morphology encompasses densely cellular neoplasms resembling solid alveolar tumours; paucicellular myxoid lesions mimicking myxoid liposarcoma; spindle cell variants with features of leiomyosarcoma or MPNST; and botryoid sarcomas with a cambium layer. The sclerosing variant has recently been added to the rhabdomyosarcoma compendium; it resembles alveolar tumours with extensive fibrosis but shows neither strong myogenin expression nor a *PAX* fusion.[10] As a result of this heterogeneity, *PAX* fusion analysis can be desirable as an ancillary test for rhabdomyosarcoma diagnosis, particularly with small biopsies. Gene array studies have indicated candidate surrogate immunohistochemical markers for diagnosis of alveolar rhabdomyosarcoma,[8,11] but additional testing is needed for confirmation.

## Ewing sarcoma/primitive neuroectodermal tumour

Ewing sarcoma and peripheral primitive neuroectodermal tumour (PNET) comprise morphologically and clinically diverse lesions that comprise a single neoplastic entity. Ewing sarcomas typically arise in bones and show patternless sheets of primitive cells with round, regular nuclei and minimal amounts of vesicular, glycogen-laden cytoplasm. PNETs usually arise in soft tissue and show cytological and histological features of a primitive neuroblastoma-like tumour, with formation of rosettes containing oblong nuclei arranged in a wreath-like fashion around a neurofibrillary core. Intermediate entities sharing features of both lesions (atypical Ewing sarcoma) commonly appear, and all variants may occur in either bone or soft tissue.

In spite of their morphological dissimilarity, Ewing sarcoma and PNET share identical genetic alterations, primarily chromosomal translocations between chromosome 22q12 and either chromosome 11q24 or chromosome 21q22.[12] These cause fusions resulting in chimeric proteins and involving the *EWS* gene on chromosome 22 with either *FLI-1* on chromosome 11 or *ERG* on chromosome 21. Both *ERG* and *FLI-1* have characteristics of the ETS gene family. Approximately 90% of Ewing sarcoma/PNETs possess an *EWS—FLI-1* fusion that can be used as a diagnostic marker; 10% show other *EWS* fusions, usually *EWS—ERG*. A host of rare fusions involving other ETS genes and *EWS* have been described, and rarely *FUS* may act as a fusion partner instead of *EWS*.[13] To add further confusion, EWS—FLI chimeras have a variable molecular weight resulting from variability in the actual fusion breakpoints. *EWS—FLI-1* fusion type has also been touted as a prognostic feature, with type 1 fusions having a better clinical outcome.[14] This feature can be identified by molecular size on an RT-PCR gel.

A variety of immunohistochemical markers have been used for ancillary diagnosis of Ewing sarcoma, including the recent addition of FLI-1 protein.[15] Like CD99, FLI-1 lacks diagnostic specificity unless a panel of stains is employed. *EWS* fusion detection offers greater specificity and sensitivity than immunohistochemical assays. Breakapart FISH for *EWS* alterations seems to offer more sensitivity than RT-PCR.[16,17] It is also important to be aware that desmoplastic small round cell tumour, clear cell sarcoma of soft tissue, extraskeletal myxoid chondrosarcoma and some forms of undifferentiated sarcoma all exhibit positive results with *EWS* breakapart FISH. Conversely, 10% of Ewing sarcoma/PNETs will be negative for *EWS—FLI-1* by studies that demonstrate both genes, such as RT-PCR. Hence, interpretation must be performed in the context of histopathological studies.

## Desmoplastic small round cell tumour

Desmoplastic small round cell tumour (DSRCT) is a highly malignant small cell neoplasm that has features similar to Ewing/PNETs, but unfortunately without the responsivity to current chemotherapeutic agents. These lesions were initially limited to the abdomen, but sporadic extra-abdominal tumours have been documented.[18] Extra-abdominal tumours occurring in the ovary and scrotum make embryological sense because of the ramifications of the developing coelomic cavity. Other locations such as the cranial cavity and extremity suggest this neoplasm does not require a mesothelial origin.

A prominent desmoplastic response, with abundant fibrocollagenous stroma enveloping and separating aggregates of