Ewing family tumours: a paediatric perspective

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Abstract

Sarcomas are malignant tumours of the connective tissues and are proportionately much more common in children than in adults. The Ewing family of tumours (EFT) is a group of sarcomas sharing rearrangement of the *EWSR1* gene on 22q12, and include Ewing sarcoma/primitive neuroectodermal tumour, desmoplastic small round cell tumour, angiomatoid fibrous histiocytoma and clear cell sarcoma. Other tumours harbouring *EWSR1* rearrangements include myoepithelial tumours, myxoid liposarcoma and extraskeletal chondrosarcoma. In addition, a group of Ewing-like primitive round cell sarcomas have been recently described in a paediatric population, further expanding the list of EFT. This review will focus on the histopathological, immunohistochemical and molecular genetic features of EFT, with an emphasis on those predominantly occurring in the paediatric population.

Keywords angiomatoid fibrous histiocytoma; clear cell sarcoma; desmoplastic small round cell tumour; Ewing sarcoma; *EWSR1*; paediatric

Introduction

Sarcomas are malignant tumours of connective tissues, and the majority occurs in either soft tissue or bone. The paediatric population is disproportionately affected by sarcomas, accounting for up to 20% of childhood solid malignancies,[1] compared with only 1% in the adult population. Clinically, paediatric sarcomas are generally divided into two major categories: rhabdomyosarcomas (RMS), which are the most common, and non-rhabdomyomatous sarcomas,[2] to which the Ewing family of tumours (EFT) belong, as well as synovial sarcoma, malignant peripheral nerve sheath tumour, osteosarcoma and others. Furthermore, paediatric sarcomas fall into two major cytogenetic categories: those characterized by relatively simple, near diploid karyotypes with a small number of consistently rearranged chromosomal loci, and those with complex karyotypes without recurrent abnormalities, suggestive of widespread genomic instability.[3] Tumours belonging to the former group include the EFT, alveolar rhabdomyosarcoma and synovial sarcoma, each of which has recurrent consistent translocations. The second group includes tumours such as embryonal rhabdomyosarcoma, malignant peripheral nerve sheath tumour and osteosarcoma.[3] Such complex karyotypes are indicative of chromosomal instability; subsequent gain-of-function mutations in oncogenes and loss-of-function mutations in tumour suppressor genes lead to tumour progression.[3]

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Classically, pathologists have focused on the morphological classification of paediatric sarcomas, and for many years this was the mainstay of sarcoma classification. However, with the discovery of subtype-specific translocations, molecular genetic techniques are playing an increasingly important role in the classification of paediatric sarcomas. This review will focus on the morphological, immunohistochemical and molecular genetic features of EFT in paediatric patients, and will also discuss some of the diagnostic tools used by pathologists in arriving at a diagnosis.

Ewing family tumours

The EFT is a group of tumours with rearrangement of the *EWSR1* gene on 22q12. Such tumours include Ewing sarcoma/primitive neuroectodermal tumour (ES/PNET), angiomatoid fibrous histiocytoma (AFH), desmoplastic small round cell tumour (DSRCT), clear cell sarcoma (CCS), extraskeletal myxoid chondrosarcoma, myxoid/round cell liposarcoma and myoepithelial tumours (Ref. [4]; see Table 1). In addition, a group of Ewing-like primitive round cell sarcomas harbouring recurrent *CIC/DUX4* fusion transcript have been described in a paediatric population.[5]

Ewing sarcoma/primitive neuroectodermal tumour

ES/PNET is the archetypal EFT. Previously divided into ES and PNET depending on the amount of neuroectodermal differentiation (more in the latter), both subtypes are now considered one and the same tumour, and the World Health Organization has labelled them as ES/PNET. For clarity, they will be referred to as 'ES' throughout the text.

The diagnosis of ES rests upon histomorphological, immunohistochemical and molecular genetic features. ES is more common in males and the incidence peaks in the second decade of life.[4,6] The majority are primary bone tumours, with up to 40% being extraskeletal.[4] The tumour comprises sheets of small round cells with a high nuclear-to-cytoplasm ratio, and some spindling of the cells may be present. The cells have clear to vacuolated cytoplasm and round, hyperchromatic nuclei with inconspicuous nucleoli. Both lighter-staining and darker-staining cells can be discerned in the majority of ES using routine haematoxylin and eosin stains, and prominent amounts of intracytoplasmic glycogen are often present[4,6,7] (Figure 1). Occasional solid rosettes may be found, the so-called Homer–Wright rosettes. Histological variants include a predominant spindle cell subtype, which often has a hemangiopericytic-like vascular pattern; a large cell or atypical subtype; a hyalinized subtype; and an adamantinoma subtype.

Immunohistochemistry shows evidence of neural differentiation (CD56, CD57, NSE). However, CD99, a transmembrane glycoprotein and the product of the *MIC2* gene,[8] is a more sensitive and specific immunostain,[6,7,9] and the vast majority of ES show strong, diffuse membranous positivity (Figure 1d). The pathologist must be aware that CD99 staining has also been reported in lymphoblastic lymphoma, mesenchymal chondrosarcoma and poorly differentiated synovial sarcoma, all of which may share morphological features with ES. In such difficult cases, molecular testing is necessary for definitive diagnosis.

Up to 98% of ES harbour diagnostic rearrangements of the *EWSR1* gene,[10] with 9 different partners recently described.[4] In

Histological, immunophenotypic and molecular genetic characteristics of the more common members of the Ewing family of tumours in pediatrics

Tumour	Histology	IHC	Translocation[a]	Fusion transcript
Ewing sarcoma	Sheets of small round blue cells; Homer–Wright rosettes; glycogen	CD99, membranous pattern	t(11;22)(q24;q12) t(21;22)(q22;q12) t(7;22)(p22;q12) t(2;22)(q33;q12) t(17;22)(q12;q12)	*EWSR1/FLI1* *EWSR1/ERG* *EWSR1/ETV1* *EWSR1/FEV* *EWSR1/ETV4*
Angiomatoid fibrous histiocytoma	Fibroinflammatory pseudocapsule; blood-filled spaces; nests of histiocytoid cells	Poly-immunophenotypic: desmin, CD99, CD68, EMA	t(2;22)(q33;q12) t(12;22)(q13;q12) t(12;16)(q13;p11)	*EWSR1/CREB1* *EWSR1/ATF1* *FUS/ATF1*
Desmoplastic small round cell sarcoma	Islands of Ewing-like cells; desmoplastic stroma	Poly-immunophenotypic:keratin, EMA, desmin, WT1 (carboxy domain)	t(11;22)(p13;q12)	*EWSR1/WT1*
Clear cell sarcoma	Nests of polygonal cells with abundant pale cytoplasm	S100, HMB45, MART1	t(12;22)(q13;q12)	*EWSR1/ATF1*

[a] Only the more common translocations are listed.

Table 1

the vast majority of cases, *EWSR1* partners with a member of the *ETS* family of transcription factors.[6] The most common partner is *FLI1* on 11q24 (85% of ES),[6] followed by *ERG* on 21q22 (10% of ES).[6] Others include *ETV1* on 7p22, *ETV4* on 17q22 and *FEV* on 2q33.[4]

The *EWSR1/FLI1* fusion gene is the most extensively studied of the translocations. Structurally, the amino terminal of *EWSR1* fuses in frame to the carboxy terminal of *FLI1*, and the resultant fusion gene acts as an aberrant transcription factor.[11] The fusion transcript has several reported breakpoints, but all contain the

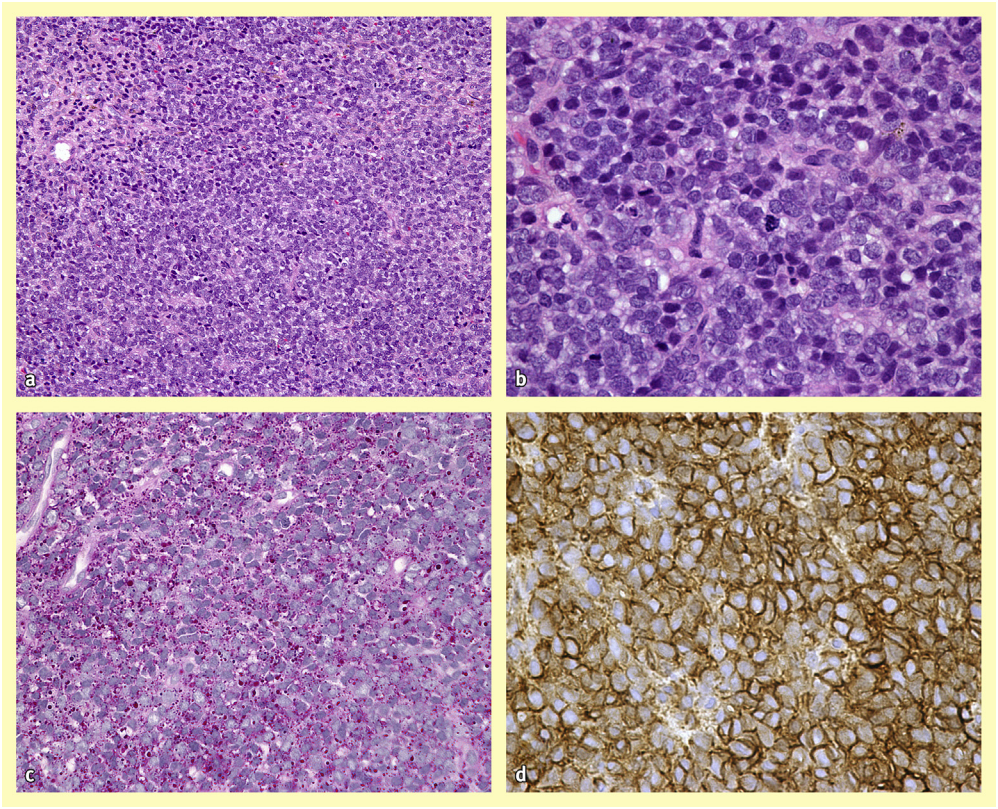

Figure 1 Ewing sarcoma/primitive neuroectodermal tumour. (**a**), low power micrograph showing sheets of round blue cells. (**b**), high power micrograph showing round cells with scant pale vacuolar cytoplasm, mild pleomorphism and finely clumped chromatin. Dark and light cells are intermixed. (**c**), PAS stain highlighting prominent amounts of intracytoplasmic glycogen. (**d**), CD99 immunostain showing a membranous pattern of staining. (**a**) & (**b**), haematoxylin and eosin, (**a**) ×200, (**b**) ×600; (**b**), PAS histochemical stain, ×400; (**d**), CD99 immunostain with haematoxylin counterstain, ×600.