Extraskeletal Ewing Sarcoma/Primitive Neuroectodermal Tumor

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Introduction

Ewing sarcoma (ES)/primitive neuroectodermal tumor (PNET) is the most common bone tumor between the age of 0–4 years [1], the second most common bone malignancy in children and adolescents [2–5], and the third most common bone sarcoma among adults [5]. ES/PNET is a family of tumor that shares hallmark molecular signature and varying degrees of neuroectodermal differentiation, which is now designated as ES by the 2013 *WHO Classification of Tumours of Soft Tissue and Bone* [3]. ES is more common among males compared to females with a male to female ratio of 3.4:1.6 [1]. The incidence of ES is higher among white population and is low among black and Asian populations [1]. Eighty to 90 % of ES affect the bony skeleton, commonly involving the diaphysis and metaphysis of long bones [3]. ES can also be seen in the pelvis, scapula, ribs, and skull. The remaining 10–20 % are extraskeletal. ES have been described in the lung [6], gastrointestinal tract [7], pancreas [8], breast [9], uterus [10], cervix [11], and pineal gland [12, 13].

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Pathogenesis

ES shows neuroectodermal differentiation with a pathognomonic translocation of the *EWSR1* gene on chromosome 22 with one of the ETS transcription factors. This specific translocation results in fusion oncogenes that is very characteristic for the Ewing/PNET (ES/PNET) pathogenesis [3], which is summarized in Table 1.

Microscopic Appearance

ES is mostly composed of small, blue, round cells with scant cytoplasm and indistinct cytoplasmic membrane. The nuclei are uniform and round with finely granular chromatin [3]. Ewing sarcoma is divided into several subtypes: (1) Classic Ewing sarcoma, which is the most common subtype. It is comprised of nests or sheets of small uniform cells with round nuclei, smooth nuclear contours, fine granular chromatin, and inconspicuous nucleoli. The cytoplasm is scant and eosinophilic. Mitosis and necrosis are infrequent. (2) PNET is composed of Ewing sarcoma with various degrees of neuroectodermal differentiation (confirmed by immunohistochemistry) together with true and pseudorosettes rendering the diagnosis of Ewing/PNET. (3) Atypical Ewing sarcoma is composed of large cells with large pleomorphic nuclei, irregular nuclear contour, coarse chromatin, and prominent nuclei. Mitosis and necrosis are common [4, 14]. Other rare ES subtypes include: (4) Adamantinoma Ewing sarcoma, which consists of nests of pleomorphic, hyperchromatic cells with peripheral palisading within a desmoplastic stroma [14, 15]. (5) Spindle cell Ewing sarcoma, this subtype is characterized by greater degree of spindling admixed with branching vasculature, reminiscent of a spindle

Table 1 Chromosomal rearrangement and fusion genes of ES [3]

Chromosomal rearrangement	Fusion gene
t(11;22)(q24;q12)	*EWSR1-FLI1*
t(21;22)(q22;q12)	*EWSR1-ERG*
t(7;22)(p22;q12)	*EWSR1-ETV1*
t(17;22)(q21;q12)	*EWSR1-ETV4*
t(2;22)(q35;q12)	*EWSR1-FEV*
t(16;21)(p11;q22)	*FUS-ERG*
t(2;16)(q35;p11)	*FUS-FEV*
t(20;22)(q13;q12)	*EWSR1-NFATC2*
t(6;22)(p21;q12)	*EWSR1-POU5F1*
t(4;22)(q31;q12)	*EWSR1-SMARCA5*
Submicroscopic inv (22) in t(1;22)(p36.1;q12)	*EWSR1-PATZ*
t(2;22)(q31;q12)	*EWSR1-SP3*
t(4;19)(q35;q13)	*CIC-DUX4*

cell sarcoma. (6) Sclerosing Ewing sarcoma, the neoplastic cells resembled the cells of typical ES but is distinguished by the presence of abundant, hyalinized, eosinophilic matrix [14].

Immunophenotype

The classic Ewing, which lacks neuroendocrine differentiation, expresses strong membranous staining for CD99 [3–16]. The FLI-1 protein expression, resulting in fusion of the *EWS* gene on chromosome 22 to the *FLI-1* gene on chromosome 11 (*EWSR1-FLI-1*), is another diagnostic marker of the classic Ewing sarcoma [16–18] (representative case shown in Fig. 1). FLI-1 has been expressed in more than 70 % of ES/PNET with greater than 90 % specificity [15]. Therefore, FLI-1 is useful for the diagnosis of ES/PNET. *NKX2*, a member of the NK2 family of

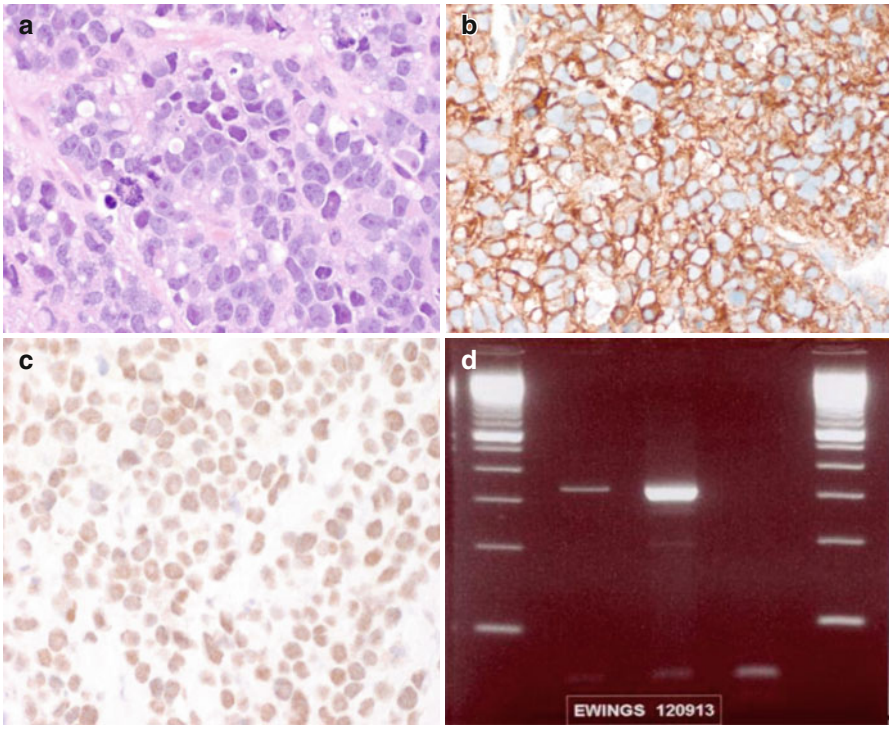

Fig. 1 A Ewing sarcoma in the right posterior shoulder of a 71-year-old man. (**a**) HE stain of the tumor exhibiting blue round cell tumor with epithelioid morphology. 200×. (**b**) CD99 immunostain reveals strong membrane reactivity. (**c**) FLI-1 immunostain is positive in the tumor nuclei. (**d**) The tumor harbors an EWSR1/FLI fusion transcript, type II, by RT-PCR confirming the diagnosis. Lane 1 and 4 DNA, size controls. Lane 3, positive control. Lane 4, patient sample

transcription, has an essential role in neuronal differentiation [19]. The *NKX2.2* gene has been recently identified as another important factor, similar to EWS/FLI in Ewing sarcoma oncogenesis [20]. For ES with *EWS-ERG* rearrangement, ERG protein expression, using the newly discovered anti-ERG antibody, is helpful for the diagnosis [21].

The PNET differentiation can be assessed by the expression of all classes of intermediate filament proteins [22], as well as synaptophysin, chromograin, CD56, CD57, CD99, neuron-specific enolase (NSE) [23].

Classic ES Versus ES/PNET

In 1991, a report by Schmidt and colleagues stated that neuroectodermal differentiation in ES (ES/PNET) has the worst prognosis and a significant decrease in disease-free survival compared to classic ES [24]. However, recent articles found that neuroectodermal differentiation in Ewing sarcoma (ES/PNET) had no prognostic impact when compared to classic ES [25]. These contradicting results may be due to the use of modern, more effective combined multiagent chemotherapy, radiation, and/or surgery which has improved the prognosis and outcome of ES/PNET dramatically [26]. Therefore, histological classification of ES is not neither necessary nor essential for proper management.

Differential Diagnosis

As small, blue, round cell tumors that may express neuroendocrine differentiation, ES overlaps morphologically and histologically with a group of malignant neoplasms that can occur in both children and adults. These tumors include neuroblastoma (NB), rhabdomyosarcoma (RMS), poorly differentiated synovial sarcomas (PDSS), lymphoblastic lymphoma (LBL), esthesioneuroblastoma (ENB), and desmoplastic round cell tumor (DRCT), mesenchymal chondrosarcoma (MCS), and blastemal predominant Wilms' tumor (WT). These tumors are histologically similar and can only be distinguished by their immunohistochemical profile and molecular profile [15, 27]. Useful immunohistochemical and molecular profiles for the above tumors are summarized in Table 2.

ES may present as a diagnostic pitfall mimicking a neuroendocrine carcinoma when neuroendocrine differentiation is expressed in conjunction with cytokeratin aberrant expression. In fact, ES can express the high molecular weight cytokeratin AE1/AE3 in almost 40 % of cases [31]. Therefore, in the proper histological and molecular setting, the expression of cytokeratin does not exclude the diagnosis of ES.

Table 2 Immunohistochemical and molecular characteristics of tumors mimic ES [3, 28–30]

Diagnosis	Translocation/molecular characteristics	Immunohistochemical positivity
Ewing-like sarcomas (undifferentiated round cell sarcomas)	*CIC-DUX4*, t(4:19)(q35;q13) or t(10;19)(q26;q13) CIC-FOXO4, t(X;19)(q13;q13.3)	CD 99 (patchy) Variable stains for CD34 Keratin, EMA, actin, and desmin
Alveolar rhabdomyosarcoma	*PAX3-FOXO1*, t(2;13)(q35;q14) *PAX7-FOXO1*, t(1;13)(p36;q14)	Desmin Myoglobin Myogenin Actin
Desmoplastic small round cell tumor	*WT1-EWSR1*, t(11;22)(p13;q12)	Multilineage markers such as: EMA and cytokeratin Vimentin and desmin NSE Desmin (dot-like) WT-1 carboxy-terminus
Extraskeletal myxoid chondrosarcoma	*NR4A3-EWSR1*, t(9:22)(q22;q12) *NR4A3-TAF15*, t(9;17)(q22;q11)	S100 (20 %) CD117 (CKIT) (30 %) Synaptophysin and NSE Loss of INI (tumors with rhabdoid features)
Clear cell sarcoma	*ATF1-EWSR1*, t(12;22)(q13;q12)	S100 HMB-45 MITF1 Other melanocytic markers

Management and Some Biomarkers

Five-year survival rates of ES were 68 % in males and 65 % in females [2, 32]. Decrease in survival rate can be due to tumor size (>8 cm) and presence of metastatic disease [32]. Management of localized disease includes wide surgical excision +/− chemotherapy to irradiate disease and prevent recurrence. Management of metastatic disease include chemotherapy, radiotherapy, and possible resection of limited metastasis [32].

Studies of additional biomarkers are underway to discover diagnostic, prognostic, and predictive information. Interestingly, the M3 and M5 subtypes of muscarinic acetylcholine receptor as well as the alpha 7 subunit of nicotinic acetylcholine receptor have been expressed in a significant number of ES/PNET family of tumors [33]. However, the clinical significance of these findings is yet to be determined. It was recently suggested that the expression of the gap junction protein connexin 43 in EWS/PNETs is reminiscent of a more aggressive clinical behavior [34].

Genomic technologies have identified genes that are modulated by EWS/FLI in Ewing sarcoma. The study of various chromosomes copy number alterations in Ewing sarcoma using karyotyping, comparative genomic hybridization, and single nucleotide polymorphism (SNP) microarray technologies has been previously reported. The summary of chromosomal aberration in Ewing sarcoma has been reviewed [35].

Tumor cells can be detected in the peripheral blood and/or bone marrow where they circulate. Circulating tumor cell (CTC) detection has been reported in epithelial neoplasms such as breast and colorectal carcinoma [36–39]; however, only few studies have been reported regarding CTC in ES. Analysis of bone marrow (BM) and peripheral blood (PB) of patients with ES using RT-PCR showed positive CTC in metastatic (46 % in BM and 22 % in PB) and nonmetastatic disease (19 % in BM and 20 % in PB) [40]. MicroRNAs (miRNAs) are short noncoding RNA which regulate gene expression, promote proliferation and differentiation, and may predispose to various human malignancies [41]. The role of microRNAs in ES pathogenesis has been investigated in the last few years. Studies have been targeted and aimed at microRNAs mediated by the *EWS-FLI-1* translocations. In vitro studies on human pediatric mesenchymal cell lines (hpMSCs) expressing EWS-FLI-1 showed that repression of miRNA-145 and the *SOX2* gene modulation induced ES pathogenesis [42]. Another in vitro study showed that hsa-miR-145 suppresses EWS-FLI-1 translation and therefore has a major role in ES pathogenesis [43]. In vivo studies on an ES mouse model showed that the *EWS-FLI-1* translocation induces CD99 expression by the inhibition of miRNA-30a-5p expression [44]. These new data may propose the utilization of miRNA as a future therapeutic target in the management of Ewing sarcoma.

Conclusion

ES should be considered as a rare but important differential diagnosis when working up a blue round cell tumor of soft tissue with neuroendocrine differentiation. Hallmark genetic marker can confirm the diagnosis, while some new immunostains are also helpful. New biomarkers are under investigation.

Abbreviations

DRCT	Desmoplastic round cell tumor
ENB	Esthesioneuroblastoma
ES	Ewing sarcoma
LBL	Lymphoblastic lymphoma
MCS	Mesenchymal chondrosarcoma
NB	Neuroblastoma

NSE	Neuron-specific enolase
PDSS	Poorly differentiated synovial sarcomas
PNET	Primitive neuroectodermal tumor
RMS	Rhabdomyosarcoma
WT	Blastemal predominant Wilms' tumor

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