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Features of Metastatic Ewing Sarcoma

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Abstract: Since its first description in 1921, Ewing sarcoma has been the subject of several morphologic and genetic investigations. Currently, the overall survival for localized Ewing sarcoma is 65–70%. However, in patients presenting with metastatic disease, the overall survival is poor, being in the range of 20–30%. There are several unknown features of Ewing sarcoma, such as its cell of origin, genetic background, chemotherapy resistance, and abnormal presentation sites, among others. A better understanding of the molecular basis of the development of Ewing sarcoma is needed to help improve survival, especially in metastatic/resistance cases. In this chapter, we provide an overview of the features of metastatic Ewing sarcoma.

Keywords: cell signaling in Ewing sarcoma; metastatic Ewing sarcoma; metastatic spread; oncogenetic origins of Ewing sarcoma; therapy of metastatic Ewing sarcoma

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INTRODUCTION

Among sarcomas, Ewing sarcoma/Primitive neuroectodermal tumor (ES/PNET) is one of the most dangerous and lethal (1). James R. Ewing, an outstanding American pathologist at Cornell, described this entity in 1921 (2–4). The incidence of this tumor is 1–3 cases per million per year (5, 6). It is an aggressive tumor that can quickly become metastatic and affect children and young adults, being more common in Caucasians than in Asians or Africans. It is also more common in males than females (7, 8). ES/PNET usually occurs in the diaphysis or metaphyseal-diaphyseal portion of the long bones, pelvis, and ribs. Radiologically, ES/PNET shows an ill-defined osteolytic lesion with permeative or moth-eaten bony destruction. An "onion-skin" like periosteal reaction, associated with pain and fever, is the most common symptom. The most common laboratory findings include anemia, leukocytosis, and an increase in erythrocyte sedimentation rate (ESR). In the early 20th century, radiotherapy was the primary therapy modality, and the overall survival was 30%. Today, the combination of chemotherapy and radiotherapy is the standard treatment approach, with surgery reserved for resistant/recurrence disease if the site of the neoplasm is amenable to surgery (9–12). Therapy and event-free survival for ES/PNET have significantly improved over the years with the 5-year survival rate being barely achieved before using neo-adjuvant chemotherapy. Today, the long-term survival rate is 30–60%, suggesting that ES/PNET is sensitive to anti-cancer agents. There are many protocols to treat this type of sarcoma. The most common drugs used are doxorubicin, cyclophosphamide, vincristine, actinomycin-D, ifosfamide, and etoposide (13).

LIGHT AND ELECTRON MICROSCOPY FEATURES

Histologically, ES/PNET is a "round blue cell tumor" that is characterized by predominantly undifferentiated sheets of cells with relatively little stroma. It may be found at the skeletal site of origin or at metastasis (Figure 1). The cell size may vary with some ES composed of small round cells with round nuclei containing fine chromatin, scanty clear or eosinophilic cytoplasm, glycogen granules highlighted by Periodic Acid Schiff (PAS) staining, and indistinct cytoplasmic membranes (Figure 2). On the other hand, some ES/PNETs are made up of larger cells with prominent nucleoli and irregular contours. The tumor cells contain abundant glycogen, which is highlighted by histochemistry (PAS). In some cases, an arrangement of the tumor cells around a pseudo lumen (Homer-Wright pseudorosettes) is seen. Necrosis is a standard feature, and non-necrotic viable areas show tumor cells with frequent perivascular distribution (7).

Almost all cases express CD99 on the cell membranes (Figure 3). Vimentin and neuron specific enolase are also frequently expressed by immunohistochemistry. The relative lack of differentiation of ES/PNET has led to difficulty in identifying the tumor cell of origin, and it probably involves more than one cell type. Various authors have tried to find the cell of origin. Many suggestions have been made. They include endothelial, perivascular lympho-endothelial, pure hematologic, mesenchymal/fibroblastic, neural crest derivatives, or stem/progenitor cells. The ultrastructural investigation of tissue specimens of ES/PNET reveals polygonal cells with

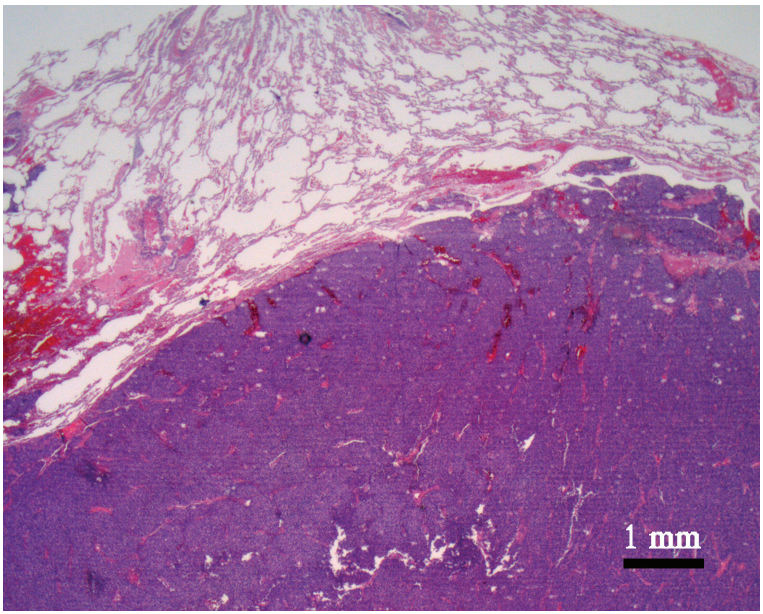

Figure 1. Blue cell tumor. The microphotograph depicts a resection specimen of the lung showing a "round" blue cell tumor involving the right lower half portion of the picture (dark blue area). The tumor is well demarcated and the intensity of the tumor cells is identifiable easily comparing to the alveolar parenchyma of the lung of the left upper half of the picture. The "roundness" of the tumor cells with round-ovoid contour is not appreciable at this magnification, but is better identifiable in figure 2 (Hematoxylin and Eosin staining x 12.5 original magnification).

slightly irregular nuclei, finely dispersed chromatin, and prominent nucleoli (14–16). Nucleoli are small-to-large and often open (nucleolonemas). However, some atypical cases of ES/PNET show more deeply indented nuclei. The tumor cells appear smooth-contoured, and aggregates of glycogen rosettes are frequently seen in the cytoplasm. Glycogen granules may also be seen free in the interstitium.

Lipid spherules may also be seen. The cytoplasm has limited cytoplasmic organelles. Microtubules and mitochondria are rare, but pseudopod-like extensions can be observed. There is little stroma between the tumor cells. Neurosecretory granules with a 100–150 nm diameter and microtubules may be seen. The cells are joined by poorly formed, so-called desmosome-type, intercellular junctions. Atypical ES/PNET shows variable amounts of glycogen. They can also demonstrate prominent cytoplasmic filaments, mitochondria, and profiles of the endoplasmic reticulum. The ultrastructural assessment of ES/PNET requires optimal preservation of the cellular and sub-cellular organization. The "en bloc" staining technique with uranyl salts results in considerable glycogen extraction, and lead staining is mandatory. The fixation with Karnovsky osmium tetroxide-potassium ferricyanide fixative enhances glycogen preservation. Finally, ES/PNET should be kept in the differential diagnosis of the ultrastructural investigation of childhood round blue cell tumors. These tumors can sometimes be difficult to differentiate on immunohistochemistry or molecular biology (17–21).

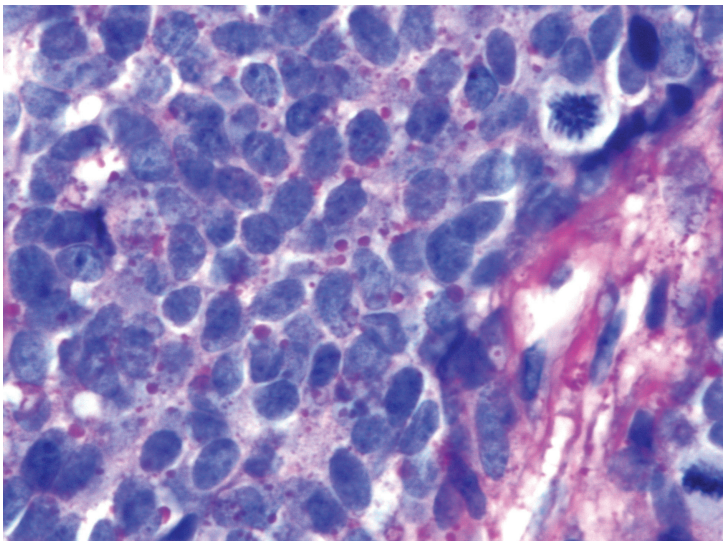

Figure 2. Glycogen deposits in ES/PNET. The microphotograph shows a high power view of the Ewing sarcoma/Primitive neuroectodermal tumor (ES/PNET) depicted in figure 1 with particulate of glycogen in the cytoplasm of the tumor cells and a high nucleus to cytoplasm ratio. Two mitoses (right upper corner and right lower corner) are also seen (Periodic acid Schiff staining x 630 original magnification).

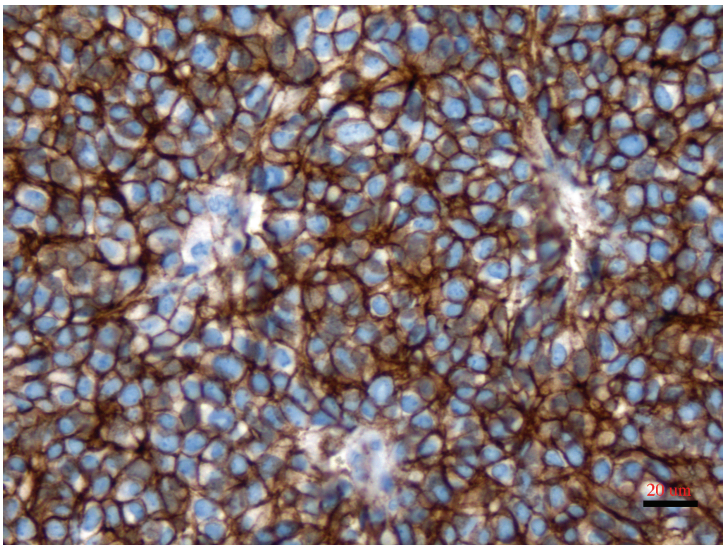

Figure 3. CD99 expression in ES/PNET. Immunohostochemistry of Ewing sarcoma/Primitive neuroectodermal tumor (ES/PNET) showing an intense membranous staining. It is imperative to emphasize that FLI1 immunohistochemistry is standard of care because of the non-specificity of the CD99 antigen. Thus, a positive CD99 staining result must be validated by a nuclear FLI1 immunohistochemical result, which is not demonstrated here. FLI1 is the surrogate marker for characteristic *EWSR1-FLI1* translocation. A minority of ES/PNET disclose a translocation involving the *EWSR1* gene and an alternative partner, most commonly the ERG gene, which is located on 21q12. (Avidin-Biotin Complex, anti-CD99 immunostaining, x400 original magnification).

ONCOGENETIC ORIGINS AND CELL SIGNALING OF EWING SARCOMA

ES/PNET is an example of a malignancy caused by fusion between oncogenes—*EWS* (Ewing's sarcoma breakpoint region 1) and *ETS* (E-twentysix family of transcription factors) (22, 23). Genetically, these fusions come from specific chromosomal translocations that yield in-frame fusion of the amino terminus of the *EWS* gene on chromosome 22 and the carboxyl terminus, including the DNA-binding domain, of an ETS gene. The most common types are t(11;22)(q24;q12) and t(21;22)(q22;q12). The t(11;22) translocation results in the fusion of the region 59 of the ubiquitously expressed *EWS1* gene to region 39 of the *FLI-1* gene. This chimeric gene product (EWS/FLI-1) is an aberrant transcription factor that contains the transcriptional domain of EWS1, which is usually involved in protein-protein interactions, and the DNA-binding domain of FLI-1. Although capable of promoting tumorigenesis, the target gene(s) of EWS/FLI-1 is not yet fully studied in detail. In a recent study involving the Children's Oncology Group (COG) trial AEWS1221, the patterns of translocation testing for newly diagnosed ES were described (24). The AEWS1221 trial was a phase III randomized study enrolling patients with newly diagnosed metastatic ES/PNET from 2014 to 2019. Three-hundred and five patients were enrolled. The most common type of molecular testing was fluorescence in situ hybridization (FISH). FISH was carried out on the primary tumor in three-fourths of the patients. Dubois et al. (24) found positive testing for an *EWSR1* or *FUS* translocation in 211, which equates almost 90% of the patients. A reverse transcription-polymerase chain reaction (RT-PCR) was carried out in one fifth of the patients. The authors recorded positive results in three-fourths of the patients. A next-generation sequencing was reported in seven patients for the primary tumor and in three patients for metastatic sites. In about 5% of the patients, a translocation testing was neither on the primary nor on the metastatic tumor reported. The lack of an abnormality consistent with a molecular diagnosis of ES/PNET was seen in about 15% of the patients.

Besides chromosomal rearrangement, two mutations are correlated with ES: p53, and Retinoblastoma *RB* pathway, with an incidence of 5–20%. The rate on *ETS/FLI-1* is in 85% of cases, *ERG* in 10% of cases, and ETV1, ETV4, or FEV in the remaining 5% of cases. Other common mutations or deregulated pathways involve the *JNK* (c-JUN oncogene) (25, 26), *uPA/uPAR* (urokinase plasminogen activator), and *PEDF* (pigment epithelium derivate factor) genes (27). The JNK pathway is related to malignant proliferation and differentiation via Mitogen-activated protein kinases (MAPKs), growth factors, and environmental stress. It occurs in response to inflammatory processes such as cytokines, being useful as biological markers to define if the tumor is at a low or high differentiation grade. The signaling consists of phosphorylation of JNK, and activation of the transcription factor c-Jun via several modulatory Serine/Threonine sites within its N-terminal transactivation domain. Activated c-Jun proceeds to homo/heterodimerize with c-Fos, generating the activator protein-1 (AP-1) transcription complex. It binds specific DNA sequences at target promoters and regulates the expression of genes (26, 28). The uPa/uPAR is involved in angiogenesis, cell migration, malignancy, and wound healing. Thus, high expression can indicate a

fast progression of ES/PNET, the chance of metastasis, and, consequently, the prognosis of the disease. This signaling works on the conversion of plasminogen to plasmin which degrades proteins such as fibrin, fibronectin and laminin, facilitating matrix degradation and breakdown of the extracellular matrix, which is a critical step in cell invasion and cancer metastasis. The PEDF is a potent antiangiogenic and antitumorigenic glycoprotein and prevents cell migration. Low expression of PEDF can contribute to tumor growth, increased aggressiveness, metastasis, and poor outcome (29–31).

The genetics of ES also interferes directly with cell signaling, deregulating many cell processes, leading to tumorgenesis. There are two common pathways deregulated in ES/PNET: tyrosine kinase and Wnt (32–35). The autocrine and paracrine activation of growth factor receptors and their corresponding ligands, such as insulin-like growth factor 1 (IGF1), determine ES proliferation and maintenance (34, 36–42). Targeting of *EWS/FLI1* by RNA interference in ES cells affected IGF1/IGF1R (insulin like growth factor receptor type 1) survival pathway and its downstream targets. Both MAPK and PI3K signaling pathways are constitutively activated in ES, probably because of the presence of IGF1R-mediated autocrine loops. Stringent clustering was observed by analyzing gene expression in 181 tumor types, including 16 classes of sarcomas with high expression levels of tyrosine kinases or receptor tyrosine kinases in the pediatric sarcomas group. Also, there is evidence that Wnt/Frizzled signaling is functional in ES/PNET cell lines (43). Canonical Wnt/β-catenin signaling enhances EFT (Ewing family of tumors) cell motility, contributing to metastasis, probably through either autocrine or paracrine modes of action of Wnt glycoproteins. These are expressed in bone, muscle, and soft tissues. Wnt-3a induces morphological changes characterized by the formation of long cytoplasmic extensions in EFT cells (44–46).

There are reports correlating the role of IGF axis with the occurrence of ES/PNET (37). Studies demonstrated a strong influence on deregulated secretion of IGF and the malignancy of tumors (40, 47–52). The genetic mechanisms involve mainly three genes: *EWS*, *FLI1*, and *WT1* (23). The WT1 proteins can suppress the transcriptional activity of IGF and its proteins. There are several ways IGF can promote growth in tumor cells: increased secretion of IGF-1, decreased production of IGFBP-3 (insulin-like growth factor-binding protein), and the development of IGF-1R on the cell membrane. The *EWS* and *WT1* gene can regulate the IGF1R activity by binding IGF1R transcription start site. As for the *EWS* and the *FLI1*, the latter, when present, can activate the *EWS* oncogene pathway, producing abnormal proteins, making the cell susceptible to abnormal growth via the IGF axis and IGF1R is considered a potential target in ES/PNET (37–39, 53–56). It has been demonstrated that resistant cells switch from IGF1-IGF1R signaling to IGF2/insulin/IRA signaling. It means that there is an activation of the proliferative downstream pathways, indicating that some responding patients with ES/PNET did not have active IRA signaling. Of the pathways detected, the IGF2/insulin/IRA and the MAPK pathways seem to be important for the resistance to IGF1R inhibition. Also, the role of the IGF2 mRNA-binding protein 3 (IGF2BP3) in IGF1R and IRA signaling needs to be better elucidated.

PATTERNS OF METASTATIC SPREAD

Metastatic spread is probably the most powerful predictor of poor survival. There are several sites that have been reportedly affected by the metastatic spread. In up to four-fifths of the patients affected by ES/PNET, metastases occur in the first two years following the primary manifestation of the tumor. Metastases appear frequently in the lung (57%) and bones (34%), while spread to the central nervous system occurs in 10–37% of cases with brain metastases; primary and metastatic ES/PNET of the skull bases have been reported (57). It seems that the occurrence of metastasis, which can be overt or subclinical, seems to be a recurring phenomenon in almost all patients. Such metastatic sites can also be quite distant and rare from the primary site of the tumor, including small bowel, oral cavity, pancreas, spine, and orbital cavities among others (57–65). In a dramatic presentation, ES/PNET metastasized into the jejunum causing intussusception, one of the most life-threatening chirurgical emergency (65). It seems that the transcription factor hepatoma-derived growth factor (HDGF) plays a crucial role in meatastatic spread (66). HDGF seems to regulate multiple metastasis-associated genes, including the activated leukocyte cell adhesion molecule (*ALCAM*)) in ES/PNET cells (66). By downregulating *ALCAM*, HDGF induces the expression and activation of many downstream effector genes, including *Rho-GTPase Rac1* and *Cdc42*. In addition, HDGF promotes actin cytoskeleton remodeling and cell-matrix adhesion, which are critical in paving the pathways for metastatsic spread.

THERAPEUTIC CHALLENGES OF METASTATIC ES/PNET

Local therapy has been proposed for some patients folllowing the examination of the primary tumor by MRI, the lungs by CT, and the entire body by ^{18}F-FDG-PET/CT/MRI. The principles, and techniques of surgery and reconstruction in primary malignant osteotumors have been defined (67). Local therapy of involved sites is crucial in controlling the outcome of patients affected with ES/PNET. Histopathological response in response to systemic therapy, assessed as >90% necrosis, has a major impact on local control rates. Additional radiotherapy following surgery has been indicated in any case of positive margins. Some protocols suggest additional radiotherapy for narrow margins and/or poor histological response (≥10% viable tumor cells in the surgical specimen assessed with histopathological examination) (67).

A combination of local therapeutic strategies may be suggested for large primary tumors with extensive soft-tissue extension. Neoadjuvant chemotherapy alone or in combination with preoperative radiotherapy has helped in reducing the tumor volume, particularly of the soft tissue component. It seems that it facilitates an adequate limb-sparing surgery. It is particularly important for subjects affected with disseminated and multifocal tumor and should probably complement systemic protocols whenever possible (67). Local treatment of both primary and metastastic tumor seems to be superior to local treatment of either the primary tumor or extrapulmonary metastases in increasing the survival of the patients.

In disseminated tumor, a combined modality treatment including surgery of both the primary tumor and extrapulmonary metastases has shown better survival than single-modality local therapy (67). It has been suggested that solitary osseous metastases may be approached by surgery, radiotherapy or both if the morbidity is considered acceptable. Patients with pulmonary metastases should be considered for the same local therapy as those without (67). The role of metastasectomy of the lung parenchyma in relapsed tumor remains controversial, with variable evidence of benefit between studies (67). It is reasonable to perform a metastasectomy in selected ES/PNET patients with resectable lung metastases and proof of adquate cardiopulmonary function. It seems that the number of pulmonary metastases, disease-free time interval, and chemotherapy-response are critical factors influencing overall survival in ES/PNET (68).

The outcome of ES/PNET patients with metastasis only in the lung is better than those with bone metastasis. In fact, patients who have both pulmonary and bone metastasis have the worst outcome (69–74). While various combinations of chemotherapeutic agents have provided beneficial event-free surivial for localized ES/PNET, benefits of chemotherapy for metatastatic ES/PNET continues to be dismal with the 3-year event-free survival being only 28–30% (75, 76). The addition of ifosfamide and etoposide to VDC (vincristine, doxorubicin, and cyclophosphamide) showed no significant benefit in patients who had netastatic diseases at diagnosis, differently from the improvement observed in patients with nonmetastatic ES/PNET (77). Various combination of other anticancer drugs such as vinblastine, celecoxib, or the anti-IGFR antibody ganitumab, with VDC/ifosfamide/etoposide have failed to improve outcomes (78, 79). High-dose chemotherapy with autologous stem cell did not provide any beneficial effect (80, 81). A recent Cochrane review concluded that when the location of primary ES/PNET metastasis, i.e., with metastatic disease at diagnosis, is other than the lungs, high-dose chemotherapy or autologous hematopoietic cell transplantation do not provide any advantage in event-free survival than those who receive conventional chemotherapy with whole lung irradiation (82). Palliative radiotherapy can provide symptom relief, especially pain, without a protracted treatment course for metastatic ES/PNET patients (83).

CONCLUSION

Ewing sarcoma is a highly malignant bone tumor with a poor prognosis. The prognosis has improved over the years for localized tumors but remains poor for patients with metastatic disease. Various molecular pathways involving JNK, uPA/uPAR, PEDF, and IGF have been identified in ES/PNET malignancy; however, their role in metastatic transformation is yet to be fully clarified. Metastatic ES/PNET is difficult to treat. The most commonly used radiation therapies and chemotherapies have very limited beneficial effect. Despite remarkable efforts in both diagnostic and therapeutic approaches, several aspects of ES/PNET remain elusive. A better understanding of the molecular mechanisms of metastatic transformation is urgently needed to develop effective treatment strategy for metastatic ES/PNET. Exploring drug repositioning may be one of the therapeutic options for advanced, refractory, or relapsed ES/PNET.

Conflict of Interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

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