Small Round Cell Tumors of Bone and Soft Tissue: An Update
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Small round cell tumors are these that contain sheets of small cells with dense high cellularity and high N/C ratio. These lesions are highly malignant as a rule, and they largely occur in children between newborn and 20 years of age. They are typically composed of primitive cells with minimal or no differentiation. When dealing with small round cell tumors, the term "small" can be relative, so that undifferentiated neoplasms may contain cells that are relatively large. When pathologists confront these lesions, they typically are aware that these represent a challenge that requires careful analysis and expert opinion.

The most common pediatric lesions in this category comprise hematopoietic neoplasms (lymphoma and leukemia), neuroblastoma, rhabdomyosarcoma, and Ewing sarcoma. Small cell tumors that occur primarily in bone include poorly differentiated chordoma, melanotic neuroectodermal tumor, mesenchymal chondrosarcoma, and small cell osteosarcoma. Small cell tumors may occur at specific sites of the body, such as desmoplastic small cell tumors that occur in the abdomen, germ cell tumors that occur in midline locations and gonads, and NUT translocation carcinomas that occur within the midline of the head, neck, and upper thorax. Small cell tumors that are specific to internal viscera include Wilms tumor, hepatoblastoma, sialoblastoma, pancreaticoblastoma, and pleuropulmonary blastoma. Some small cell tumors are relatively rare but occur in diverse sites: These include synovial sarcoma and rhabdoid tumors. Finally some neoplasms still resist categorization and are best termed by the diagnosis "undifferentiated sarcoma, not otherwise specified". With further genetic study, this last category will likely show a continuing decrease. It is the purpose of this presentation to focus on some of the newer findings in this latter category.

Small round cell tumors may be associated with a variety of genetic factors, including constitutional mutations, deletions and epigenetic factors such as loss of imprinting, loss of heterozygosity, methylation abnormalities, histone acetylation errors, and dysregulation of non-coding RNAs. These lesions may also occur as acquired mutations, including "second hits", clonal progression, and translocations that produce chimeric proteins and altered promoter regions.

Ewing sarcomas

Ewing sarcomas are poorly differentiated or undifferentiated neoplasms that arise mostly in bone and often in soft tissue. Rarely, they occur in viscera such as the kidneys. These neoplasms are characterized by genetic fusions of *EWS* with ETS family genes. Rarely *FUS* is substituted for *EWS*. These lesions are characterized by a marked predilection for Caucasians and a relative rarity in African-

Americans. They mostly occur within adolescents and young adults, but young children and older adults can also be affected. They are slightly more common in boys. Any bone of the body may be affected. Among visceral organs, kidneys rank as the most commonly affected. Overall, Ewing sarcoma is the second most common primary pediatric cancer in both bone and soft tissue.

Bony Ewing's sarcomas occur mostly within the diaphysis and are typically associated with a large soft tissue component. Favored locations include the paravertebral region, retroperitoneum, chest wall, and extremities. Radiographs show a destructive, lytic lesion with endosteal erosion and periosteal reaction, often with cortical breakthrough and formation of a soft tissue mass.

There are 3 major histologic varieties of Ewing's sarcomas: classical Ewing's sarcoma, atypical Ewing's sarcoma, and peripheral primitive neuroectodermal tumor (PNET). Classical Ewing's sarcomas contain sheets of even round cells with minimal amounts of cytoplasm, sometimes vacuolated, and round nuclei with even chromatin. Mitoses can be surprisingly uncommon. Often, there is a mixture of "light" and "dark" cells that appear to be viable and effete cells. There may be cytoplasmic vacuolization, which represents abundant glycogen. The large cell variant of Ewing sarcoma contains larger cells with more pleomorphism, prominent nucleoli, and mitotic activity. PNET should by definition contain rosettes of either the Homer Wright or Flexner-Wintersteiner variety. On rare occasions, PNET contains S100-positive spindle cells.

Electron microscopy is not often used in current practice for diagnosis of Ewing's sarcoma. Besides the pools of glycogen, one may see evidence of primitive neural differentiation, such as neurosecretory granules, microtubules, and cytoplasmic processes. A more consistent finding is the presence of intercellular junctions, which cause cells to adhere on touch preparations. Immunohistochemistry has become a standard part of diagnosis. Markers used include CD99 and FLI1, which are almost always positive. CD56 can be used as a negative marker and typically stains other neural tumors. Chromogranin is typically negative because of the dearth of neurosecretory granules. Cytokeratin is positive in minority of cases. Focal CD57 expression is often present.

Because of the non-specificity of Ewing's sarcoma markers, genetic testing has become the standard part of diagnosis. About 85% contain a t(11;22) that fuses *EWS* and *FLI1*. Another 10-15% contain an alternate translocation, a t(21;22) that fuses *EWS* and *ERG*. Rare Ewing's sarcoma translocations fuse *EWS* with other ETS genes, including *FEV, EWS, ETV1*, and *E1AF.* Genetic diagnosis can be performed by karyotyping, FISH, and RT-PCR. Karyotyping has the advantage of discovering new fusion variants. However, karyotyping is relatively imprecise, as it does not identify the fusion site at the molecular level. Unfortunately, results may be false negative and are often only available after the diagnosis has already been made.

RT-PCR has much more sensitivity and precision than standard karyotyping, but diagnosis requires recognition of the entire fusion site, as a portion of both gene partners must be present. This requires consensus primers for reagents, and multiplex reaction panels are needed to detect multiple translocations. RT-PCR has the advantage of detecting the variety of fusion lengths, formally felt to have prognostic significance; however, more recent studies no longer show clinical significance.

FISH testing recognizes rearrangement of *EWS*, which generally indicates the presence of a translocation. This is perhaps the most sensitive technique, but it suffers from the fact that it does not identify the partner gene. As a result it is relatively nonspecific, as a variety of non-Ewing's tumors may show *EWS* translocations. These include clear cell sarcoma of soft tissue, extraosseous myxoid chondrosarcoma, myoepithelial carcinoma of soft tissue, myxoid liposarcoma, and angiomatoid fibrous histiocytoma. Thus, FISH testing must always be used in combination with immunohistochemistry and histologic evaluation.

The majority of cases tested with both FISH and RT PCR are positive in both. Some cases may be FISH positive and RT-PCR negative. This frequently is caused by the presence of an alternate fusion partner other than the ones tested on the RT-PCR panel. Another possibility is a "low expressor" that does not produce enough RNA to be detected by routine RT-PCR. More sensitive techniques such as the nested PCR reveal low expression of RNA in these cases. Finally, some tumors may be FISH negative and RT-PCR positive. This may indicate a problem with processing of formalin fixed paraffin-embedded tissues, particularly failure to properly indicate the location of tumor on a slide, leading to a normal FISH study. Thus, it is always incumbent on the pathologist to carefully indicate the location of tumor with a marking pen prior to FISH testing.

In rare cases, instead of the usual EWS *FUS* forms a fusion with an ETS gene. *FUS* partners have included *ERG* and *FEV*, and others will undoubtedly be discovered. As a member of the TET family of genes, *FUS* shares homology with *EWS*. This situation occurs with a variety of other sarcomas that also show substitution of *FUS* for *EWS* such as myxoid liposarcoma, angiomatoid fibrous histiocytoma, and low-grade fibromyxoid sarcoma.

The prognosis and outcome of patients with Ewing's sarcoma has improved greatly in recent years, particularly following the addition of ifosfamide and etoposide to the treatment regimen. At the present time, there is a 70% survival for patients with non-metastatic tumors. Unfortunately, patients with metastatic tumors still suffer from a poor outcome, with a survival of approximately 20%. Negative factors that affect outcome include tumor size (greater than 8 cm), tumor site (more proximal tumor locations), and age (greater than 18 years old). The histological effect of chemotherapy has been said to be related to prognosis in a fashion similar to osteosarcoma, with the presence of total necrosis being a good prognostic sign. However, the data for this latter observation are less compelling, and more studies are needed. Controversial prognostic factors include tumor histology and bony versus soft tissue primaries. In older reports, PNETs had a worse outcome, but this has not been verified in more recent studies. People have also argued about whether soft tissue tumors should be treated as bony neoplasms, but this has been a standard way of treating these lesions for over a decade. In the United States, soft tissue Ewing's sarcomas were originally treated as rhabdomyosarcomas, with outcomes similar to those of embryonal rhabdomyosarcoma.

Differential diagnosis of Ewing's sarcomas include a variety of lesions. In bone, one should also consider lymphoma, osteosarcoma, mesenchymal chondrosarcoma, invasive soft tissue sarcomas, and undifferentiated "Ewing like" sarcomas. CD45 may be negative in lymphoblastic lymphoma, and CD99 is often positive. As result, a wider panel of lymphoma markers that includes reagents such as CD3, CD20,

CD43, and TDT should be considered. Rhabdomyosarcomas may be excluded by use of myogenin, but desmin may be positive in rare Ewing's sarcomas.

Undifferentiated Ewing-like sarcomas

There is a growing body of knowledge about "undifferentiated Ewing like sarcomas", which show a variety of genetic aberrations. These include those with *EWS* fusions with non-ETS family genes and those lacking *EWS* fusions but showing a similar genetic profile. These lesions may partner *EWS* with either a transcription factors or non-transcription factors, show typical or atypical Ewing histology, occur either in bone or soft tissue, and show variable CD99 and neural marker positivity. The clinical setting and therapeutic responses of these lesions are heterogeneous and at the present time unpredictable. The rarity of these lesions has precluded extensive understanding, so a fragmented medical approach is often necessary to their diagnosis and treatment.

EWS-NFATC2 sarcomas fuse *EWS* with a non-ETS gene transcription factor. Although *NFATC2* is not part of the ETS family of genes, it shares similar DNA binding properties. As result, the protein product of the fusion gene activates the same downstream pathway as *EWS-FLI1* fusions. Of note, these fusions show recurrent gene amplification, so that one may discover a large number of signals on FISH studies.

EWS-PATZ1 and *EWS-SP3* sarcomas fuse *EWS* with a transcription factor unlike ETS family genes. Both of these fusion genes fuse *EWS* to a zinc finger gene, similar to the *EWS-WT1* occurring in desmoplastic small round cell tumor. These Ewing-like sarcomas also show polyphenotypia, aggressiveness, and drug resistance similar to desmoplastic small round cell tumor. Of note, rarely one may see small round cell tumors with the polyphenotypia and desmoplasia of desmoplastic small round cell tumor but containing an *EWS-FLI1* fusion.

EWS and *SMARCA5* form a variant fusion that links *EWS* with an epigenetic factor rather than a transcription factor. SMARCA5 is a chromatin remodeling protein similar to INI1. This suggests a relatedness of this tumor to rhabdoid tumor, but nothing has been proven to date.

Some have described a Ewing like sarcoma that contains a fusion of *EWS* with *POU5F1,* which produces OCT4, a transcription factor that regulates stem cells. This fusion gene binds *EWS* to a transcription factor gene entirely unlike those of the ETS family. These tumors show a heterogeneous morphology, with areas containing the nested polygonal and spindle cells, and they express S100. Thus, they more likely represent a form of soft tissue myoepithelial tumor rather than a Ewing like sarcoma (*EWS* fusions have been reported in the former lesion).

Some Ewing like sarcomas contain fusions that are unlike *EWS-ETS* but lead to overexpression of a similar downstream group of genes. These include *CIC-DUX4* tumors, containing balanced translocations between chromosomes 4 and 19 or 10 and 19, and *BCOR-CCNB3* fusion tumors, containing a paracentric inversion of the X chromosome.

CIC-DUX4 sarcomas are usually extraskeletal and arise most commonly in the extremities. They show an aggressive course with early metastasis. They have histologic features in common with atypical Ewing

sarcoma, such as prominent nucleoli, more abundant cytoplasm, and extensive necrosis and mitotic activity. CD99 positivity can be variable, and this lesion should be considered in any Ewing like sarcoma that lacks CD99 staining. Of note, they are frequently WT1-positive. The CIC protein contains a binding site for TLE1 protein, similar to that seen with synovial sarcoma fusions. *CIC-DUX4* transcribes a DNA binding site that up-regulates several ETS family genes. Alternate partners for *CIC* have been described in recent papers. *CIC-DUX4* appears to be particularly common among EWS fusion-negative Ewing like tumors.

BCOR-CCNB3 sarcomas usually arise in bone and resemble classic Ewing's both clinically and morphologically. These are rare neoplasms that represent less than 5% of undifferentiated sarcomas. Often they contain areas with relatively low grade appearing myxoid stroma and lower cellularity. This may lead to problems with limited biopsies. These tumors contain fusions of *BCOR* with *CCNB3,* an epigenetic regulator. This fusion amplifies the ability of CCNB3 to drive cell cycle events, and the loss of function of BCOR causes epigenetic instability. *BCOR* mutation may also occur as a constitutional mutation that leads to skeletal dysplasia, the oculofaciocardiodental syndrome. This leads to increased osteogenic potential of mesenchymal stem cells and bony overgrowth. Somatic mutations of *BCOR* are also occur in AML, myelodysplasia, medulloblastoma, endometrial stromal sarcoma, and ossifying fibromyxoid tumor.

Desmoplastic small round cell tumor (DRSCT) is a high grade malignancy that resembles Ewing's sarcoma but contains abundant fibrous stroma. These lesions predominantly occur in intra-peritoneal organs but may be seen elsewhere, particularly in the paratesticular region. They contain small cells that are surrounded by abundant fibroplasia and express multiple anagen types typical of epithelial, neural, and mesenchymal tissues. Dot-like desmin positivity is a common feature. Some lesions do not have typical histology. As noted above, DRSCT contains an *EWS-WT1* fusion that links *EWS* with a gene encoding a zinc finger protein. This leads to dysregulation of WT1, causing tumor cell proliferation that features aggressive behavior, poor chemotherapy response, and peritoneal seeding and metastasis, not unlike ovarian carcinoma. DSRCT cells may show features of epithelial differentiation such as glands, neural differentiation such as rosettes, and rhabdoid cells. Many show CD99 positivity, sometimes with a Ewing-like membrane pattern. Mesenchymal markers positive in these lesions include vimentin, desmin, smooth muscle actin, and muscle specific actin. Epithelial markers include cytokeratin and epithelial membrane antigen. Positive neural markers may include neuron specific enolase, CD57, synaptophysin, chromogranin, and NB84. Miscellaneous markers that also may be positive include CD15, WT1, CD99, CA 125, and BER-EP4.

Conclusion

To summarize, we are rapidly moving towards an era of genetic diagnosis for small cell tumors. It is hoped that concomitant advances in personalized medicine and gene based therapy will lead to better patient outcomes.

Recommended reading:

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