Ewing Sarcoma With Extensive Neural Differentiation

A Clinicopathologic, Immunohistochemical, and Molecular Analysis of Three Cases

Annikka Weissferdt, MD, FRCPath, Neda Kalhor, MD, and Cesar A. Moran, MD

From the Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston.

Key Words: Sarcoma; Neural; Ewing sarcoma

ABSTRACT

Objectives: Three patients with Ewing sarcomas showing extensive neural differentiation are presented.

Methods: The patients were two women and one man between the ages of 15 and 35 years. Anatomically, one tumor was in the lung, one in the testis, and one in the cervix uteri. The symptoms were determined by the location of the neoplasm and included respiratory symptoms, testicular pain, and pelvic manifestations, respectively. Complete surgical resection of the tumors was performed.

Results: Histologically, all neoplasms showed similar characteristics—namely, a neoplastic cellular proliferation arranged in sheets and composed of small blue cells with round to oval nuclei and inconspicuous nucleoli typical for Ewing sarcoma. In addition, in two cases, there were areas characterized by the presence of neuropil, ganglion cells, and small cells most compatible with ganglioneuroblastoma, while in one tumor, the neural component was characterized by the presence of small cells with prominent perivascular pseudorosettes more closely resembling ependymoma. Immunohistochemical studies in all cases and molecular analysis in two tumors were in keeping with a diagnosis of Ewing sarcoma.

Conclusions: The recognition of such histologic variants is important in the diagnostic assessment of these tumors to avoid misinterpretation, especially in small biopsy specimens.

Ewing sarcoma is the current acceptable nomenclature for a group of tumors that, over the years, have received different designations, including primitive neuroectodermal tumors, neuroepithelioma, small round cell tumors of the thoracopulmonary region, and Askin tumor.1-5 Most of these tumors occur in the bone or soft tissue; Ewing sarcoma occurring outside of those areas can be considered real rarities in tumor pathology. Nevertheless, the latter have been well documented in the literature, and Ewing sarcomas have been described in unusual areas such as the testis, cervix uteri, and lung, among others.6-11 Therefore, the cases presented in this study do not represent a novelty in terms of anatomic location, but the fact that these three neoplasms contained an additional tumor component characterized by extensive areas of neural differentiation in keeping with the histologic features of ganglioneuroblastoma or ependymoma. In this context, it is important to point out that none of our patients had received any medical treatment prior to surgical resection of their tumors. This report highlights the occurrence of prominent neural differentiation in Ewing sarcoma and discusses the possible implications with regard to diagnostic accuracy in small biopsy samples as well as the potential role of such a phenomenon for the clinical outcome.

Materials and Methods

This study was approved by the MD Anderson Institutional Review Board. Three cases of Ewing sarcoma represent the basis for this report. The cases were identified in the files of the pathology department at MD Anderson Cancer Center in Houston, Texas. H&E-stained sections were available for
review in all cases, ranging from eight to 12 per case. Representative paraffin blocks or unstained slides were available in all cases for immunohistochemical studies. Deparaffinized tissue sections were incubated with antibodies directed against vimentin (1:900; Dako, Carpinteria, CA), CD99 (1:300; Dako), CD56 (1:100; Invitrogen, Carlsbad, CA), CD117 (1:400; Dako), Neu N (1:50; Bio SB, Santa Barbara, CA), glial fibrillary acidic protein (GFAP) (1:7,000; BD Biosciences, San Jose, CA), synaptophysin (1:200; Dako), chromogranin (1:100; Dako), S100 protein (1:900; Biogenex, Freemont, CA), smooth muscle actin (SMA) (1:80,000; Sigma, St Louis, MO), desmin (1:100; Dako), and pancytokeratin (1:100; Dako) using the polymeric biotin-free horseradish peroxidase method. Appropriate positive and negative controls were run for all antibodies tested. Fluorescence in situ hybridization (FISH) analysis was performed on formalin-fixed, paraffin-embedded tumor tissue of two cases using the LSI EWSR1 dual-color break-apart probe covering 22q12 (Abbott Molecular, Des Plaines, IL). Clinical information and follow-up were obtained by reviewing the patients’ clinical charts.

**Results**

**Clinical Features**

The most important clinical features are depicted in Table 1. The patients were two women and one man between the ages of 15 and 35 years (mean, 26 years). The symptoms varied depending on the location of the tumor. The young female patient with an intrapulmonary mass had symptoms of chest pain, cough, and shortness of breath. Radiographically, a mass was discovered in the left lower lobe, and surgical lobectomy was performed. The only male patient had groin pain and swelling of the right testis. Clinically, the intratesticular mass was believed to represent a germ cell tumor. Right testicular orchiectomy was performed. The patient with the cervical neoplasm had symptoms of pelvic pain and vaginal bleeding, and at gynecological examination, a mass was palpated in the cervix uteri. Clinically, the mass was believed to represent a carcinoma, and the patient underwent total hysterectomy.

**Histologic Features**

Morphologically, all three tumors showed similar histologic features. The low-power view of the lesions showed two distinct tumor components: a solid component composed of cords and subtle nests of tumor cells grouped in sheets with focal areas of crush artifact mimicking the so-called Azzopardi phenomenon. In addition, the tumors showed areas of hemorrhage and necrosis. High-power view of these areas showed a neoplastic cellular population composed of rather small cells with round to oval nuclei and inconspicuous nuclei [Image 1A]. Prominent nuclear atypia and mitotic activity were easily identified. The second component in two of the tumors was a rather loose matrix of neuropil that spread and merged with the solid areas of the tumors. This fibrillary matrix contained a cellular component of small cells with round to oval nuclei and inconspicuous nuclei histologically different from the small cells of the solid component [Image 1B]. In addition, the fibrillary matrix also contained larger cells with round nuclei and prominent nuclei. These features were compatible with a ganglioneuroblastomatous component [Image 1C]. In the testicular tumor, the second component, although similar, showed less of a fibrillary matrix but a more prominent nested growth pattern and extensive areas of cells arranged in a neuroendocrine or organoid pattern along with the marked presence of perivascular pseudorosettes. These features were closely resembling ependymomatous areas [Image 1D].

**Immunohistochemical Features**

Numerous immunohistochemical stains were performed in these cases. All tumors strongly expressed vimentin, predominantly in the round cell component of the tumor. The tumor cells in the solid cell component of all cases were also notably positive for CD99 [Image 2A] and focally positive for CD56, S100 protein, and in one case for CD117; however, the neural component (ganglioneuromatous and ependymal) did not show significant staining. Neu N showed variable staining ranging from strong focal positivity to weak focal positive staining in the fibrillary component and negative staining in the round cell component of the tumor. GFAP was essentially negative in all cases. Synaptophysin showed a strong positive reaction in the neuropil component.
and focal positive staining in the round cell component of the tumor. All tumors were also negative for SMA, desmin, chromogranin, and pancytokeratin.

Molecular Analysis

FISH was performed in two cases and demonstrated positive rearrangement of the EWSR1 gene at the 22q12 locus in both tumors.

Follow-up

Clinical follow-up in the three patients showed that they remain alive and well on adjuvant treatment 1 to 2 years after the initial diagnosis.

Discussion

Ewing sarcomas are ubiquitous in distribution, and although their occurrence in the soft tissue and bone is more common, similar tumors have been described in unusual sites, including the testis, cervix, and lung.6-11 When these tumors occur in the more common sites, the diagnosis may be suspected clinically since they affect predominantly the younger age group. However, when similar tumors occur in less common areas, the diagnosis may pose significant problems not only clinically but also histologically. Another interesting fact is that this type of tumor may also exhibit additional areas of differentiation that may pose further
diagnostic problems, thus requiring in many circumstances complete surgical resection to arrive at a more complete and accurate interpretation. One of those unusual events associated with Ewing sarcoma includes the finding of (ganglio) neuroblastomatous differentiation.12-19 A possible explanation that has been presented to account for the existence of these tumors in the soft tissue is the possible migration of neural crest cells during embryogenesis to the peripheral soft tissues,12 and such possibility may also account for the occurrence of these tumors in the testis, lung, or cervix. This theory may also explain the presence of ganglion or ependymal differentiation in these tumors. In addition, it should not be surprising to encounter (ganglio)neuroblastomatous differentiation in these lesions since neuroblastomas and Ewing sarcomas represent the two main peripheral neuroectodermal tumors, with neuroblastoma differentiating along the autonomic nerve cell lineage.17 Nevertheless, regardless of their similar origin, Ewing sarcoma containing ganglion cells or (ganglio)neuroblastomatous or ependymomatous differentiation has been reported only rarely in the literature,12-19 and those tumors were mostly located in the soft tissues where such differentiation poses less of a problem than for those tumors in more unusual areas such as the lung or genital organs. Hasegawa et al17 studied 11 small cell sarcomas under the designation of atypical primitive neuroectodermal tumors. The authors separated five tumors as peripheral neuroepitheliomas based on the presence of Homer-Wright rosettes and fibrillary or neurite-like processes and suggested that those tumors may show more advanced neuronal differentiation. The authors added that two tumors classified as Ewing sarcoma did not show evidence of neuronal differentiation on ultrastructural studies. In addition, four lesions classified as undifferentiated tumors with atypical features showed certain neuroectodermal characteristics such as ganglion cell differentiation, perivascular pseudorosettes, and neuron-specific enolase reactivity. It is very possible that, based on our experience with the cases herein described, some of the latter tumors may actually represent cases of Ewing sarcoma with ganglioneuroblastomatous or ependymomatous differentiation.

Modern adjunct tools available such as molecular analysis were of crucial importance in our cases to arrive at such interpretation. Also, Ushigome et al13 reported the finding of Homer-Wright rosettes and/or at least foci of ganglion differentiation in three Ewing sarcomas of soft tissue and bone. The report by Ushigome et al highlights that most of these patients had received chemotherapy and radiation therapy. If we compare these cases with those presented in the current report, our cases are notable for the presence of extensive areas of ganglioneuroblastomatous or ependymomatous differentiation, contrary to only foci as described by Ushigome et al.13 Collini et al14 and Maeda et al15 also reported similar cases in a 17-year-old and 12-year-old female patient, respectively. Both patients had bone tumors and received preoperative chemotherapy and/or radiation treatment. In Collini et al,14 the tumor strongly resembled a neuroblastoma posttreatment, but the presence of EWSR1-FLI1 fusion transcript was confirmed in the pre- and post-therapy samples. In Maeda et al,15 the tumor was nearly completely replaced by ganglion cells after the patient had received neoadjuvant chemoradiation therapy.

Our cases, contrary to those described in previous reports, are different in some respects. The cases herein
described occurred in very unusual anatomic locations—testis, cervix, and lung—and none of our patients had received any type of treatment prior to the resection of the tumors. More important, the tumors were composed of extensive areas of ganglioneuroblastomatous or ependymomatous differentiation, which renders them unique. However, this also raises the possibility of difficulty when dealing with small biopsy specimens, especially since any tumor in the lung or the uterine cervix will likely be biopsied prior to resection. In such cases, the differential diagnosis will largely depend on the tumor location. For instance, for a lung neoplasm in which the biopsy specimen shows fibrillary areas with ganglion cell differentiation, the possibility of primary pulmonary ganglioneuroblastoma would need to be considered. Pulmonary ganglioneuroblastomas are rare neoplasms that have been described only sporadically in the literature. Complete surgical resection of the tumor would obviously lead to a different interpretation. On the other hand, a cervical biopsy specimen may pose a different problem if the tissue sampled represents the characteristic small round cell component of Ewing sarcoma due to the potential confusion with a small cell carcinoma of the cervix. However, if on the contrary, the fibrillary material were sampled, then other possibilities would need to be considered, including teratomatous lesions. For testicular tumors, with or without biopsy, the most important differential diagnosis would include a germ cell tumor with the presence of neural components. In either one of the above-mentioned settings, the most important issue is extensive sampling, short of submitting the entire tumor for accurate interpretation.

Another important issue that still needs to be addressed is the role of such unusual differentiation in Ewing sarcoma. Does ganglion cell, ependymomatous, or neuroblastomatous differentiation play any significant part for clinical behavior? Are these patients associated with better clinical outcome? Or, is it a more ominous feature, and should these patients be treated more aggressively? Currently, there are not enough cases described in the literature to draw more meaningful conclusions, nor is the clinical follow-up in those cases long enough to make such determination. However, it is important to at least recognize that such differentiation exists, especially in Ewing sarcomas outside of their more typical locations, and not to misinterpret them for other more common lesions in these anatomic areas.

In short, we have described three cases of Ewing sarcomas in the testis, cervix, and lung that showed extensive ganglioneuroblastomatous and ependymomatous differentiation. Inclusion of these tumors into the differential diagnosis of otherwise undifferentiated tumors in these sites is crucial so that measures, such as molecular techniques, can be employed to clarify the process and to eventually arrive at the correct diagnosis.

References


Address reprint requests to Dr Weissferdt: Dept of Pathology, MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030; aweissferdt@mdanderson.org.

