Rhabdomyosarcomas in Adults and Children
An Update

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Context.—Rhabdomyosarcomas comprise a relatively common diagnostic entity among childhood cancers and a relatively rare one among adult tumors. They may possess a variety of histologies that generally differ among age groups. These lesions appear to be separate biologic entities as well as morphologic categories, with embryonal tumors having genetic lesions related to loss of heterozygosity and aberrant parental imprinting, alveolar tumors containing genetic fusions between PAX and forkhead genes, and pleomorphic tumors showing an accumulation of genetic lesions similar to other adult high-grade sarcomas.

Objective.—To present guidelines for diagnosis of rhabdomyosarcoma and recent finding concerning the biology and classification of these lesions.

Data Sources.—Review of recent and older published literature and distillation of the authors’ experience.

Rhabdomyosarcomas constitute a unique group of soft tissue neoplasms that share a propensity to undergo myogenesis, a well-defined biologic process that primarily occurs during embryonal and fetal development.1 As a result, these neoplasms tend to resemble stages of muscle formation more akin to prenatal than postnatal life. This often striking resemblance manifests not only by their histopathology but also by their pathobiology, as shown by recent genetic studies of the tightly orchestrated process of muscle development.1–4 Thus, these tumors may be considered lesions that initiate myogenic differentiation but fail to disconnect their constituent cells from the proliferative cycle that terminal differentiation normally quenches.2

Another unusual feature of rhabdomyosarcoma, perhaps related to its linkage with somatic development, is its propensity to affect children, primarily infants, toddlers, and preschoolers.5,6 Older children and adolescents may also be affected, so that rhabdomyosarcomas comprise the most common single soft tissue sarcoma among children and adolescents by a striking majority. On the other hand, rhabdomyosarcomas are distinctly unusual in adults,7 who generally have tumors with pleomorphic, high-grade cytologic features and differing biologic characteristics.8

Conclusions.—Infants and young children tend to have embryonal rhabdomyosarcomas, adolescents and young adults tend to have alveolar rhabdomyosarcomas, and older adults tend to have pleomorphic rhabdomyosarcomas, although there is some overlap. Newer rare entities, including spindle cell rhabdomyosarcoma and sclerosing rhabdomyosarcoma, have been described in children and adults. Fusion-positive tumors have a distinct molecular signature with downstream activation of a number of myogenic and tumorigenic factors. Genetic testing may be successfully used for diagnosis and may guide therapy in future clinical trials. Differential diagnosis has become simpler than in previous years, because of use of myogenic factors in immunohistochemistry, but classification based solely on histologic features remains challenging.

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CLINICAL FEATURES

Rhabdomyosarcomas may arise from a wide variety of locations.9 These may be usefully separated into extremity and axial lesions, because the extremity tumors cause local symptoms related to mass formation, infiltration, and destruction of adjacent tissues. Axial tumors most com-
monly arise from the head and neck, the paraspinal region, and the genitourinary system, but tumors from other abdominal and thoracic sites of origin occur. Among head and neck lesions, orbital tumors occur most commonly. Other sites include the nasal passages, paranasal sinuses, mouth, pharynx, parotid region, temporal region, pterygoid region, and cheek. Rarely, intracranial meningeal tumors can be seen. Genitourinary tract lesions comprise those affecting the urinary bladder, prostate, perineum, vagina, cervix, uterus, and paratesticular soft tissues. Paratesticular tumors are among the most common and often affect older children and adolescents. Abdominal lesions include those of the retroperitoneum, abdominal wall, and biliary tract. Thoracic tumors primarily affect the chest wall. One must be particularly cautious with myogenic tumors of the lung and pleura, because these more often represent pleuropulmonary blastomas than true rhabdomyosarcomas. Rare tumors arise from the skin.19

As might be expected from their diversity of anatomic sites, rhabdomyosarcomas have a host of clinical manifestations. As a rule, these tumors present as bulging, infiltrative, growing soft tissue masses that may be fungating when they present in external locations such as the conjunctiva and vagina. Obstructive features typically occur with lesions of the genitourinary tract and biliary system, causing urine or bile retention. Orbital lesions usually cause proptosis and diplopia. Paraspinal tumors may have neural manifestations if nerve roots are involved.

One striking feature of rhabdomyosarcomas is their association with familial cancer syndromes. Initial studies by Li and Fraumeni20 on familial rhabdomyosarcoma led to description of a cancer susceptibility syndrome later proven to be caused by constitutional mutations of the TP53 gene. This syndrome is characterized by a high risk for early breast cancer, adrenal cortical neoplasia, gliomas, hematopoietic cancers, and other bone and soft tissue sarcomas. Independent observations by Beckwith21 and Wiedemann22 lead to the recognition of a syndrome characterized by somatic overgrowth and a propensity to develop embryonal tumors including rhabdomyosarcoma, hepatoblastoma, and Wilms tumor. Other features include organomegaly, placentomegaly, macroglossia, omphalocele, and adrenal cortical cytomegaly. The genetic study of this disease has yielded a fascinating array of observations on the relation between tumor development and non mendelian genetics, as discussed later. The sarcomatous tendencies of patients with neurofibromatosis 1 (NF1) have long been recognized, and rhabdomyosarcomas are along the list of tumors that have been described as affecting them.23 However, whether these are “true” rhabdomyosarcomas or a variant of malignant triton tumors has not be conclusively demonstrated. A subset of rhabdomyosarcomas with patched gene mutations24-25 appear related to the syndrome described by Gorlin in patients with heritable basal cell carcinoma, odontogenic keratocysts, and medulloblastoma.

HISTOLOGIC FEATURES

As may be surmised from the previous discussion, the major histologic feature of rhabdomyosarcomas is their resemblance to developing muscle, but some may offer no evidence of differentiation by routine stains and others may show abundant differentiation and resemble rhabdomyomas.26 The key cell to recognize by routine microscopy is the rhabdomyoblast (Figure 1), a cell with an eccentric round nucleus and variable amounts of brightly eosinophilic cytoplasm. Rhabdomyoblasts assume a variety of shapes that have been likened to straps, tadpoles, tennis racquets, and spiders. Occasional tumors (less than 30%) contain terminally differentiated myoblasts with cross striations. In our experience, these can be more easily recognized by increasing the light intensity of the microscope and lowering the condenser. More often, however, the tumors are largely or entirely composed of undifferentiated cells with round to oval nuclei with minimal cytoplasm and stellate borders. As they differentiate, rhabdomyosarcoma cells undergo fusion, giant cell transformation, and tandem nuclear displacement, similar to normally developing myoblasts.

Of critical importance is recognition of the basic histologic pattern, which separates rhabdomyosarcomas into subtypes. Determination of the pattern may be difficult if not impossible on limited biopsies, and one must also carefully observe the cytologic features of the tumor cells. The most common subtype, embryonal rhabdomyosarcoma, shows a loose and dense pattern created by variable condensation of tumor cells, separated by a loose, myxoid stroma often rich in connective tissue mucins (Figure 2). This tumor must be separated from alveolar rhabdomyosarcoma, which typically forms cellular nests separated by fibrovascular septa (Figure 3). Alveolar rhabdomyosarcomas are highly cellular and often contain densely populated fields of small round blue cells. When this is the predominant or sole feature (more likely seen in small samples), then the term solid variant applies. Unfortunately, this concept has engendered much confusion because of the difficulties in separating solid foci of alveolar rhabdomyosarcoma from the dense portion of embryonal rhabdomyosarcoma. This becomes particular problematic in embryonal tumors in which dense foci predominate.

Cytologically, embryonal rhabdomyosarcoma cells tend to have ovoid shapes with oval nuclei and relatively bland chromatin, whereas alveolar rhabdomyosarcoma cells tend to be larger and contain round, Ewing tumor-like nuclei with central nucleoli and less cytoplasm. Solid variants in particular may closely resemble non-Hodgkin lymphomas. Cytologic features may be helpful in separating densely cellular embryonal rhabdomyosarcomas from alveolar rhabdomyosarcomas, but one should not hesitate to request additional studies such as molecular genetics in problematic cases. It is not surprising that nuclear morphometry alone has been successfully used to predict outcome in rhabdomyosarcoma.27

IMMUNOHISTOCHEMISTRY AND ELECTRON MICROSCOPY

Ancillary studies are often critical with rhabdomyosarcomas, because of the frequency of poorly differentiated tumors. The diagnosis should be considered with all small round cell neoplasms and an appropriate immunohistochemical battery included in the workup. Fortunately, muscle cells have a relatively unique phenotype, because developing muscle expresses unique transcription factors that initiate myogenesis. As a result, a small battery of 1 or 2 stains can be included in a larger workup and reveal myogenic potential in virtually all appropriately fixed and stained material.

A large number of articles have touted various markers, ranging from myasthenia gravis serum to dystrophin, as potentially useful in diagnosis of myogenic tumors. In our
Figure 1.  Rhabdomyoblasts (arrow) with eccentric nucleus and bright eosinophilic cytoplasm. This cell type is the histologic feature that defines rhabdomyosarcoma at the morphologic level (hematoxylin-eosin, original magnification ×200).

Figure 2.  Embryonal rhabdomyosarcoma showing oblong cells with perivascular condensation lying in a myxoid stroma (hematoxylin-eosin, original magnification ×200).

Figure 3.  Alveolar rhabdomyosarcoma showing small round blue cells lining fibrovascular septa (hematoxylin-eosin, original magnification ×200).

Figure 4.  Diffuse nuclear myogenin staining in an alveolar rhabdomyosarcoma (myogenin immunostain, original magnification ×400).

Figure 5.  Focal nuclear myogenin staining in an embryonal rhabdomyosarcoma (myogenin immunostain, original magnification ×400).

Figure 6.  Botryoid rhabdomyosarcoma with a cambium layer, defined by the cellular condensation near the epithelial lining. At a casual glance, this lesion might be mistaken for an inflammatory process (hematoxylin-eosin, original magnification ×200).
own practice and in working with the Children's Oncology Group (COG), we have found 2 markers to be most useful—desmin and myogenin. Desmin is one of the intermediate filaments that separate cells into broad phenotypic groups; others include vimentin (mesenchyme), cytokeratin (epithelial), glial acidic protein (glia), and neurofilaments (neurons). Like other members of this family of proteins, desmin exhibits occasional nonspecificity, particularly with smooth muscle cells and myofibroblasts. Another problem is desmin positivity in other small round cell tumors, particularly desmoplastic small round cell tumor. Thus, desmin should always be used as part of a panel and never as a sole marker for diagnosis.

Transcription factors comprise a broad group of proteins that directly interact with the DNA strand and control the expression of genes by binding their promoter regions upstream of RNA transcription sites. These proteins include the myogenic factors, composed of a helix-loop-helix motif that intercalates into the DNA groove. Excellent monoclonal antibody reagents for 2 of these proteins, MyoD (myogenic determination factor) and myogenin, have been developed for immunohistochemistry. They both show excellent specificity and sensitivity with certain caveats. MyoD immunostains work best with freshly cut sections, because antigen reactivity fades with slides stored at room temperature. Also, one must be cautioned to consider only nuclear staining as truly positive, because nonspecific cytoplasmic positivity may be seen with a variety of other tumors. These caveats do not appear to affect myogenin staining to the same extent as that of MyoD. Both immunostains strongly and diffusely decorate the nuclei of alveolar rhabdomyosarcoma, although this phenomenon is more pronounced with myogenin stains (Figure 4). In contrast, embryonal rhabdomyosarcomas usually stain in a more heterogeneous fashion (Figure 5), which yields a clue to subclassification. Of note is that a number of authors complained about a lack of correlation between classification and clinical outcome.

Because of its distinctive gross features, botryoid rhabdomyosarcoma was initially recognized as early as 1854. Named for their resemblance to a bunch of grapes, these tumors are characterized by fleshy, nodular, polypoid excrescences that fill the lumen of a hollow viscus. Characteristic locations include the urinary bladder, vagina, and extrahepatic bile ducts, but on occasion they arise from unusual sites such as conjunctiva or ear. Microscopically, these lesions produce a “cambium layer” as a key feature; this comprises a subepithelial condensation of primitive cells evoking the hypercellular zones that produce growth rings in trees (Figure 6). Botryoid rhabdomyosarcomas can be relatively hypocellular, innocuous appearing lesions that may initially be misdiagnosed as chronic inflammation or developmental anomaly, so close examination is advisable when dealing with myxoid juxtaglomerular swellings in children. Botryoid lesions are considered to be a variant of embryonal rhabdomyosarcoma, but they have a superior prognosis. Whether this relates to their site specificity, which also affects outcome of rhabdomyosarcoma, is not clear.

Embryonal rhabdomyosarcomas are so-named because of their remarkable evocation of developing skeletal muscle. As such they are characterized by variable zones of condensation that produce alternating foci of hypocellularity and hypercellularity. Like embryonic muscle, the dense zones typically contain areas of more overt myogenesis, whereas loose areas more closely resemble primitive mesenchyme and lie in a loose gelatinous matrix. Cellular condensation results from an interconnected expression of cell matrix proteins, adhesion molecules, and cell surface receptors, initiated by expression of protooncogenes such as C-MET. C-MET expression in turn is regulated by PAX3 or PAX7, transcription factors that also initiate MyoD expression and have been called the master switches of myogenesis. In embryonal rhabdomyosarcomas, precursor cells appear to reawaken quiescent impulses to develop muscle, resulting in a neoplasm called the rhabdopoietic sarcoma by Masson. The exact cell of origin is unclear, although a muscle stem cell has been suggested.

Alveolar rhabdomyosarcomas comprise a distinct and relatively well-characterized subgroup that tends to arise in extremities and axial musculature, as opposed to the more heterogeneous origin of embryonal rhabdomyosarcomas. These lesions affect all ages and are more common in adolescents and young adults, whereas the incidence of embryonal and botryoid rhabdomyosarcoma precipitously drops after children reach school age. Of note is that some alveolar rhabdomyosarcomas arise from the nose and

CLASSIFICATION

Pathologic classification of subtypes of rhabdomyosarcoma has been the source of considerable debate, because a number of distinct morphologic variants have been described. The first schema was that of Horn and Enterline, who subdivided rhabdomyosarcomas into botryoid, embryonal, alveolar, and pleomorphic subtypes. This initial system depended purely on morphologic features, without the benefit of immunohistochemical or genetic confirmation. Thus, the definition of alveolar rhabdomyosarcoma was overly strict because of the need to clearly distinguish it from other round cell tumors. As a result, a number of authors complained about a lack of correlation between classification and clinical outcome.

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paranasal sinuses, similar to the head and neck localization of many embryonal tumors, and tend to concentrate in parameningeal regions. Alveolar rhabdomyosarcomas typically produce fibrovascular septa that outline clusters and nests of primitive cells. The cells produce central aggregates that appear to float in the middle of alveolar spaces, hence the name. Cells with early myoblastic differentiation tend to hang from the fibrovascular septa in a picket row fashion, and a peripheral “cracking” artifact is common at the borders of the cell clusters. Some alveolar rhabdomyosarcomas contain myogenic tumor giant cells, analogous to the stage of myoblast fusion that normally precedes myofiber development. Other examples contain no obvious differentiation whatsoever by routine stains, so that Riopelle and Thériault45 seized on the alveolar pattern as a means of distinguishing these lesions from lymphoma or Ewing sarcoma in the earliest description of this tumor. Later cytogenetic studies indicated that many of these lesions contained a unique reciprocal translocation, the t(2;13)(q35;q14), as discussed later.46

Pleomorphic rhabdomyosarcomas were first described by Stout, as pleomorphic sarcomas arising in the muscularature of adults, and they were then included in the original classification of Horn and Enterline.37 As the name implies, pleomorphic rhabdomyosarcomas comprise high-grade sarcomas composed of large, interlacing spindle cells containing irregular, hyperchromatic nuclei and numerous mitoses. With the advent of the diagnosis malignant fibrous histiocytoma, these neoplasms became vanishingly rare, but the diagnosis was resurrected by Gaffney et al.48 These lesions contain cells with copious, eosinophilic cytoplasm, which demonstrate muscle filaments by electron microscopy. Immunohistochemistry confirms the myogenic nature of these tumors, which demonstrate positivity for desmin and MyoD.49,50 These tumors typically arise in the skeletal musculature of adults and are distinctly uncommon in children.51 When they occur in children, embryonal foci may be found.51 Pleomorphic rhabdomyosarcomas are aggressive lesions with a poor outcome, similar to other pleomorphic sarcomas of adults.52 Cytogenetic studies reveal complex karyotypes, with rearrangements and evidence of gene amplification, that offer no distinctions from other adult pleomorphic sarcomas.52

Following its publication, dissatisfaction with the Horn-Enterline classification became evident, with Stout and Lattes53 stating that because of the uniform fatality of rhabdomyosarcoma it was unnecessary, and Bale and Reye54 stating that it did not adequately predict outcome. However, with the improvements in clinical outcome that came with multiagent chemotherapy, it became apparent that some subtypes behaved significantly better than others, with alveolar rhabdomyosarcomas showing a poor outcome and botryoid rhabdomyosarcomas a superior one. The poor prognosis of alveolar rhabdomyosarcoma was confirmed by Gonzalez-Crussi and Black-Schaffer,56 but they felt that the classification disregarded the morphologic heterogeneity of rhabdomyosarcoma.

Fresh from their grading experience with childhood renal tumors, Beckwith and Palmer57 took a different approach in retrospective studies, looking at cytologic features of rhabdomyosarcoma rather than their histology. Palmer published a series of abstracts that documented a strong association between tumor cytology and outcome of a large cohort of Intergroup Rhabdomyosarcoma Study (IRS) patients.58–60 Unfortunately, this work was never published in article form, but it gave an impetus to further modifications in rhabdomyosarcoma classification that culminated in the International Classification of Childhood Rhabdomyosarcomas.61,62 Of particular note was Palmer’s description of “anaplastic” and “monomorphous round cell” tumors associated with poor clinical outcome and “spindle cell” tumors associated with a superior outcome.

Anaplastic rhabdomyosarcomas shared the cytologic features of anaplastic Wilms tumors, that is, foci of cells containing enlarged, hyperchromatic nuclei and atypical, multipolar mitoses (Figure 7).57 An independent, retrospective study of anaplastic rhabdomyosarcomas was subsequently published by IRS pathologists and confirmed the aggressive nature of these neoplasms, particularly when anaplastic foci occurred in clusters or large sheets.63
Molecular studies of these tumors indicate that they contain evidence of gene amplification, particularly involving the locus for the insulin-like growth factor type 1 receptor. Of note is that approximately one fourth of alveolar rhabdomyosarcomas and embryonal rhabdomyosarcomas display gene amplification, with those in embryonal tumors occurring almost exclusively in anaplastic subtypes. Preliminary studies indicated that TP53 expression also corresponds to anaplastic histology. A more recent prospective study of IRS-V patients has confirmed the relatively aggressive behavior of embryonal rhabdomyosarcomas with anaplasia, despite modern chemotherapy (S. Qualman, MD, unpublished data, 2006). This suggests that anaplasia is associated with drug resistance, similar to that seen in anaplastic Wilms tumor.

Monomorphic round cell tumors constitute the second high-risk rhabdomyosarcoma group in Palmer’s cytologic classification system. This term basically refers to cells with round hyperchromatic nuclei and even nuclear margins, similar to those seen in Ewing sarcoma or Burkitt lymphoma. Nuclear morphometric studies have confirmed the poor outcome of rhabdomyosarcomas with these features. Morphometric studies also indicate that this feature is significantly associated with alveolar rhabdomyosarcoma, so that tumors with solid sheets of monomorphic round cells but lacking fibrous septa might be considered solid variants. If one carefully scrutinizes the original description of alveolar rhabdomyosarcoma by Riopelle and Thériault, one finds a description of these features, but the lack of ancillary techniques precluded definitive proof of myogenic potential in the tumors without septa. Also, septa may be focal in alveolar rhabdomyosarcomas and not seen with limited biopsies. Thus, Tsokos et al added a new histologic wrinkle to the diagnosis of alveolar rhabdomyosarcoma and found that these lesions had poor outcome regardless of whether or not septa were present. Subsequent studies also described the 1(2;13) in solid variants, further confirming that they are alveolar rhabdomyosarcomas.

Spindle cell rhabdomyosarcomas comprised the other end of the cytologic spectrum for Palmer and Foulkes, being composed of elongate spindle cells arranged in tight fascicles with variable amounts of intervening collagen, often reminiscent of leiomyosarcomas (Figure 8). Palmer also found their behavior to be converse, with a prognosis superior to typical embryonal rhabdomyosarcoma. A group of these lesions were consequently described by Cavazanna et al who confirmed their superior prognosis and found that the majority occurred in the paratesticular region. An independent retrospective review by IRS pathologists also confirmed the observations made by Palmer and noted a tendency for spindle cell rhabdomyosarcomas to occur in paratesticular regions. Both groups noted that spindle cell rhabdomyosarcomas tended to be composed of relatively more differentiated rhabdomyoblasts, as judged by morphologic and immunohistochemical studies.

Spindle cell rhabdomyosarcomas may arise in adults, in whom they may have a worse prognosis. In the series by Nascimento and Fletcher, they most commonly arose in the head and neck rather than paratesticular soft tissue. In the setting of adult neoplasia, they can pose a diagnostic dilemma because of their resemblance to smooth muscle tumors and other spindle cell lesions. Immunohistochemistry and/or electron microscopy confirms the diagnosis. Of note is that among Nascimento and Fletcher’s cases, 3 had focally marked stromal sclerosis, suggesting a relationship to sclerosing rhabdomyosarcoma. However, the question of whether these are the same or different tumors must await further biologic studies.

Following various modifications of the classic Horn-Enterline system, Newton convened an international panel of rhabdomyosarcoma experts, including proponents of the various systems, to formulate a “working classification” for a large group of IRS cases was assembled for expert review and correlation, and the results were analyzed for statistical reproducibility. Following this mammoth effort, Newton et al proposed a new classification for rhabdomyosarcomas that modified the Horn-Enterline system and incorporated portions of the newer schemas. This system is listed in the Table. It should be noted that botryoid and spindle cell rhabdomyosarcomas are currently considered to be variants of embryonal rhabdomyosarcomas and no inherent biologic differences have been identified to date. Second, anaplasia was not originally included in the International Classification, because the reproducibility of the Palmer system by the expert panel was poor; however, it has been included in subsequent modifications as a poor prognosis lesion. Current pediatric sarcoma protocols of the COG (which has replaced the IRS) do not currently upgrade tumors based on the presence of anaplasia. Finally, solid variant alveolar rhabdomyosarcomas were included with the alveolar group, which incorporates the monomorphic round cell tumors described by Palmer.

As part of the classification, Newton et al included 2 additional diagnoses: sarcoma, not otherwise specified (NOS), and undifferentiated sarcoma. Sarcoma, NOS, was defined as a soft tissue neoplasm resembling rhabdomyosarcoma but whose definitive diagnosis was hampered by poor histology and/or a limited sample. This category has essentially disappeared with the advent of immunostains such as myogenin and MyoD, so that these are largely now called rhabdomyosarcoma, NOS. Undifferentiated sarcomas comprised round cell neoplasms with no evidence of myogenesis by ultrastructural or immunohistochemical molecular studies. Patients with these tumors have a poor outcome, similar to alveolar rhabdomyosarcoma. Because undifferentiated sarcomas neither respond to current rhabdomyosarcoma protocols nor possess phenotypic markers of muscle, they will be treated as nonrhabdomyosarcomatous soft tissue sarcomas in future COG studies.

One should note that pleomorphic rhabdomyosarcomas are not included in the International Classification, which is limited to pediatric patients, who rarely have this variant. This diagnosis has been largely supplanted by “anaplastic” rhabdomyosarcoma, recognizing that the 2 lesions have some differences. As defined by Stout and Lat-
tes, pure pleomorphic rhabdomyosarcoma is a spindle cell lesion that does not contain differentiating, embryonal elements or primitive small blue cells. However, it is our personal observation that spindle cell rhabdomyosarcomas may on rare occasions show diffuse anaplasia and appear to be indistinguishable from adult, malignant fibrous histiocytoma-like, pleomorphic rhabdomyosarcomas.

Rare variants of rhabdomyosarcomas exist that are not included in the International Classification. These include “lipid-rich” or “clear cell” rhabdomyosarcoma and “sclerosing” rhabdomyosarcoma. Cells with vacuolated cytoplasm, resembling lipoblasts, characterize both lipid-rich and clear cell tumors. By electron microscopy, however, lipid-rich tumor cells contain lipid droplets, where clear cell tumor cells contain large amounts of glycolgen, reminiscent of Ewing sarcoma. At any rate, pathologists should be aware of these unusual histologic variants and not confuse them with either liposarcomas or Ewing sarcomas. Of note is that rhabdomyosarcomas contain variable amounts of glycolgen, so that periodic acid–Schiff stains have no diagnostic significance. Liposarcomas are distinctly uncommon in children, and it has been our experience that lipid-rich rhabdomyosarcomas may be misdiagnosed as such.

Sclerosing rhabdomyosarcoma is a relatively new entity, described by Mentzel and Katencamp in 2000 in a small series of 3 adults and by Folpe et al in 4 adults in 2002. These lesions contain an abundant, hyalinizing matrix that surrounds and entraps tumor cells and can impart a chordoid or osteoid appearance. Following description of sclerosing rhabdomyosarcoma in adults, several articles described pediatric cases, which in retrospect resemble the lesion Bale and Reye described as “carcinoma-like” rhabdomyosarcoma. Although sclerosing rhabdomyosarcomas may resemble alveolar rhabdomyosarcomas with pronounced fibrous septa, they demonstrate a weaker expression of myogenin and typically contain no genetic evidence of the t(2;13). Thus, they appear to be a unique variant that may be related to embryonal rhabdomyosarcoma. Interestingly, both fat and fibrous tissue, as well as a variety of other cell types, can be induced from muscle stem cells, suggesting that both “lipid cells” and “sclerosing cells” might similarly arise from rhabdomyosarcoma stem cells.

**MOLECULAR BIOLOGY AND DIAGNOSIS**

A singular discovery in rhabdomyosarcoma biology was that the t(2;13)(q35;q14) found in alveolar tumors resulted in a fusion between the PAX3 gene on chromosome 2q35 and the FKHR gene (also known as FOXO1) on chromosome 13q14. As noted previously, PAX3 encodes a DNA transcription factor that plays a major role in the initiation of embryonic myogenesis within the myotome. FKHR encodes a molecule that regulates the aging process and myoblast conjunction. The PAX3-FKHR fusion gene is a highly potent activator of a number of downstream events leading to synthesis of cell cycle and apoptosis proteins as well as myogenesis.

As a result, fusion-positive rhabdomyosarcomas display a strikingly homogeneous molecular signature with gene expression profile studies, whereas fusion-negative tumors, regardless of histologic classification, show a relative lack of homogeneity. Keller et al generated PAX3-FKHR “knock-in” mouse models that must be conditional in order for the embryos to survive. Tumors identical to alveolar rhabdomyosarcomas arise when expression of the fusion is delayed until postnatal life and combined with a conditional knock-out of either Trp53 (TP53) or Ink4a-ARF (which encodes p14 and p16). Thus, the generation of tumors from the PAX3-FKHR appears to depend on a “second hit” analogous to Wilms tumor or retinoblastoma. This hypothesis also applies to the fusion molecules associated with leukemogenesis.

To date, the occurrence of the PAX3-FKHR fusion has been essentially limited to alveolar rhabdomyosarcoma, in which an alternate fusion appears in 20% of cases. This alternate fusion, the PAX7-FKHR, results from the t(1;13)(p36;q14) reciprocal translocation. Patients with metastatic rhabdomyosarcoma having the PAX7-FKHR fusion gene appear to have a substantially better prognosis than those with the PAX3-FKHR, perhaps because increased expression of the former tends to occur as a result of fusion gene amplification rather than enhanced transcriptional activity. Early articles on tumors with the t(1;13) fusion gene amplification rather than enhanced transcriptional activity. Early articles on tumors with the t(1;13) reciprocal translocation. These fusion-negative tumors comprise approximately 30% of alveolar rhabdomyosarcomas and appear to be a heterogeneous group by molecular studies. Some appear to be low expressors, because they do not show fusion gene expression by routine reverse-transcriptase polymerase chain reaction testing. These fusion-negative tumors comprise approximately 30% of alveolar rhabdomyosarcomas and appear to be more sensitive nested reverse-transcriptase polymerase chain reaction but it can be detected by the more sensitive nested reverse-transcriptase polymerase chain reaction. In some cases, fluorescence in situ hybridization can detect the actual gene fusion, but no RNA products may be detected by polymerase chain reaction methods. Rare cases seem to contain alternate fusions that substitute other members of the forkhead gene family for FKHR. Finally, some cases exist in which no evidence of a fusion can be detected whatever. Of particular note is the occurrence of the latter phenomenon in tumors with mixed alveolar-embryonal histology, suggesting that in some cases “mixed histology” represents emergence of aggressive embryonal clones. Further work on this phenomenon is in progress.

Histologic features of alveolar rhabdomyosarcoma do not appear to predict the presence of a gene fusion or the type of fusion, but the total absence of the typical pattern with fibrous septa and nesting predicts a high probability of fusion negativity. Genetic testing should thus be considered in these latter cases. On the other hand, roughly one half of fusion-negative alveolar rhabdomyosarcomas exhibited typical alveolar features in a recent review. Fusion-negative cases had an outcome intermediate between those with either a PAX7-FKHR fusion or a PAX3-FKHR fusion in 1 series of metastatic tumors, whereas fusion status did not predict outcome in locoregional disease.

The potential value of genetic testing becomes clearer as we begin to recognize the biologic homogeneity and aggressive nature of fusion-positive alveolar rhabdomyo-
sarcomas. Genetic testing has particular application in those tumors with mixed alveolar-embryonal histology (Figure 9) as well as those with a solid alveolar pattern, particularly if the material is limited. Tumors with mixed histologies have long been recognized and continue to plague pathologists and protocol reviewers. Of particular note is that the International Classification includes tumors with any evidence of alveolar features among the alveolar group, whereas earlier IRS studies required at least 50% alveolar histology for that diagnosis. This has likely increased the number of alveolar tumors and may affect the stringency of the diagnosis. A relatively high rate of disagreement already exists between institutional pathologists and protocol reviewers for the diagnosis of alveolar rhabdomyosarcoma; only 70% agreement was seen in the recent IRS-IV study for nonmetastatic tumors. This factor led to a “rapid review” system for entry of patients onto former IRS and current COG soft tissue sarcoma protocols. The existence of genetic testing can only serve to refine the diagnosis and improve the rate of agreement, particularly with solid and mixed histology alveolar tumors. If a biology-based treatment is devised that specifically attacks fusion-positive cells, then genetic testing will assume primary importance for therapy.

In contrast to alveolar rhabdomyosarcomas, embryonal rhabdomyosarcomas possess no distinct molecular signature, although like other embryonal tumors they tend to exhibit loss of allelic heterozygosity and abnormalities in parental imprinting. Loss of allelic heterozygosity particularly affects loci at chromosome 11p15, which contains the gene involved in Beckwith-Wiedemann syndrome. Because parental imprinting occurs by gene methylation, our laboratory investigated the latter phenomenon in rhabdomyosarcomas and found differences in PAX3 and MyoD methylation patterns between alveolar and embryonal tumors. Of note is that the MyoD gene is also located at the chromosome 11p15 locus. Hypermethylation of tumor suppressor genes also occurs in rhabdomyosarcomas, similar to other sarcomas. Although they are interesting from a biologic basis, methylation abnormalities have not been usefully exploited as diagnostic tools in rhabdomyosarcomas.

**DIFFERENTIAL DIAGNOSIS**

The differential diagnosis of rhabdomyosarcomas includes 2 categories: the small round cell tumors of childhood and myogenic tumors of various types. Small round cell tumors have been the source of numerous reviews. Alveolar rhabdomyosarcomas often appear to be composed of sheets of undifferentiated small round cells. Rare tumors may have features of lymphomas on histologic and cytologic examination, and B-cell characteristics rarely may even be expressed. This phenomenon is more likely to occur with rhabdomyosarcomas of unknown primary that present with a leukemia-like picture. Fortunately, leukemic presentation of rhabdomyosarcoma is a rare occurrence, for it carries a dire prognosis and often poses a diagnostic dilemma. The diagnosis may become apparent with therapy, which typically induces myogenesis, or cytogenetic findings may yield a clue. At any rate, use of myogenic markers, particularly myogenin, usually clarifies this dilemma, even with microchip analysis. Although at times very challenging, diagnostic dilemmas in small cell tumors are not immune from legal action.
Distinguishing rhabdomyosarcoma from extraskeletal Ewing sarcoma/peripheral neuroectodermal tumor poses a more common dilemma. Of note is that initial IRS studies included extraskeletal Ewing sarcomas, and the affected patients achieved a cure rate similar to embryonal rhabdomyosarcoma. However, because of the benefits of ifosfamide and etoposide, more recent COG studies include extraskeletal Ewing sarcomas in their bone tumor regimens. With Ewing sarcoma, use of myogenic markers, particularly myogenin, may be helpful, noting that desmin positivity may occur in rare peripheral neuroectodermal tumors. CD99, often used as a Ewing marker, may be positive in rhabdomyosarcoma, whereas FLI-1 was negative in them in a series. The problem is compounded by the existence of biphenotypic sarcomas known as primitive ectomesenchymomas that express both myogenin and neural features, even with coexisting myoblasts and ganglion cells. At least some of these tumors contain molecular rearrangements typical of Ewing sarcoma, but others are negative. These lesions evoke the diagnosis to mind, particularly in NF1 patients. It should be noted that rhabdomyosarcoma may arise in pleuropulmonary blastoma may be particularly problematic. Of note is that initial IRS studies included extraskeletal Ewing sarcomas, and the affected patients achieved a cure rate similar to embryonal rhabdomyosarcoma. However, because of the benefits of ifosfamide and etoposide, more recent COG studies include extraskeletal Ewing sarcomas in their bone tumor regimens. With Ewing sarcoma, use of myogenic markers, particularly myogenin, may be helpful, noting that desmin positivity may occur in rare peripheral neuroectodermal tumors. CD99, often used as a Ewing marker, may be positive in rhabdomyosarcoma, whereas FLI-1 was negative in them in a series. The problem is compounded by the existence of biphenotypic sarcomas known as primitive ectomesenchymomas that express both myogenin and neural features, even with coexisting myoblasts and ganglion cells. At least some of these tumors contain molecular rearrangements typical of Ewing sarcoma, but others are negative. These lesions evoke the diagnosis to mind, particularly in NF1 patients. It should be noted that rhabdomyosarcoma may arise in pleuropulmonary blastoma may be particularly problematic.

Another lesion with a mixed phenotype, including that of mesenchymal, neural crest, and epithelial, is the desmoplastic small cell tumor, which primarily occurs within the abdomen. This lesion may be confused with rhabdomyosarcoma because of 2 features: frequent desmin positivity and an alveolar pattern (Figure 10) imparted by dense sclerosis that separates nests of small tumor cells. However, myogenin stains are typically negative, and no overt myogenesis occurs either at the light microscopic or at the ultrastructural level. In addition, these tumors possess an EWS-WT1 fusion unlike that of alveolar rhabdomyosarcoma and detectable by fluorescence in situ hybridization or reverse-transcriptase polymerase chain reaction.

Coexistence of neural and myogenic features within a tumor is also seen in malignant peripheral nerve sheath tumor with myogenous differentiation, the so-called malignant triton tumor (Figure 11). These lesions occur in patients with NF1, as well as sporadically in non-NF1 patients. They are high-grade sarcomas that rarely occur in children. The occurrence of lesions arising in nerve or in association with neurofibromatous foci should call the diagnosis to mind, particularly in NF1 patients. It should be noted that rhabdomyosarcoma may arise in NF1 patients, as noted previously, but the relationship of these lesions to triton tumors is uncertain. At any rate, there are no apparent recurring cytogenetic findings.

Triton tumors illustrate the problem of distinguishing rhabdomyosarcomas from other myogenous tumors. Of note is that a wide variety of embryonic tumors may show myogenesis. These include Wilms tumor, hepatoblastoma, and pleuropulmonary blastoma. Of these, distinction between rhabdomyosarcoma of the chest wall and pleuropulmonary blastoma may be particularly problematic. Similar to Wilms tumor, rhabdomyosarcomas may express WT1, although immunohistochemical staining is cytoplasmic rather than nuclear. Conversely, Wilms tumors often stain with antibodies after heat retrieval, and they may express MyoD. This phenotypic overlap among embryonal neoplasms also occurs at a genetic level. Fortunately, embryonic tumors are generally organ-specific, so that differential diagnosis with rhabdomyosarcoma is usually resolved if sufficient histologic and radiographic studies are available. Spindle cell rhabdomyosarcomas may pose a problem because of their resemblance to other spindle cell neoplasms, notably fibroblastic and peripheral nerve sheath tumors. However, they should be sufficiently cellular, mitotically active, and atypical not to be confused with benign proliferations. Desmin and actin stains may be misleading in fibroblastic lesions, because they are also expressed in myofibroblasts. Similarly, smooth muscle actin may be expressed in rhabdomyosarcomas, so that it is not useful in differential diagnosis with smooth muscle lesions. MyoD and myogenin stains can be useful, but one must be aware that positive entrapped muscle fibers may be confusing and that only nuclear staining should be considered “true positive.” These markers may also prove useful in the limited biopsies one may receive following rhabdomyosarcoma to determine whether residual tumor is present.

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