Molecular or morphology? Challenges in pathologic diagnoses

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October 2021—Recent molecular genetic advances have dramatically expanded diagnostic options, thus revolutionizing the diagnosis of many tumor types, especially those of soft tissue and bone. Advances in the discovery of molecular alterations underlying neoplastic pathogenesis have also provided insights into novel therapeutic targets and prognostic biomarkers. These improvements have led to the reclassification of a growing list of previously established tumor types, resulting in significant challenges for practicing pathologists, as exemplified herein.

First, many different tumor types demonstrate no identifiable differentiation on histomorphology and, frequently, immunophenotyping, but they harbor unique molecular genetic alterations yielding a specific diagnosis.[1] Undifferentiated small round cell sarcomas of bone and soft tissue are among the best examples in this regard, as outlined in the recent (fifth edition) *WHO Classification of Tumours: Soft Tissue and Bone Tumours*. The Ewing sarcoma/PNET family of tumors was historically thought to be different entities, but these tumors are now generally accepted as one and the same fundamental neoplastic process as they share common genetic abnormalities. This emerging change has resulted in improvement in the correct classification of this group of lesions. While diagnosing a small blue round cell as Ewing sarcoma is no longer as great a challenge given its recurrent *EWSR1* gene rearrangement in more than 90 percent of all cases (available in virtually all FISH and molecular laboratories), a small subset of Ewing sarcomas we've come to now understand harbor a fusion between a member of the FET family of genes other than *EWSR1* (i.e. *FUS*) and a member of the ETS family of transcription factors other than *FLI1*, including *ERG, ETV1, ETV4,* and *FEV*, thus necessitating molecular testing to reach an accurate diagnosis.

A greater challenge and diagnostic pitfall resides in a small group of entities previously regarded as Ewing-like sarcomas. These lesions share various degrees of similarity with Ewing sarcoma clinically, histologically, and immunophenotypically. However, they are characterized by *EWSR1*-non-ETS fusions and thus are lacking the pathognomonic molecular hallmark of Ewing sarcoma. The reported fusion partners thus far include *NFATc2, PATZ1, SMARCA5*, and *SP3*. It remains unclear whether these tumors represent one or more standalone pathologic entities or are better classified as variants of Ewing sarcoma. Their rarity thus requires additional examples to ensure accurate classification. Furthermore, a number of emerging Ewing-like undifferentiated round cell sarcomas have been identified with overlapping immunophenotypes (i.e. CD99), including *CIC*-rearranged sarcomas and sarcomas with *BCOR*-genetic alterations (most commonly *BCOR-CCNB3*). While most are treated similarly to Ewing sarcoma, *CIC*-rearranged sarcomas generally show unfavorable outcomes. The prognosis of *BCOR-CCNB3* sarcomas appears similar to that of Ewing sarcoma, whereas other tumors in the *BCOR* family are not well characterized.

Second, with increasingly identified molecular alterations, it is not uncommon to encounter different entities with seemingly similar histomorphology, overlapping immunophenotypes, and cytogenetic characteristics. Aneurysmal bone cyst, a giant cell-rich neoplasm of the bone characteristically harboring the *USP6* gene rearrangement, may rarely originate within soft tissue, and thus can be confused with other soft tissue lesions radiologically and histologically.[2] *USP6* rearrangements have also been found in cases with classic radiological and histologic features of myositis ossificans; however, an extended clinical course suggested that they might be better classified as evolving aneurysmal bone cyst (i.e. local recurrence after complete resection).[3] Settling this issue requires the relationship between these neoplasms to be better characterized. Even more confusing to the classical morphologist, nodular fasciitis shares the same *USP6* rearrangement but looks nothing like these other lesions

radiologically or under light microscopy.

Recurrent *PHF1* gene rearrangements have been detected in a significant proportion of ossifying fibromyxoid tumor (OFMT), while those with a *PHF1-TFE3* fusion are associated with an aggressive clinical behavior.[4] The same fusion has also been found in soft tissue myoepithelial carcinoma[5] and malignant chondroid syringoma (cutaneous mixed tumor).[6] These entities also have overlapping immunophenotypes, including variable expression of keratins, EMA, S-100 protein, SOX10, and GFAP, as well as myogenic markers including calponin, SMA, and desmin, and thus are difficult (if not impossible) to distinguish, especially when presented as a cytologically atypical or malignant form. These findings may provoke further reclassification of the group of lesions with a *PHF1-TFE3* fusion to reflect their frequent malignant biologic behavior, whether of myoepithelial or of OFMT origin.

The same challenge is not limited to mesenchymal tumors but can also be encountered in epithelial neoplasms. Adnexal-type and salivary gland-type tumors may rarely arise in breast parenchyma. Distinguishing a clear cell hidradenoma and a low-grade mucoepidermoid carcinoma of the breast poses a significant diagnostic challenge. They demonstrate overlapping histomorphologic features, including squamoid differentiation, mucus cells and clear cells, cyst formation, and, rarely, a papillary growth pattern. Both neoplasms also have significant overlapping immunophenotypes (co-expression of low- and high-molecular-weight keratins and p63, albeit with different patterns) and share identical cytogenetic alterations, principally *MAML2* gene rearrangement.[7] Thus, interpretation of the molecular findings should be closely incorporated with the salient histologic features (i.e. cytologic atypia) and the pattern of biomarker expression to reach the correct diagnosis. Inclusion of these rare tumor types in the WHO classification may prompt their recognition and avoid overdiagnosis and overtreatment.

Third, different tumor types may share identical molecular alterations. One such example is low-grade fibromyxoid sarcoma/sclerosing epithelioid sarcoma. These tumors consistently show a similar immunophenotype (MUC4) and frequently have either *FUS-CREB3L2* or *FUS-CREB3L1* gene fusions. In some cases, tumors with hybrid features are present, reflecting the overlap between the two entities. Another instance is the identification of t(1;10)(p22;q24) or *OGA* and *TGFBR3* rearrangement in a number of entities, including pleomorphic hyalinizing angiectatic tumor (PHAT), hemosiderotic fibrolipomatous tumor (HFLT), and myxoinflammatory fibroblastic sarcoma (MIFS).[8,9] These tumors most commonly involve the ankle and foot. Tumors showing histologic overlap with HFLT and MIFS (so-called hybrid HFLT-MIFS) have been reported. Moreover, changes identical to those seen in HFLT are present at the periphery of some PHATs (also known as early PHAT). The findings suggest a similar pathway in the pathogenesis among these tumor types and thus merit further exploration.

In short, a growing number of benign and malignant neoplasms share many histomorphologic similarities and immunophenotypes but carry characteristic genetic alterations, leading to specific diagnoses of some entities with potential novel therapeutic targets emerging. On the other hand, distinct neoplasms with diverse histologic features may harbor identical molecular characteristics, suggesting a similar histogenesis. This has resulted in a great deal of diagnostic challenge in current pathology practice. Recognition of salient histologic features and astute use of biomarkers are crucial. Molecular genetic studies are often needed, especially for those entities with an unusual histomorphology and typical histologic features but unusual clinical presentation, or with an uncommon immunoprofile. The advances in molecular techniques are certain to lead to further changes and refinement of classifications in the pursuit of precision medicine.

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