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**LOOK-ALIKES IN SPINDLE AND EPITHELIOID TUMORS:  
ULTRASTRUCTURAL VALUE AND PITFALLS**

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Due to the variable light microscopic appearance and overlapping morphologic patterns of spindle cell/epithelioid tumors, there are a large number of neoplasms that may need to be considered in the differential diagnosis depending on the particular situation. In a number of difficult cases, the surgical pathologist must approach the differential diagnosis using ancillary diagnostic techniques and should do so in a proper, efficient fashion, recognizing that arriving to the correct diagnosis is without doubts the most important goal. It is also imperative to take into account the expense and time that may be involved in arriving to the definitive diagnosis. Therefore, the most reasonable route to address the differential diagnosis and to make a final unequivocal interpretation should be taken. Whether immunohistochemistry, electron microscopy, cytogenetics and/or molecular diagnostics should be employed in the case in question becomes the dilemma. In some cases a combination of the above mentioned techniques is important not only to solidify the diagnosis but also to provide important additional information regarding prognosis, treatment and other important factors.

Differentiation of sarcomas from sarcomatoid carcinomas, spindle and pseudo sarcomatous reactions can create challenging diagnostic dilemmas. Differentiation of these entities by light microscopy alone can be almost impossible in some cases. Keratin expression by immunohistochemistry is quite variable in poorly differentiated carcinomas in general, where the entity of sarcomatoid carcinoma usually falls. While keratin staining using a variety of cocktails may be of value, it is not uncommon for immunoreactivity to be weak and focal or entirely absent.

A similar difficult diagnostic dilemma arises when differentiating sarcomatoid mesothelioma vs sarcoma. Most immunohistochemical markers that are expressed by epithelial mesotheliomas are not present in the sarcomatoid variety. The “positive” mesothelioma markers: podoplanin, calretinin, keratins CK 5 and 6, WT1 protein, thrombomodulin and mesothelin are usually not present in the sarcomatoid variety of mesotheliomas. Of these, calretinin is the one most commonly expressed in sarcomatoid mesotheliomas.

Another differential diagnosis that electron microscopy is very helpful in addressing is that of pseudomesotheliomatous adenocarcinoma of the lung associated with florid stromal (spindle-cell) reaction vs. mesothelioma. The differential with mesothelioma arises from the finding of an apparent biphasic neoplasm microscopically and the gross appearance encasing the lung, as is typically seen in mesotheliomas from where the descriptive name “pseudomesotheliomatous” originates. A significant number of these pseudomesotheliomatous adenocarcinomas reveal immunohistochemical profiles that are not characteristic, creating diagnostic controversies. Some of the characteristic lung adenocarcinoma markers such as TTF-1, B72.3, MOC-31 and carcinoembryonic (CEA) antigen may be expressed weakly and/or focally. Furthermore, some of these adenocarcinomas (as well as other adenocarcinomas originating in other sites) express mesothelioma-markers such as calretinin, cytokeratins 5/6 and WT-1 making the diagnostic interpretation based on immunohistochemical profiles confusing and at times basically impossible. Mucin is most commonly seen in adenocarcinomas but can also be found, albeit rarely, in mesotheliomas. These situations have medico legal ramifications, as cases in which a diagnosis of mesothelioma is considered may end up in litigation.

Electron microscopy plays an important role in addressing the above mentioned differential diagnoses and, in the hands of experienced electron microscopists, is considered the preferred ancillary diagnostic tool to establish an unequivocal diagnosis. The long (tall)-ratio of height to width greater than 10-15-, sinuous, bushy and complex microvilli in mesotheliomas surrounding glandular spaces differ dramatically from the short, non-branching and blunt microvilli typical of adenocarcinomas in general. Epithelial mesotheliomas often have tonofilaments in the cytoplasm-often perinuclear- of the neoplastic cells and long, well developed desmosomes, further supporting the diagnosis. The differentiating features may be focal and a careful and sometimes extensive evaluation is needed to establish a definitive diagnosis in some cases. Morphological parameters provide clear and irrefutable evidence that can be of great value in defending (confirming) the diagnosis.

Pleomorphic spindle cell / epithelioid neoplasms may also be a source of diagnostic confusion with poorly differentiated carcinomas (see below) and occasionally melanomas. In this situation a panel of immunohistochemical stains may be quite helpful in narrowing the differential diagnoses or solidifying a final diagnosis. Electron microscopy can then be reserved for those cases in which confusion still exists after the panel of initial immunohistochemical stains is reviewed. Distinguishing some sarcomas from melanoma can be quite difficult by light microscopy. Both are composed of poorly cohesive cells sometimes with abundant eosinophilic cytoplasm. This differential diagnosis arises predominantly in those melanomas lacking identifiable melanin by light microscopy. In this particular situation, immunohistochemical markers for melanoma can be used to make a definitive diagnosis. Melan A and HMB-45 expression can be definitive in making an unequivocal diagnosis. There are cases where ultrastructural evaluation may be needed to solidify the diagnosis or to resolve discrepant or unclear immunohistochemical findings. It should be remembered that benign and malignant peripheral nerve sheath tumors can have melanosomes and express melanocytic markers,

and in this situation the presence of a well defined basal lamina surrounding neoplastic cells can be definitive in supporting schwannian differentiation.

All of the above described situations represent possible indications to perform electron microscopy to make a definitive diagnosis. There are certain ultrastructural features that are particularly useful in the differential diagnosis of spindle cell / epithelioid lesions vs. look-alikes. In the differential diagnosis of sarcomas from poorly differentiated carcinomas, careful evaluation of cell junctions may be of importance. In sarcomas, tight junctions and rudimentary cell junctions can be seen; however, true desmosomes are only found in synovial sarcomas and epithelioid sarcoma. True lumens are also absent in the great majority of sarcomas with the exception of biphasic synovial sarcomas where tonofilaments can also be seen. Tonofilaments are not present in any other sarcomas. On the other hand, these epithelial characteristics (i.e. true desmosomes and rudimentary but well defined lumens lined by microvilli and with apical tight junctions) in a poorly differentiated spindle cell neoplasm may be all that is needed to make a definitive diagnosis of synovial sarcoma. Specific electron microscopic features of certain types of spindle cell tumors in the proper clinicopathologic setting can also be helpful in arriving to a definitive diagnosis. Among these are myofilaments with spindle densities or dense bodies as evidence of smooth muscle differentiation, thin and thick filaments, Z lines (discs or band material), myosin-ribosome complexes and/or rudimentary sarcomere structures to establish a diagnosis of rhabdomyosarcoma, abundant rough endoplasmic reticulum and fibronexus for fibroblastic differentiation, interdigitating cell processes, pseudo-mesaxons, external basal lamina and pinocytotic activity as features of schwannian lineage, glycogen "lakes" in Ewings sarcoma and Weibel-Palade bodies, pinocytotic vesicles and basal lamina for endothelial differentiation, just to mention a few of the important ultrastructural criteria that are routinely employed for diagnostic purposes. It is important to emphasize that the overall evaluation of cellular details in the neoplastic cellular elements, rather than individual findings, is the best way to approach the diagnosis from an ultrastructural point of view. Not only what is seen in the neoplastic cells, but also what is absent is of importance when making a diagnosis.

Another unique role for electron microscopy in the differential diagnosis of spindle / epithelioid tumors is in the evaluation of fine needle aspirates. In this situation, neoplastic cells are usually scanty (small numbers) and pattern recognition is mostly absent. Immunohistochemistry in fine needle aspirates may be difficult to interpret, especially if specimens are bloody and/or the number of neoplastic cells available for evaluation is small. Electron microscopy can provide accurate determination of cell type by identifying specific morphological markers, even when only a handful of cells are available in the sample. Ultrastructural evaluation can also be very important in the evaluation of fine needle aspirates obtained from epithelioid neoplasms presenting in soft tissues, abdominal, mediastinal or retroperitoneal locations, as the differential diagnoses of these soft tissue lesions include carcinomas, sarcomas or lymphomas. Accurate assessment of fine needle aspirates may be extremely important and decisive in establishing a definitive diagnosis which will have an immediate impact on patients' management and treatment and may result in avoiding unnecessary invasive surgical procedures.

A factor to be considered is the availability of some of the ancillary diagnostic techniques. In some practices immunohistochemistry is the only readily available diagnostic tool and it is reasonable that in such situations an initial immunohistochemistry work-up be requested to approach the differential diagnosis. However, many spindle cell / epithelioid neoplasms do not have distinctive immunohistochemical findings or so-called specific phenotypes. The risk is that since the immunohistochemical findings are generally not diagnostic of specific entities but rather consistent with a diagnosis considering the particular differential diagnosis that is being addressed and the clinical setting the wrong diagnosis may be rendered. This may result when a given immunohistochemical profile is considered to fit the favored light microscopic diagnosis. One situation that is particularly vulnerable to mistakes, is when the correct diagnosis is not even suspected on the basis of the light microscopic evaluation, as the requested immunohistochemical panel may not include markers that if expressed may suggest the unexpected diagnosis. Electron microscopy, not only serves to rule out specific entities but the findings may be suggestive or diagnostic of an entity not previously considered in the differential diagnosis.

Cytogenetics may play an important role in selected instances. Some spindle cell neoplasms (i.e. sarcomas) display specific chromosomal aberrations. Good examples are synovial sarcoma which characteristically shows a typical reciprocal translocation involving chromosome X and 18. (X;18) (p11.2;q11.2), Ewings sarcoma (EWS-Fli1), clear cell sarcoma (eWS-ATF1) and myxoid liposarcoma (FUS-CHOP), among other sarcomas. But there are many others, as reviewed by Miettinen in the reference given below. Nevertheless the molecular signatures of various tumors are quite often not specific but rather shared with other neoplasms with different differentiation.

Sending a difficult case to a regional electron microscopy laboratory should be always a serious consideration when difficulties in making a definitive diagnosis arise in poorly differentiated tumors. For ultrastructural evaluation to be of most value, proper tissue fixation is very important. Even though tissue obtained from paraffin blocks may retain enough differentiating ultrastructural features that is often not the case. It is quite inappropriate and risky to address difficult differential diagnoses with electron microscopy without adequate tissue preservation that allows proper evaluation of fine ultrastructural details. Tissue fixation in formalin, provided that the tissue is cut in small pieces which assures penetration of the fixative is quite adequate for electron microscopy. It is imperative that areas with necrosis be avoided. Sampling of several areas of a sarcoma is recommended, as the appearance and differentiation of the neoplasm may vary from one area to another. If the specimen available for ultrastructural evaluation is from only a particular area of the tumor, precise classification may be compromised. Data obtained from molecular diagnostics is often devoid of morphological parameters (with the exception of FISH in certain instances) and although quite useful at times, caution should be employed in the interpretation of this information. Careful correlation with morphological parameters is a must and if a discrepancy arises, there should be no hesitation to proceed with additional work up to clarify the situation.

There are pitfalls in the ultrastructural evaluation of spindle / epithelioid neoplasms. Since electron microscopy cannot always differentiate benign from malignant cells, it is important not to interpret normal tissue entrapped in the tumor. Fixation should be good enough for preservation of diagnostic findings making possible an accurate interpretation. The electron microscopist must be aware of the entire spectrum of ultrastructural findings of various tumors, as overlap in ultrastructural features may be a problem or findings indicating certain differentiation in the neoplastic cells may be shared by a variety of tumors. Finally, consideration of the light microscopic findings is crucial in interpreting the electron microscopic features in a given situation.

In many of the cases where a difficult differential diagnosis is being investigated, it is highly recommended that a combined diagnostic approach using immunohistochemistry and electron microscopy be used combined with pertinent molecular diagnostic techniques when appropriate, taking advantage of the additive diagnostic power of these ancillary diagnostic techniques.

The comprehensive diagnostic approach with use of several diagnostic techniques as soon as the workup of the case begins generally provides a solid unequivocal diagnosis in the shortest period of time. Even though this is perceived by some as a more expensive approach and; therefore, less desirable, the savings to the health care industry that result from a prompt and accurate diagnosis more than justify the additional expense incurred in the pathologic work up of a given case. This approach is also by far the safest in terms of obtaining the correct diagnosis.

#### PERTINENT REFERENCES:

Abutaily, AS, Addis, BJ, Roche, WR: Immunohistochemistry in the distinction between malignant mesothelioma and pulmonary adenocarcinoma: a critical evaluation of new antibodies. *J Clin Pathol* 2002; 55:662-668.

Cin, PD, Rao, U, Sani-Sait, S et al: Chromosomes in the diagnosis of soft tissue tumors. I. Synovial sarcoma. *Mod Pathol* 1992; 5:357-362.

Dardick, I, Herrera, GA: Diagnostic electron microscopy of neoplasms. *Hum Pathol* 1998; 29: 1335-1338.

Dickersin, GR: The electron microscopic spectrum of nerve sheath tumors. *Ultrastruct Pathol* 1987; 11:103-146.

Dickman, DS, Triche, TJ. Extrasosseous Ewing's sarcoma versus primitive rhabdomyosarcoma: diagnostic criteria and clinical correlation. *Hum Pathol* 1986; 17:881-893.

Erlandson, RA: Peripheral nerve sheath tumors. *Ultrastruct Pathol* 1985; 113-122.

Erlandson, RA: The ultrastructural distinction between rhabdomyosarcoma and other undifferentiated sarcomas. *Ultrastruct Pathol* 1987; 11:83-101.

Franchi, A, Massi, D, Santucci, M: The comparative role of immunohistochemistry and electron microscopy in the identification of myogenic differentiation in soft tissue pleomorphic sarcomas. *Ultrastruct Pathol* 2005; 29:295-304.

Hammar, SP, Bockus, DE, Remington, FL et al: Mucin-positive epithelial mesotheliomas: A histochemical, immunohistochemical, and ultrastructural comparison with mucin-producing adenocarcinomas. *Ultrastruct Pathol* 1996; 20:293-325.

Herrera, GA, Reiman, BEF, Salinas, JA et al: Malignant schwannomas presenting as malignant fibrous histiocytomas. *Ultrastruct Pathol* 1982; 3:253-261.

Herrera, GA, Pinto de Moraes, H: Neurogenic sarcomas in patients with neurofibromatosis (von-Recklinghausen's disease). Light, electron microscopy and immunohistochemical study. *Virchows Arch A* 1984; 403:361-374.

Herrera, GA: The search for an accurate pathologic assessment of melanoma: Where do we stand now?. *J Histotechnol* 2003; 211-212.

Herrera, GA: Metastatic melanoma: Ancillary diagnostic techniques. *J Histotechnol* 2003; 26:217-224.

Herrera, GA, Turbat-Herrera, EA: Current role of electron microscopy in the diagnosis of pigmented tumors. *Sem Diag Pathol* 2003; 20:60-71.

Mertens, F, Fletcher, CD, Dal Cin, P et al: Cytogenetic analysis of 46 pleomorphic soft tissue sarcomas and correlation with morphologic and clinical features: a report of the CHAMP Study Group. *Genes Chromosomes Cancer* 1998; 22:16-25.

Mierau, GW, Favara, BE: Rhabdomyosarcoma in children: ultrastructural study of 31 cases. *Cancer* 1980; 46:2035-2040.

Miettinen, M: From morphological to molecular diagnosis of soft tissue tumors. *Adv Exp Med Biol* 2006; 587:99-113.

Ordonez, N: What are the current best immunohistochemical markers for the diagnosis of epithelioid mesothelioma?. *Hum Pathol* 2007; 38:1-16.

Robinson, BW, Creaney, J, Lake, R et al: Mesothelin-family proteins and diagnosis of mesothelioma. *Lancet* 2003; 362:1612-1616.

Tos, AP: Classification of pleomorphic sarcomas: where are we now?. *Histopathol* 2006; 48:51-62.

Turbat-Herrera, Herrera, GA: Electron microscopy renders the diagnostic capabilities of cytopathology more precise: An approach to everyday practice. *Ultrastruct Pathol* 2005; 29:475-482.

Velez, E, Turbat-Herrera, EA, Herrera, GA: Malignant epithelial mesothelioma vs. pulmonary adenocarcinoma- A pathologic dilemma with medico-legal implications. *Pathol Case Rev* 2002; 7:234-243.

Weeks, DA, Beckwith, JB, Mierau, GW: Rhabdoid tumor. An entity or a phenotype?. *Arch Pathol Lab Med* 1989; 113-114.

Herrera GA: Metastatic melanoma: Ancillary diagnostic techniques. *J Histotechnol* 26:217-224, 2003.

Herrera GA, Turbat-Herrera EA: Sarcoma look-alikes: The important role of ultrastructural evaluation. *Ultrastruct Pathol* 32: 43-50, 2008.

#### BULLETS:

Immunohistochemical profiles in the work-up of many spindle cell / epithelioid neoplasm often overlap and are not specific of a given entity

Electron microscopy not only addresses the differential diagnosis in question but may provide crucial information that suggests or is diagnostic of a totally unexpected entity

Electron microscopic evaluation should take into account not only the findings present but also those absent in a given case

Electron microscopy has a significant advantage over immunohistochemistry in fine needle aspirate specimens from soft tissue masses

Proper fixation is imperative for ultrastructural evaluation to be able to be most helpful

A combined diagnostic approach using more than one ancillary diagnostic technique is highly recommended, especially in difficult cases