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Electron microscopy in the diagnosis of inherited connective tissue diseases

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Martan syndrome is a dominantly inherited disorder characterized by Cardiovascular, ocular and skeletal abnormalities. The major cardiovascular complications of ascending aorta dilatation and dissection often lead to premature death in the absence of treatment and justify the requirement of relevant diagnostic criteria in the early years of life. The pathogenetic role of fibrillin has been confirmed by the identification of mutations in the fibrillin gene FBN1 in patients with classical Martan syndrome. Immunohistochemical and immunohistochemical analysis converged to demonstrate defective synthesis, secretion and extracellular matrix formation of fibrillin by cultured dermal fibroblasts. The skin biopsy thus appeared

as a noninvasive Clinical requirement allowing immunochemical, immunohistochemical and genetic assessments of fibrillin deficiency in early Marfan syndrome diagnosis.

Fibrillin is a large 350kDa matrix protein associated with one of the major subgroups of microfibrils morphologically characterized by 10-13 nm diameter, a hollow cross-section and a beaded appearance with some periodicity. Considered as integral components of elastic elements, these microfibrils are constituents of three types of fibers, namely oxytalan, elaunein and elastic. Oxytalan fibers consist mostly of microfibrils arranged in an orderly manner, although they contain some elastin. Elaunein fibers are present beneath the oxytalan fibers and contain both microfibrils and amorphous elastin. In all the probands the arborescent distribution of the elastic and elaunein fibers was lost and the oxytalan fibrils were scarce, with variable patterns of microfibril alteration.

These patterns of microfibril alteration extended to the elaunein and elastic fibers in which the peripheral microfibrillar apparatus appeared either condensed with vanishing of the microtubular structure or scarce with preservation of ultrastructure. In any case, the close interconnection between the elastic fibers and the collagen fiber bundles supported by microfibrils appeared ruptured or slender, thus emphasizing the discontinuity between the tensile elastic and architectural collagenic frameworks of the dermis in the Marfan patients. The dermal microvasculature was variably affected in relation to the decreased density of microfibrils anchoring the elastic network to the perivascular basement membrane, and displayed gradual constrictive features.

While fibrillin immunodetection in cultured skin fibroblasts allows pathologists to appreciate the level of fibrillin expression deficiency, ultrastructural tissular immunolabeling renders account of the pleomorphism of the pathological implications in the dermal connective matrix and the microvasculature.

Electron microscopy of melanin-synthesizing tumors

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With the availability of relatively new antibodies such as HMB-45, HMB-50 or MART-1 (Melan-A), the identification of melanocytic phenotype in an undifferentiated neoplasm has become much easier. These markers, however, are often negative in many (and sometimes in all) of the cells in a given tumor. In addition, there are situations in which the identification of melanin-synthesizing cells is not enough to reach a correct diagnosis. Electron microscopy may play a contributory role in many of these cases. In this part of the course, the application of electron microscopy in four specific settings will be reviewed, namely: i) poorly differentiated tumors, either primary or metastatic, in which malignant melanoma enters the differential diagnosis; ii) soft tissue tumors with melanocytic differentiation; iii) pigmented tumors of the central nervous system (mostly arising in the meninges); and iv) complex neoplasms with aberrant

melanin production (angiomyolipoma, 'sugar tumor' and lymphangioliomyomatosis).

Poorly differentiated tumors

Before the advent of immunohistochemistry, the diagnosis of metastatic – and particularly of amelanotic – malignant melanoma was supported by the ultrastructural identification of melanosomes and premelanosomes showing variable degrees of melanin deposition in the striated or zigzag strands of tyrosinase. This could become a time-consuming search, until it was recognized that the usual or "classic" configuration might be lacking in many cases (particularly in malignant cases), containing the so-called aberrant melanosomes; these abnormal melanosomes can show many appearances, from those reminiscent of their 'normal' counterpart to structures nearly indistinguishable from primary lysosomes. Two main clues may be used to identify these structures as melanosomes: the usual variable electron density from one to another in a given cell (indicating variable accumulation of melanin) and the occasional and focal presence of striations (particularly when underfocussed and lightly contrasted electron micrographs from very thin sections are examined). The presence of tubular aggregates in the cisternae of rough endoplasmic reticulum was found to be an additional feature that could be helpful in recognizing metastatic amelanotic malignant melanoma with very scanty melanosomes. Although this same structure was subsequently found in myxoid chondrosarcoma, it is still a helpful finding in the right context.

In the early immunohistochemical panels, the coexpression of S-100 protein and vimentin was considered virtually diagnostic of malignant melanoma. It was soon realized that carcinomas from certain sites, such as the breast, can be S-100 protein positive. Vimentin can be present in undifferentiated or sarcomatoid carcinomas and cases of keratin-positive melanoma have been reported; therefore, adequate comprehensive panels of antibodies should be used in problematic cases with epithelioid appearance. The same is also true in spindle cell proliferations, as there is an extensive list of tumors in this group in which antibodies against S-100 protein can be positive and therefore malignant melanoma can enter the differential diagnosis. The second generation of "melanocytic" antigens (mainly HMB-45) is highly specific but far less sensitive and relatively wide panels of antibodies are still required to reach the correct diagnosis, even in cases with light microscopic appearance highly reminiscent of malignant melanoma.

It is obvious that combining electron microscopy with immunohistochemistry increases diagnostic accuracy but there are practical and economical reasons for using only one of them. Although immunohistochemistry is usually preferred over electron microscopy, pathologists should be aware that there are many instances in which it is better to start by doing electron microscopy: some cases will already be solved and, in the few remaining ones, the number of antibodies required to complete the diagnosis will be significantly reduced or more selectively chosen. One of the settings in which this approach can prove particularly helpful is that of the undifferentiated neoplasms (either made up of epithelioid, spindle, or small round blue cells). Of course, it requires a fast and reliable electron microscopic processing but this is no longer a problem in the era of sophisticated technological developments. Not all institutions have an electron microscopy facility but properly fixed tissue can always be sent to electron microscopy laboratories in other hospitals. These cases are almost always first investigated by immunohistochemistry in the referring hospitals. In this situation, the use of electron microscopy

after immunohistochemistry can prove particularly helpful in cases with nonspecific (*i.e.*, vimentin-only) or paradoxical immunophenotype (*i.e.*, cytokeratin and vimentin, cytokeratin and S-100 protein or, more exceptionally, cytokeratin and HMB-45).

Soft tissue tumors with melanocytic differentiation

Pigmented dermatofibrosarcoma protuberans, so-called Bednar tumor, is composed of cells identical to those of other fibrohistiocytic lesions intermingled with cells that contain melanosomes and premelanosomes; there is no general agreement regarding the true neoplastic or reactive nature of the latter. Bednar tumor is rare but usually easy to identify.

Clear cell sarcoma (CCS) of soft parts is the paradigmatic neoplasm in this group. It is also known as melanoma of soft parts and it might be so classified from its phenotype. Several studies, however, have shown that genetic and molecular abnormalities in the cells of this tumor are different from those of malignant melanoma. In addition, CCS does not display the variable histological appearance of malignant melanoma and affects younger patients. Synovial sarcoma, fibrosarcoma and neurofibrosarcoma, as well as metastatic spindle cell malignant melanoma, are included in the differential diagnosis of CCS. Although rare cases of synovial sarcoma have been reported to express S-100 protein, distinction from CCS on the basis of light and electron microscopic appearance, combined with immunohistochemistry, will rarely be problematic. CCS can only be confused with fibrosarcoma if the diagnosis is based in hematoxylin and eosin sections and the clear cell appearance resulting from cytoplasmic glycogen is not appreciated.

Conversely, the differential diagnosis of pigmented nerve sheath tumors, metastatic spindle cell melanoma or neurotropic melanoma can prove extremely difficult in some cases, even with the aid of electron microscopy and immunohistochemistry. Tumor cells in all these neoplasms express S-100 protein and may show HMB-45 positivity. Also, melanosomes in different stages of development are present in all of them. External lamina and mesaxon formation are constantly found surrounding individual cells and cell processes in benign pigmented nerve sheath tumors and can be focally present not only in pigmented neurofibrosarcoma but also in neurotropic malignant melanoma. It is the relative predominance of any of these features, combined with the careful evaluation of the clinical data and the relationship of the tumor with surrounding structures that can lead to a correct diagnosis. This group of tumors exemplifies the importance of an integrated approach to the diagnosis of soft tissue tumors.

Genetic and molecular studies are already helpful in soft tissue tumors and will become an even more relevant tool in the near future. CCS is an example of how molecular data can help in separating related entities (CCS vs. malignant melanoma, olfactory neuroblastoma vs. pigmented primitive neuroectodermal tumor (PNET), skeletal vs. extraskeletal chondrosarcoma) or by unifying apparently different tumors (Ewing's sarcoma and PNET, mesoblastic nephroma and fibrosarcoma, malignant solitary fibrous tumor and synovial sarcoma). It is still unclear how this new body of knowledge will influence the actual management of soft tissue tumors and the role that will remain for the phenotypic characterization of these lesions.

Pigmented tumors of the central nervous system

Malignant melanoma can spread to the central nervous system and meninges and is probably the most common pigmented tumor that

can be found in these sites. Meningeal melanocytoma has generally been accepted as a well-defined clinicopathological entity potentially arising from meningeal melanocytes, most commonly located in the spinal canal and posterior fossa. Cervical and thoracic regions are usually involved, as residual melanocytes may remain in these regions after normal embryogeny. They are made up of a proliferation of pigmented spindle cells that tend to arrange around blood vessels with occasional palisading.

The differential diagnosis of these two tumors includes other pigmented proliferations. Most of them share a similar immunocytochemical profile: antibodies against vimentin and 5-100 protein are usually positive, while cytokeratin, epithelial membrane antigen, glial fibrillary acidic protein and neuron-specific enolase are negative. On the other hand, HMB-45 can be focally positive in meningeal melanocytoma, melanocytic nevi and pigmented schwannoma and is sometimes negative or weakly positive in malignant melanoma. Electron microscopy is very useful in distinguishing meningeal melanocytoma from Schwann cell tumors, PNET, pigmented medulloblastoma, meningioma and malignant melanoma. All of these tumors can have melanin pigment and spindle or polygonal cells with cell processes resulting in a fibrillary appearance. However, unlike Schwann cell tumors, several cells and cell projections are enclosed by a single rim of external lamina in melanocytoma; in addition, this tumor does not display mesaxon formation. PNET are made up of rather undifferentiated cells with cell projections that have longitudinally oriented microtubules and lack external lamina. Furthermore, in some cases, dense-core granules and synaptic vesicles can be encountered. The many intermingling cell processes of meningioma are not lined by external lamina. Instead, they are joined by many well-developed desmosomes. Although the nevoid arrangement of melanocytoma, with several cells and cell processes surrounded by every single external lamina, can sometimes be found in malignant melanoma, this is usually an isolated ultrastructural finding: Furthermore, melanosomes in melanocytoma are typical, *i.e.*, similar to normal skin melanosomes, while metastatic malignant melanoma often contains aberrant melanosomes. Consequently, in spite of the axiom that electron microscopy is not able to distinguish benign from malignant lesions, it can provide indirect data that may be potentially useful in differentiating meningeal melanocytoma from malignant melanoma.

In summary, this is a differential diagnosis in which immunohistochemistry can be particularly unrewarding and accurate analysis of the fine structure is often the only way to complement detailed histological examination.

Complex neoplasms with aberrant melanin Production

Classical angiomyolipoma (AML) is a heterogeneous proliferation composed of an admixture of blood vessels, smooth muscle cells, adipocytes and peculiar "myoid" cells with variable melanin synthesis. Not all these elements are present in every single case, the most constant finding being the presence of well developed or 'aberrant' melanosomes by ultrastructural examination. AML has been reported typically in the kidney and liver but it can also be encountered in many other locations, such as the lymph nodes, skin, uterus and lung. An epithelioid variety of AML has been reported in some of these sites, where it can easily be confused with a primary or metastatic epithelial neoplasm. 'Sugar tumor' (ST) is currently regarded by some authors as a clear cell epithelioid variant of AML. It was first reported in the lung and is made up of an indolent, monomorphous, epithelioid or hemangiopericytoma-like proliferation of clear cells containing large glycogen pools. Both HMB-45 positiv-

ity and melanin synthesis have also been identified in these cells. Recently, similar tumors have been found to arise in the pancreas and uterus.

The many similarities between AML, ST and lymphangiolo-myomatosis would favor the hypothesis of variable phenotypic modulation in a common precursor cell. This hypothetical cell of origin could be related to perivascular cells that give rise to both adipocytes and perivascular smooth muscle during embryogenesis. In patients with tuberous sclerosis, a genetic abnormality has been identified that would explain the "ectopic" melanin synthesis occurring in these cells as regions linked to the tuberous sclerosis complex 1 gene locus (9q34) map to the position of dopamine- β hydroxylase, which takes part in the conversion of phenylalanine to melanin.

Clear cell epithelioid AML (or ST) arising in the lung can be misdiagnosed as a metastatic clear cell carcinoma and, in the kidney, it can easily be confused with the much more prevalent clear cell renal carcinoma. In fact, several reported cases of monotypic epithelioid AML were initially misdiagnosed, as immunohistochemical or ultrastructural studies had not been found indicated in what had been interpreted as conventional clear cell carcinomas. Cases of epithelioid AML are increasingly being recognized and pathologists should include them in the differential diagnosis of epithelioid neoplasms. Tumors in which expression of HMB-45 cannot be demonstrated will benefit from a careful ultrastructural examination. Aggressive cases, usually of the epithelioid variety and arising in the kidney, have been reported.

These tumors illustrate how electron microscopy can provide insights into the understanding of specific entities – just as the presence of melanosomes in all these lesions was first established by ultrastructural examination – and the convenience of performing electron microscopy studies when dealing with peculiar, complex or difficult cases.

In conclusion, there are many situations in which a given pigmented tumor can benefit from ultrastructural examination. In some cases, it will be wise to use electron microscopy as the initial special study and complement it with immunohistochemistry or molecular studies. In other instances, it will help to understand paradoxical immunohistochemistry findings, pathogenic mechanisms, or the histogenesis of complex tumors. Awareness of the potential information that can nowadays be obtained from electron microscopy would improve the pathological diagnosis in these situations.

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