after a follow-up of 1, 4 and 7 years; and three patients with lymph node metastases were alive after a follow-up of 6, 7 and 10 years. More patients need to be studied and followed to see if the prognosis is what would be predicted by the depth of the lesion or slightly better than that. However, there is no doubt that some patients do develop metastases and can die from such nevoid melanomas.

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Immunohistochemistry and molecular biology in the management of melanocytic lesions

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Melanocytic lesions constitute a very important part of surgical pathology material. Melanoma kills more patients than any other cutaneous malignancy and early detection is crucial in its clinical management. Additionally, melanocytic lesions are involved in a high percentage of malpractice lawsuits. Although histological exam of hematoxylin and eosin slides remains the main component in the diagnosis of melanocytic legions, in recent years, immunohistochemistry and, to a lesser extent, other molecular pathology techniques have been applied to help in the diagnosis of this group of lesions.

Immunohistochemistry

Because melanin pigment may be confused with the brown chromogen diaminobenzidine, immunohistochemical interpretation is sometimes problematic. Approaches to circumvent this problem include use of other immunohistochemical chromogens (fast red, etc.), use of Giemsa or azure B counterstain to stain melanin green, and bleaching of melanin. With bleaching techniques, reactivity for 5-100 protein, gplOO (HMB-45), the NKI-C3 antigen, CD34, and 0020 (L26) are unaffected, while certain other antigens may show enhancement or abolition. Therefore, any use of bleaching techniques in immunohistochemistry should be carefully worked up in each particular laboratory.

As in other areas of pathology, immunohistochemistry should be pertormed using a panel of antibodies to avoid misdiagnosis. For example, occasional cytokeratin expression occurs in melanoma, especially in metastases; if only anticytokeratin antibodies are used in such a case, a diagnosis of carcinoma can be entertained.

S-100 protein remains the most sensitive (if nonspecific) marker of melanocytic differentiation. Most cases initially considered 5-100-negative become positive on additional testing. In the differential diagnosis between spindle-cell melanomas and malignant peripheral nerve sheath tumors, the former usually express 5-100 protein in most tumor cells.

HMB-45 is a monoclonal antibody that detects a protein in early melanosomes. HMB-45 labels malignant melanoma of soft tissues (clear cell sarcoma), the sugar tumor" of the lung, angiomyolipomas and lymphangiomyomatosis. Because these lesions rarely occur in the skin, HMB-45 is very useful in detecting melanocytic differentiation in skin biopsies. HMB-45 labels immature or activated intraepidermal melanocytes (melanocytes of fetal and neonatal skin, proliferating melanocytes in inflamed skin or in skin adjacent to diverse neoplasms), blue nevi, the superficial portions of other nevi, and most primary melanomas. HMB-45 detects a pattern of "maturation' with labeling of the top but not of the bottom of most melanocytic nevi. Such a pattern is usually absent in melanomas, thus HMB-45 may be helpful in this differential diagnosis. The use of antigen retrieval techniques and high-sensitivity detection systems (such as streptavidin, Envision®, etc.) increases the sensitivity of HMB-45; with such techniques, up to 75% of primary spindle cell melanomas are positive, at least in a few scattered cells.

Other markers for melanocytic differentiation are under study. Peripherin, an intermediate filament involved in growth and development of the peripheral nervous system, shows a pattern of expression similar to that seen with HMB-45. Melan-A (MART-i) and tyrosinase are expressed in both nevi and melanoma. NKI-C3 is an antibody that labels melanocytic lesions as well as cellular neurothekeomas and macrophages.

The analysis of cell proliferation markers such as Ki-67 helps distinguish between benign and malignant lesions. Common, dysplastic and compound Spitz nevi exhibit reactivity in <6% of cells, generally disposed at the dermal-epidermal junction or in the more superficial dermal compartment, with an orderly gradient with progressive loss of Ki-67 expression in proportion to the dermal depth of the cells. In contrast, melanomas contain a higher count of reactive cells and do not show that orderly pattern, but instead have a random pattern of immunoreactivity. In a study with 112 lesions, the analysis of Ki-67 expression helped detect those lesions with systemic progression (recurrence or metastasis). However, in a different study, Ki-67 expression did not correlate with development of metastasis in thin melanomas. Analysis of Ki-67 expression is also

important in the distinction between desmoplastic (sclerotic) nevi and desmoplastic melanomas; desmoplastic nevi contain a mean of eight cells/mm^2 expressing Ki-67 vs. 105 cell/mm2 in desmoplastic melanomas.

There is current investigation on other markers to determine their usefulness in distinguishing nevi from melanoma. It has been reported that melanomas, and not most nevi, express TGF-13; substance P (a potent mast-cell secretagogue); cyclin A, cyclin B, and p34cdc2 (proteins involved in the cell cycle); mci-i and bcl-xl (anti-apoptotic factors); p21; CD39 (an ectoadenosine triphosphate diphosphohydrolase that may enable tumor cells to escape from immune recognition); and D-1 (another melanoma-associated antigen). CD44v5 splice variant is expressed in melanomas but not in dermal and "dysplastic" nevi.

Other markers are usually are usually lost in melanomas: nm23 [nucleoside diphosphate (NDP) kinase, a purported metastasis-suppressor gene], c-kit (tyrosine-kinase receptor), and p1 6/CDKN2A and p27Kipl [two cyclin-dependent kinase (cdk) inhibitors]. 0D26 (dipeptydil-peptidase IV, that may bind and degrade some components of the papillary dermis) and moesin (a protein involved in the association of actin filaments with the cytoplasmic membrane), are reduced in deeply invasive and metastatic melanomas.

Investigation has also been centered on the tumor vessels. F-selectin, an endothelial antigen that acts as an adhesion molecule for lymphocytes to extravasate, is absent in tumor vessels of primary and metastatic melanoma, and may participate in a reduced immune reaction against melanoma. Endoglin, a homodimeric cell surface component of the transforming growth factor-beta (TGF-It) family appears to be involved in the process of melanoma angiogenesis.

Some markers may be helpful in predicting prognosis. Expression of hsp70 in the primary lesion and HLA-DR in the lymphocytes around the tumor correlate with a better prognosis. Increased serum S100 β correlates with development of distant metastases. In uveal melanomas, coexpression of vimentin and low-molecular-weight keratins 8 and 18 results in increased *in vitro* invasiveness through extracellular matrix and may correlate with a higher rate of metastases. HMB-45-positive spindle cell melanomas have a worse prognosis than their negative counterparts.

Spindle cell melanomas express p75, a neurotrophin receptor **(p75NTR),** which may explain the greater tendency of spindle cell melanomas to undergo neurotropic spread. Immunohistochemistry for this marker may help to detect perineural invasion and to evaluate surgical margins.

Immunohistochemical detection of S100 protein and gplOO (HMB45 antigen) increases detection of micrometastasis in sentinel node biopsy for melanoma. More sensitive molecular techniques have also been proposed (see below). A potential pitfall in sentinel node biopsies is the detection of nodal nevi. These clusters of benign melanocytes are present in as high as 22% of yinphadenectoiny specimens. A key difference between metastatic melanoma and nodal nevi is that nevi typically occur in capsular rather than subcapsular location, and moreover, nodal nevi should not react with HMB-45, demonstrate significant cytologic atypia, or show evidence of cellular proliferation (mitotic figures or significant Ki-67 expression). Obviously, concurrent review of the patient's primary melanoma specimen with a view to comparing cytologic features is crucial in this evaluation.

Molecular biology

Application of microdissection techniques has provided a method to obtain tumor cells without contaminating elements such as fibroblasts or endothelial cells. The resulting sample can be studied for possible molecular changes.

Several genetic anomalies have been reported in families with high melanoma rates. The gene of the familial melanoma syndrome is present in chromosome lp36 (OMMi). A second gene (CMM2) is present in 9p, corresponding to the cell cycle regulator p16/INK4. A cyclin-dependent kinase (CDK4, in chromosome 12q14) is also present in melanoma-prone families.

Microsatellite instability has been reported to be relatively frequent in metastasizing melanomas as well as in metastatic melanoma but not in clear cell sarcoma (melanoma of soft parts). In contrast, detection of the t (12;22)(q13;q12) translocation (Ewing's sarcoma and ATF-1 gene fusion) is characteristic of clear cell sarcoma but not of cutaneous melanoma.

Using the polymerase chain reaction technique (FOR), detection of mRNAs of tyrosinase, p97, MUC18, and MAGE3 (melanocytic markers) in blood samples from patients with melanoma correlates with a higher stage of disease and progression of disease. Similarly, PCR detects tyrosinase mRNA in some sentinel lymph nodes negative with immunohistochemistry.

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