Overview

Melanoma continues to represent one of the greatest diagnostic challenges in surgical pathology and is an important source of litigation. Both as a primary lesion in the skin and in metastatic sites, this neoplasm is capable of assuming many different macroscopic and histologic appearances that mimic other diseases, both benign and malignant. The four main histologic phenotypes of primary melanoma include (1) superficial spreading, (2) lentigo maligna, (3) acral lentiginous mucosal, and (4) nodular melanoma (Figs. 7.1 to 7.4). Other morphologic variants include nevoid, balloon (clear) cell, pleomorphic, sarcomatoid, spindle cell/desmoplastic/neuroid, small cell (neuroendocrine-like), signet-ring cell, myxoid, metaplastic, and rhabdoid.1–3 All of these variants may be amelanotic in nature, which may render the morphologic differential diagnosis difficult. Accordingly, electron microscopy, immunohistology, and cytogenetic analysis have become exceedingly important in the accurate recognition of melanoma. This discussion focuses on the second of these investigative modalities and is directed principally at diagnostic questions concerning melanocytic tumors in general.4 In addition, we discuss the use of immunohistochemistry (IHC) and the newer ancillary techniques to help determine the prognosis and, possibly, aid in screening for targeted therapies.

Biology of Antigens and Antibodies

Filamentous Proteins in Melanocytic Neoplasms

Intermediate filament protein (IFP) analysis has been an important cornerstone of IHC evaluation for nearly 30 years. Immunoanalysis for keratins, vimentin, desmin, neurofilament proteins, and glial fibrillary acidic protein (GFAP) are broadly capable of distinguishing between histologically similar classes of neoplasms with dissimilar lineages.5,6 Regarding melanocytic neoplasms, nevi and melanomas typically express only vimentin, and only rarely do they express keratins or the other IFPs (Fig. 7.5).6–10 Moreover, the density of vimentin in melanogenic tumors is high and yields intense immunoreactivity for that marker in most instances. Due to the widespread expression of vimentin by most mammalian cells, some authors have suggested to use it to determine whether the tissue antigenicity is appropriately preserved. However, in our opinion, the majority of antibodies used in the skin already have built in internal controls (e.g., Langerhans and intraepidermal melanocytes for S100, MART1, SOX10, MITF expression; sebaceous cells and perineural cells for epithelial membrane antigen [EMA] expression) and therefore there is no appreciable need for the use of vimentin as a control of adequacy for immunohistochemical studies of the skin. GFAP and desmin have been reported in a small minority (<1%) of melanomas,2 usually tumors that demonstrate “metaplastic” sarcomatoid microscopic features or, conversely, desmoplastic and neuroid characteristics. For practical purposes, and in specific reference to studies on paraffin sections for these IFPs, more than 95% of melanocytic neoplasms are labeled solely for vimentin or S100 (see also later) even after application of techniques such as heat-mediated epitope retrieval.

Muscle-specific actin, recognized by monoclonal antibody HHF-35; α-isoform, or smooth muscle actin (SMA), recognized by antibody 1A4; and caldesmon are also preferentially seen in nonepithelial, nonmelanocytic, nonglial tissues. They are rarely detected in melanocytic lesions but can be
Immunohistology of Melanocytic Neoplasms

The principal exceptions in the latter group include germ cell tumors, adrenocortical proliferations, and hepatocellular neoplasms. An EMA-like moiety also may be observed in selected lymphoid and plasmacellular tumors. Melanocytic proliferations are rarely reactive for this marker. It may be observed within foci of melanomas that border zones of geographic necrosis, but those areas should be considered as likely to be artificial. Another possible pitfall is the observation of EMA expression in plasma cells, which are present in a number of melanomas and may be misinterpreted to be positive melanoma cells.

Carcinoembryonic Antigen

Carcinoembryonic antigen (CEA) characterizes a family of glycoproteinaceous cell membrane constituents present mainly in tissues and neoplasms. The principal exceptions in the latter group include germ cell tumors, adrenocortical proliferations, and hepatocellular neoplasms. An EMA-like moiety also may be observed in selected lymphoid and plasmacellular tumors. Melanocytic proliferations are rarely reactive for this marker. It may be observed within foci of melanomas that border zones of geographic necrosis, but those areas should be considered as likely to be artificial. Another possible pitfall is the observation of EMA expression in plasma cells, which are present in a number of melanomas and may be misinterpreted to be positive melanoma cells.

Cell Membrane Proteins

A diversity of proteins associated with cell membranes come into play in relationship to the differential diagnosis of melanoma. These fall into two broad categories: those associated with epithelial cells and those that relate to hematopoietic elements.

Epithelial Determinants

Epithelial Membrane Antigen

EMA, a family of glycoproteins related to the milk fat globule proteins, is expressed by a variety of epithelia and their neoplasms. The principal exceptions in the latter group include germ cell tumors, adrenocortical proliferations, and hepatocellular neoplasms. An EMA-like moiety also may be observed in selected lymphoid and plasmacellular tumors. Melanocytic proliferations are rarely reactive for this marker. It may be observed within foci of melanomas that border zones of geographic necrosis, but those areas should be considered as likely to be artificial. Another possible pitfall is the observation of EMA expression in plasma cells, which are present in a number of melanomas and may be misinterpreted to be positive melanoma cells.

Carcinoembryonic Antigen

Carcinoembryonic antigen (CEA) characterizes a family of glycoproteinaceous cell membrane constituents present mainly in tissues and neoplasms with endodermal differentiation.
epithelioid vascular tumors; but melanocytic neoplasms should be negative for these three glycoproteins. Therefore IHC studies against this group of adjunctive epithelial markers are useful supplements to others for keratin, EMA, and CEA in helping to exclude epithelial neoplasms when considering the differential diagnosis of melanoma. It can safely be stated that virtually all somatic carcinomas should be reactive for at least one of the membrane determinants cited earlier in this discussion, in addition to keratin, in contrast to those rare melanomas that focally express keratin but lack the other markers.

Placental-Like Alkaline Phosphatase

Placental-like alkaline phosphatase (PLAP) is an isozyme commonly synthesized by neoplastic germ cells and certain somatic epithelial malignancies. As such, it is a useful screening marker for gonadal tumors such as seminoma, embryonal carcinoma, and yolk sac carcinoma, all of which may enter the differential diagnosis of melanoma. In contrast, melanocytic proliferations are consistently negative for PLAP, although we have encountered a melanoma lesion with focal PLAP expression.

Hematopoietic Markers

Selected cell-surface antigens typically associated with hematopoietic cells and neoplasms also are potentially seen in melanocytic cells and neoplasms. Among others, these include CD10, CD44, CD56, CD57, CD59, CD68, CD74, CD99, CD117 (c-kit protein), CD146, class II major histocompatibility antigens (MHC2As; human leukocyte antigen [HLA]-DR, HLA-DP, and HLA-DQ), β-2-microglobulin (B2M), and Bcl-2 protein. These markers may be seen in some examples of Spitz nevus (epithelioid and spindle cell), architecturally disordered (dysplastic) nevus, and melanoma. Particularly interesting is the expression of CD10 by some melanocytic lesions because this marker is commonly expressed in atypical fibroxanthoma, a lesion in the differential diagnosis with spindle cell melanoma. Conversely, melanocytes uniformly lack other hematopoietic determinants that may enter into differential diagnostic evaluations of melanoma, such as terminal deoxynucleotidyl transferase (TdT), factor XIIIa, myeloperoxidase, and CD15, CD20, CD21, CD23, CD30, CD35, CD43, CD45, and CD138.

The expression of MHC2A proteins and B2M by melanocytic proliferations appears to be a property confined to inflamed intradermal or architecturally disordered nevi and occasional melanomas. Interestingly, mucosal melanomas (MMs) that escape immune surveillance have been noted to downregulate their expression of B2M and histocompatibility antigens over time through mutations in the corresponding gene complexes and other mechanisms. Because of the immunophenotypic heterogeneity seen in melanocytic lesions, as well as other tumors, for MHC2A and B2M, such determinants should not be used to distinguish or rule out a melanocytic lesion.

The ability of melanomas to express Bcl-2 protein and CD10, CD68, CD56, CD57, CD99, and CD117 creates a possible diagnostic pitfall in that such markers may lead the clinician to interpret these as lymphomas, histiocytic lesions, primitive neuroectodermal or neuroendocrine neoplasms, and gastrointestinal stromal tumors. As usual, the application of carefully constructed panels of antibody reagents,
The function of S100 has not been determined with precision; however, it is thought to function in intracellular calcium trafficking or microtubular assembly or both.\(^\text{37,38}\) It has a loose physiologic relationship to calmodulin, another calcium flux protein.\(^\text{34}\) Two subunits to S100, α and β, yield three possible dimeric forms: α-α, α-β, and β-β.\(^\text{39}\) Melanocytes synthesize only the first of these combinations. Immunoreactivity for S100 protein is both nuclear and cytoplasmic. Immunoelectron microscopic studies have confirmed that this marker is present in both intracellular compartments in normal and neoplastic melanocytes.\(^\text{40}\)

There are many antibodies against S100, some of which are dimer-specific monoclonal products.\(^\text{41}\) In general practice, most clinical laboratories still use heteroantisera that recognize all three isotypes of the protein against this marker, yielding a high-sensitivity screening tool. In this context, the author’s experience has been that more than 98% of melanomas can be labeled for S100 at least focally, regardless of histologic subtype, as long as the tissue is properly preserved (see below).

Smoller\(^\text{42}\) also reported a similar experience with S100 protein (97.4%). Other S100 protein–positive tumor types that enter into differential diagnosis include various carcinomas (e.g., breast carcinomas), selected histiocytic proliferations, gliomas, peripheral nerve sheath tumors (PNSTs), and Langerhans histiocytosis.\(^\text{40,42–46}\) It should be obvious, then, that S100 is most valuable in this setting as an initial screening reagent for melanocytic tumors rather than as a specific marker for such neoplasms.

Various isoforms of S100 protein (A2, A6, A8/A9, and A12) have also been considered diagnostically regarding various melanocytic lesions. Ribe and McNutt\(^\text{47}\) suggested that S100A6 was differentially expressed by Spitz nevi and melanomas. All Spitz tumors were A6 reactive, whereas only 33% of melanomas showed positivity. Moreover, differences in the scope of labeling were observed, with Spitz nevi being globally A6 reactive; in contrast, melanomas showed weak and patchy staining. Another possible application for this monoclonal marker (S100A6) is secondary to its expression in cellular neurothekeomas.\(^\text{48}\)

Possible pitfalls are related to S100 protein expression. The antibody S100 protein in formalin-fixed tissue may not work on frozen sections, alcohol-fixed tissue, or on formalin-fixed paraffin-embedded (FFPE) sections of previously frozen tissue. Furthermore, if the tissue is overfixed or underfixed, S100 expression may be impaired. At our institution, to optimize S100 (and other antigens) detection, we require for skin biopsies to be fixed in a manner similar to breast biopsies. The protocol requires at least 6 hours fixation in formalin for a maximum of 48 hours. As another pitfall, it has been suggested that S100 expression may be decreased in sun-damaged melanocytes.\(^\text{49}\)

Calretinin
Calretinin is a cytoplasmic 31-kDa protein first isolated from CNS tissues.\(^\text{50}\) It is also seen in peripheral nerves in the skin and elsewhere.\(^\text{51}\) Otherwise, this polypeptide is rather restricted in distribution, having been detected thus far only in mesothelium,\(^\text{50}\) germinal-surface epithelium of the ovary,\(^\text{50}\) and certain adenocarcinomas, most notably a subset in the colon, rectum, and skin.\(^\text{52–54}\) Also in the skin, some of the cells in the adnexa (eccrine and apocrine) may express this

\[\text{FIGURE 7.7} \text{ Intense nuclear and cytoplasmic immunoreactivity for S100 protein in primary cutaneous melanoma.}\]
antigen recognized by T-cells—was named specifically for that property; it is recognized by two monoclonal antibodies, A103 and M2-7C10, and is also known as Melan-A (Figs. 7.8 and 7.9).62–73

Nucleotide sequence analysis performed by Adema and colleagues59 has shown by alternative splicing that the cDNAs for gp100 and PMel-17 emanate from a single gene. This conclusion gained further support by their observation that gp100 and PMel-17 are consistently expressed concomitantly by both nonneoplastic and neoplastic melanocytic populations.74 The protein target of human melanoma black (HMB)-45 is probably a unique, premelanosome-related polypeptide.75,76

**Melanocytic Tumors**

Melanocytic tumors are not included in the list of neoplasms that show potential calretinin immunoreactivity.

**Melanocyte-Specific Monoclonal Antibodies**

Beginning in the early 1980s, monoclonal antibody technology was applied to a quest for a melanoma-specific reagent, not only for diagnostic, but also for therapeutic utility.55

A variety of antimelanocyte hybridoma products have been described, some of which are applicable to routinely processed tissue specimens.42 Products with activity restricted to frozen tissue substrates include PAL-M1 and PAL-M2, 691-13-17 and 691-15-Nu4B, and MEL (melanoma) series antibodies 1 through 4. In contrast, human melanosome-associated antigen (HMSA)-2, 2-139-1, 6-26-3, KBA62, 1C11, 7H11, MEL-CAM, MEL-5, and SM5-1 do have immunoreactivity with FFPE specimens and are therefore more useful in routine specimens.42,56,57 Products that have been used in a clinical context are considered in the following sections.

**gp100 and Pmel-17–Related Monoclonal Antibodies**

Several monoclonal antibodies were raised against a glycoprotein antigenic group restricted to cells of melanocytic lineage. This group is designated “gp100,” and its corresponding complementary DNA (cDNA) has been cloned.58,59 Two proteins are encoded by that nucleic acid sequence: gp100 itself, with a molecular weight of 100 kDa, and gp10, with a molecular weight of 10 kDa.59 The translational product related to gp100-C1 cDNA is closely homologous, but not identical, to yet another melanocytic protein, Pmel-17.60 Both are localized to the inner membranes of types 1, 2, and 3 premelanosomes; they similarly serve as potential targets for cytotoxic T lymphocytes, probably in concert with MHC2A.61

Indeed, one marker in this group—MART-1, melanoma antigen recognized by T-cells 1—was named specifically for that property; it is recognized by two monoclonal antibodies, A103 and M2-7C10, and is also known as Melan-A (Figs. 7.8 and 7.9).62–73

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Interestingly, gp100 transcripts are not specific for melanocytes and have been observed in other tissue types. Nonetheless, translation of the mRNA in question apparently occurs only in melanocytic elements. This finding strongly suggests that immunohistology is the most practical technologic method for assessment of gp100 as a melanocyte marker (Figs. 7.10 and 7.11) and that nucleic acid–based procedures, such as in situ hybridization (ISH) and polymerase chain reaction (PCR), are likely to be positive in other, nonmelanocytic pigment-producing cells.

Antibodies in the gp100/PMel-17 group include NKI-beteb and NKI/C3, HMB-45 and HMB-50, and MART-1/melan-A. These demonstrate variable levels of specificity and sensitivity for melanocytes, nevi, and melanomas vis-à-vis other cell lineages and tumor types. In practice, HMB-45 and MART-1 have thus far enjoyed the greatest use as agents to confirm the identity of S100 protein–positive neoplasms as melanocytic in nature.

Unfortunate peculiarities exist that relate to the commercial distribution of HMB-45. Dr. Mark Wick evaluated the original supernatant product from the HMB-45 clone, received as a gift from Dr. Allen Gown, one of the developers of the antibody, in the mid-1980s. He then found HMB-45 to be more than 95% sensitive for melanoma and essentially 100% specific for that diagnosis among non–spindle cell malignancies. Afterward, HMB-45 was sold to a commercial firm that marketed an impure form of the hybridoma product. It showed a much lower degree of specificity and was seen to label a variety of tumors other than melanomas. When other firms subsequently assumed distributorship of HMB-45, its specificity improved.

HMB-45, HMB-50, MART-1, and other gp100-related reagents can also label angiomyolipomas, lymphangioleiomyomatosis, clear-cell myomelanocytic “sugar” tumors of the lung, and perivascular epithelioid cell tumors (PEComas). These lesions manifest ultrastructural evidence of premelanosome synthesis and therefore have at least partial melanocytic differentiation. Hence, gp100-related antibodies are not manifesting cross-reactivity in labeling such pathologic entities; that affinity is merely a biologic extension of their specificity for premelanosome–associated proteins.

One true exception to the last statement is represented by the ability of MART-1/Melan-A (but not HMB-45, NKI-beteb, or HMB-50) to label the tumor cells of a subset of adrenocortical carcinomas and sex-cord tumors of the gonads. No evidence of true melanocytic differentiation has been seen in such neoplasms, and MART-1 antibodies are presumably recognizing an antigenic epitope in those steroidogenic proliferations, which is shared with the gp100/PMel-17 molecules.

**FIGURE 7.10** Labeling with HMB-45 in a nevus (A) and in a primary melanoma (B). The deep portion of the nevus has fewer positive cells than the upper portion (maturation). In contrast, both superficial and deep areas in a melanoma show similar numbers of positive cells (HMB-45, diaminobenzidine, and hematoxylin).

**FIGURE 7.11** Intense cytoplasmic positivity with HMB-45 in epithelioid melanoma metastatic to a lymph node (HMB-45 and diaminobenzidine).
Microphthalmia Transcription Factor Protein

The microphthalmia gene encodes a transcription factor that is necessary to the survival and development of melanocytes during embryogenesis. Microphthalmia transcription factor (MITF) is a nuclear, basic helix-loop-helix leucine zipper moiety that, with the PAX3 and MSG1 gene products, plays a role in controlling the activity of melanogenic enzymes by upregulating the cyclic adenosine monophosphate pathway.119 (Incidentally, another related gene, TFE, has similar properties, and its transcription factor may emerge in the future as an additional melanocyte marker.120)

These reagents are fairly sensitive and specific for the identification of melanocytic differentiation. As is true of most other markers in this general category, except for S100 protein, MITF typically labels epithelioid melanocytic lesions (Fig. 7.13) and, to a lesser degree, spindle cell/desmoplastic melanomas.121–123 Because of its nuclear localization, one situation in which we favor the use of

KEY DIAGNOSTIC POINTS OF THE GP100 ANTIBODY GROUP

- HMB-45 and Melan-A, both highly specific for melanocytic cell types, have sensitivities in the 60% to 80% range.
- The gp100 antibody group regularly reacts with cells of angiomyolipoma, sugar-cell tumors, and lesions of the PEComa group of neoplasms.
- Anti–Melan-A decorates adenocortical carcinomas and sex-cord tumors of the ovary.
- Only a minority of desmoplastic/spindle cell melanomas show reactivity with the gp100 antibody group.

Tyrosinase-Related Antibodies

In normal melanogenesis, the amino acid tyrosine is hydroxylated to form 3,4-dihydroxyphenylalanine (“dopa”), which is then oxidized to dopa-quinone. The latter moiety is polymerized to form melanin, thereafter combining with melanosprotein to form a stable complex within premelanosomes and melanosomes.108 Tyrosinase plays a central role in this process by catalyzing the first step in the stated sequence.109 As such, it is a specific marker for melanocytic differentiation. This premise has been affirmed by studies that show tyrosinase gene transcripts are confined to melanin-producing cells and therefore might be used to detect isolated tumor cells by PCR.110

T311 and monoclonal antityrosinase (MAT-1) are the two antityrosinase monoclonal antibodies that have been best analyzed in surgical pathology.111–118 The second of them is an immunoglobulin G (IgG) reagent raised by using a synthetic peptide, corresponding to the carboxy terminus of human tyrosinase as an immunogen.117 Both antibodies show a high level (>80%) of sensitivity and virtually absolute specificity for melanoma of the non–spindle cell type among all malignant tumors (Fig. 7.12). Interestingly, nevi are said to be nonreactive with MAT-1 in many instances, as are nonneoplastic melanocytes.116 Hence, the epitope it recognizes is presumably related to melanocytic maturation and differentiation. In light of that finding and because of the excellent specificity of antityrosinase antibodies, it would seem logical and permissible to use them diagnostically as mixtures, so-called cocktails. Thus in our institution, we use two such cocktails, one with anti–Melan-A, anti–MART-1, HMB-45, and antityrosinase and a second one with HMB-45 and antityrosinase.
MITF is in the analysis of intraepidermal melanocytic proliferations on sun-damaged skin. The nuclear pattern is relatively easy to distinguish from the melanin contained in the atypical melanocytes of pigmented actinic keratosis. Similar to other melanocytic markers, MITF is also positive in angiomyolipoma, lymphangioleiomyomatosis, and so-called sugar tumors. Based on this expression in at least a fraction of cells in desmoplastic melanoma, detection of MITF expression in such spindle cell lesions supports the diagnosis of melanoma over that of malignant PNST (Fig. 7.14). Interestingly, cellular neurothekeoma, a nonmelanocytic lesion that may enter the differential diagnosis of melanoma, also expresses MITF.

**p16**

Upregulation of p16\(^{INK4a}\) inhibitors melanocyte growth in culture, and it inhibits loss of replicative potential in melanocytic nevi. Oncogene-induced senescence seems to inhibit further nevus growth and thereby inhibits formation of cutaneous melanoma. Expression of p16 is preserved in most nevi, both standard and Spitz, and may be lost in melanomas (Fig. 7.15). However, its possible diagnostic utility to distinguish Spitz nevi from spitzoid melanoma has been questioned by Mason and colleagues. In our institution, we use p16 as a screening tool to determine the possible homozygous loss of p16 gene (9p21) to trigger fluorescence in situ hybridization (FISH) analysis.

**PNL2**

In 2003, Rochaix and colleagues described the clinical use of a new monoclonal antibody, PNL2, which had been raised against a fixative-resistant melanocyte antigen. In that study, which encompassed a spectrum of benign and malignant melanocytic lesions, the authors observed little if any labeling of banal melanocytes in the dermis, whereas junctional nevus cells were PNL2 positive. Melanomas were also immunoreactive in all cases, except for tumors with a desmoplastic appearance. PNL2 has also been tested against nonmelanocytic neoplasms, and it appears to have adequate specificity to justify its use in clinical settings.

**SOX9**

SOX9 is a transcription factor that participates in chondrocyte differentiation, originally described in chondrocytes. This marker is routinely expressed in cartilaginous lesions.

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**FIGURE 7.14** (A) Desmoplastic melanoma with epithelioid and spindle cells within dense stroma in the dermis. (B) Nuclear expression of microphthalmia transcription factor (MITF) in the deep dermis. ([B] MITF; anti-MITF with diaminobenzidine and hematoxylin.)

**FIGURE 7.15** Metastatic melanoma with nevoid features and involving the dermis. (A) Epithelioid cells in the dermis with focal cytologic atypia and apparent maturation (loss of pigment with depth in the dermis). (B) Loss of p16 expression. ([B] p16; anti-p16 with diaminobenzidine and hematoxylin.)
Soluble Adenylyl Cyclase

Soluble adenylyl cyclase (sAC) is an enzyme that generates cyclic adenosine monophosphate, a molecule involved in regulating melanocyte function. R21, a mouse monoclonal antibody against sAC, shows nuclear expression in melanoma in situ, lentigo maligna type, but is mostly negative in benign melanocytes. Thus it may be helpful in distinguishing melanoma in situ from melanocytic hyperplasia in sun-damaged skin.

Ki-67

Ki-67 is a nuclear marker expressed in proliferating cells. Its pattern of expression, similar to gp100, highlights the presence or absence of maturation. Common nevi and dysplastic nevi exhibit reactivity in less than 1% of cells, generally disposed at the dermal-epidermal junction or in the more superficial dermal compartment. In contrast, melanomas do not show this “maturation” pattern, but rather contain positive cells throughout the dermal component, with a mean proliferative fraction of more than 10%, particularly at the deep edge of the lesion (Fig. 7.18). Similarly, desmoplastic melanomas have a much higher proliferation rate, as detected with Ki-67 antibody, than do desmoplastic nevi.

Neuroendocrine Markers in Melanocytic Lesions

The association of both melanocytic and neuroendocrine proliferations with the neuroectoderm suggests that these neoplasms may demonstrate immunohistologic homologies. Proteins that are restricted anatomically to neurosecretory granules and neurosynaptic vesicles, such as chromogranin and synaptophysin, are occasionally present in melanocytic lesions. In addition, it is apparent that some lesions that are basically neuroendocrine or neuroectodermal neoplasms (e.g., neuroendocrine carcinomas, paragangliomas, PNETs, PNSTs) may exhibit a variable degree of melanocytic differentiation. These lesions include pigmented carcinoid,149,150 pigmented paraganglioma,151 melanotic neuroectodermal tumor,152,153 and melanotic schwannoma,154 both benign and malignant (Fig. 7.19). In these lesions, some of the cells have the same immunophenotype as cutaneous nevi or MMs (i.e., S100P+, HMB-45+, MART-1+, tyrosinase+) in addition to having monodifferentiated epithelial, neural, or schwannian cells. The latter cells lack melanocytic markers altogether and produce a mostly dimorphic and mutually exclusive immunophenotype.

SOX10

SOX10 is a transcription factor seen in cells from the neural crest. It is involved in maturation and maintenance of Schwann cells and melanocytes. SOX10 nuclear expression has been found in a majority of melanomas and nevi, in benign neural lesions, in most schwannomas and neurofibromas, and in almost half of malignant PNSTs. SOX10 appears to be strongly and diffusely expressed in spindle cell/desmoplastic melanoma and is therefore helpful in distinguishing melanoma from scar. A possible pitfall is its expression in some epithelial cells from eccrine and apocrine glands and in proliferating Schwann cells in dermal scars. In both situations, the presence of such positive cells in the scar of a prior procedure may be interpreted as invasive (desmoplastic) melanoma (Fig. 7.17).

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Other neuroendocrine determinants that may be seen in melanoma are, in fact, synthesized by a broad repertoire of cell types. They include, but are not limited to, melanocytes, neuroendocrine epithelial cells, neuroblasts, primitive mesenchymal neuroectodermal cells, and Schwann cells. The markers in question are principally represented by neuron-specific (γ dimer) enolase, neural-cell adhesion molecule (CD56), CD57, and CD99 (MIC2 protein).155-160 With the exception of CD99, these markers tend to be observed preferentially in melanocytic proliferations with neuroid features, such as neurotized intradermal nevi and desmoplastic/neurotropic melanomas.158 Because

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Several authors have expressed points of view against the performance of this technique. These authors question the utility of SLN because it has not been yet demonstrated that removal of SLN improves survival. These authors also indicate a paucity of treatments for patients with metastatic melanoma. However, SLN should be considered a procedure to help determine the prognosis of patients with cutaneous melanoma; therefore analysis of SLNs is important in the clinical management of patients with melanoma.

At our institution, we “bread-loaf” the SLN without performing frozen sections and then examine one hematoxylin and eosin (H&E) section. If that slide is negative, we cut three sections in the block (after ~200 µm), and then we stain one with H&E and another with a cocktail that includes four antibodies: anti–MART-1, A-103, HMB-45, and antityrosinase. We examine any possible positive cells in the immunoslides and determine whether they have morphology consistent with metastatic melanoma, possibly by comparing them with the cells from the primary lesion. The most important differential diagnosis is with isolated macrophages and capsular nevi. Some macrophages can be labeled with the antibodies used in the cocktail and therefore may constitute a diagnostic pitfall. In addition, as many as 20% of lymphadenectomies may contain benign melanocytes (nodal nevus) (Fig. 7.20). These cells are mostly located in the capsule but may also involve the parenchyma.

If the morphology is still unclear between melanoma cells and nevus cells, IHC may be helpful because most nodal nevi are negative for HMB-45 and have negligible proliferation rates with anti–Ki-67.

Most clinical studies confirm that examination of SLN provides significant prognostic information. Our study on 237 positive SLNs out of 1417 patients suggests a stratification in three groups with progressively worse prognoses: (1) involvement of one or two SLN and metastasis size of 2 mm or less in the largest nest and no ulceration in the primary...
in the deep reticular dermis are either negative or only weakly positive. This pattern recapitulates the pattern of maturation of nevi (see Fig. 7.10). A second, less frequent pattern is that of diffuse and strong labeling in blue nevi, cellular blue nevi, deep penetrating nevi, and some Spitz and Reed nevi; most Spitz and Reed nevi show patterns of maturation.

In nevi, Ki-67 is expressed in patterns similar to HMB-45 and thus shows maturation. Intraepithelial and superficial melanocytes express it, whereas deeply located melanocytes are negative for Ki-67. In contrast, melanomas express Ki-67 in a patchy manner throughout the lesion, without showing the zonal distribution of nevi maturation (see Fig. 7.18).

Proliferative Dermal Nodules in Congenital Nevi Versus Melanoma

Several papers have reported on the use of immunohistology for the separation of benign and malignant melanocytic lesions. Because no IHC markers are exclusively expressed in either nevus or melanoma, these markers are used in a scaled manner rather than for a positive/negative result. For example, HMB-45 labels melanocytic lesions in patterns different for benign and malignant melanocytic lesions. In nevi, HMB-45 shows two patterns: it most commonly labels the intraepithelial and superficial cells, with decreased labeling with depth in the dermis; cells located in the deep reticular dermis are either negative or only weakly positive. This pattern recapitulates the pattern of maturation of nevi (see Fig. 7.10). A second, less frequent pattern is that of diffuse and strong labeling in blue nevi, cellular blue nevi, deep penetrating nevi, and some Spitz and Reed nevi; most Spitz and Reed nevi show patterns of maturation.

In nevi, Ki-67 is expressed in patterns similar to HMB-45 and thus shows maturation. Intraepithelial and superficial melanocytes express it, whereas deeply located melanocytes are negative for Ki-67. In contrast, melanomas express Ki-67 in a patchy manner throughout the lesion, without showing the zonal distribution of nevi maturation (see Fig. 7.18).
power, it seems that PMNs are well demarcated from the adjacent, benign-appearing, small melanocytes, an intimate relationship exists between the two types of cells. In contrast, melanomas that arise in congenital nevi cells and often have a different stroma with more fibrosis and lymphocytic infiltrates. Herron and colleagues have systematically examined a series of congenital nevi that contain PMNs, finding that the tumor cells in those foci paradoxically expressed putatively mutant p53 protein and Bcl-2 protein, both of which are antiapoptotic, with Bax protein, a proapoptotic mediator. CD117 was also usually retained in PMNs as opposed to its relatively common absence in melanomas. The specified immunoprofile may therefore prove to be helpful in distinguishing PMNs in congenital nevi from malignant melanocytic lesions.

Primary Versus Secondary Intracutaneous Melanoma

Cutaneous melanoma has a well-known ability to metastasize back to the skin; when it does, that tumor may even demonstrate apparent intraepidermal growth and pagetoid spread. Although most melanoma metastases to the skin present as circumscribed dermal or subcutaneous nodules, unfortunately, no reliable morphologic features can distinguish primary melanoma from secondary so-called epidermotropic melanoma. Guerriere-Kovach and colleagues assessed a group of cases in which both of the latter possibilities were represented by using antibodies to Bcl-2 protein, mutant p53 protein, Ki-67, proliferating-cell nuclear antigen (PCNA), SMA, and CD117 (c-kit protein). Although some trends were observed toward greater labeling of metastatic melanomas for mutant p53 protein and Ki-67 but with diminution of CD117 reactivity, that pattern was not consistent.

In summary, at the present time, there is no absolute immunohistologic solution to this specific problem. It is thus necessary to establish clinical-pathologic correlation that should include determination as to whether single or multiple lesions are present, whether the lesion appeared recently, or whether it is located close to a previous melanoma. Then the clinician should look for possible vascular invasion, which is much more common in metastatic than in primary melanoma; obvious mitotic figures; and an irregular (patchy) pattern of HMB-45 and high proliferation with anti–Ki-67. Our opinion is that anti–MART-1 is very helpful in this differential diagnosis because this potential pitfall may be avoided by counting the bodies of the melanocytes regardless of the area immunolabeled. Another marker that may be helpful in this diagnosis is MITF. Because of its nuclear pattern of expression, it is relatively easy to distinguish positive cells (melanocytes) from adjacent, pigmented keratinocytes (Fig. 7.21).

Melanocytic Neoplasms Versus Histiocytic Proliferations

In the skin and elsewhere, it may be histologically difficult to separate amelanotic melanocytic lesions from histiocytic proliferations such as epithelioid histiocytoma, foam-cell–poor xanthogranuloma, atypical fibroxanthoma, and reticulohistiocytoma. Even though occasional histiocytic tumors may label for S100 protein, they are consistently nonreactive with HMB-45, HMB-50, MART-1, antityrosinase, and anti-MITF. In contrast, both histiocytic lesions and melanocytic neoplasms may be reactive for factor XIIIa and CD68, so-called histiocytic markers. We prefer CD163 as a marker of monocyte-macrophage differentiation because it is lacking in melanocytic proliferations. A possible pitfall is the interpretation of normal dendritic dermal cells that express S100 protein as part of the neoplasm, rather than stromal cells; and thus consider the lesion to be melanocytic. We have seen at least one case of an epithelioid fibrous histiocytoma diagnosed as melanoma based upon its epithelioid morphology and the presence of dermal mitotic figures (Fig. 7.22).

Another lesion of putative “fibrohistiocytic” differentiation is cellular neurothekeoma. These tumors usually are found on the head, neck, and upper extremities, more commonly in children and young women. Cellular neurothekeomas usually behave in a benign fashion regardless of the degree of cytologic atypia. These lesions are negative for S100 protein but are strongly positive for S100A6, NKI-C3, neuron-specific enolase (NSE), SMA, protein gene product 9.5 (PGP9.5), and MITF (Fig. 7.23).

Recognition of Rhabdoid and Sarcomatoid Malignant Melanomas

Rhabdoid and sarcomatoid melanomas include desmoplastic, myxoid, neurotropic, and osteochondroid subtypes, and these may demonstrate antigenic deletion or aberrancy. Others and we have seen rhabdoid melanomas that lack melanocytic markers while expressing keratin or desmin. Obviously, an extended panel of reagents is needed to support the diagnosis of these melanomas. Sarcomatoid melanomas are consistently S100 protein positive, but only 3% to 10% express other melanocytic markers (Fig. 7.24). Furthermore, such lesions may express CD56, CD57, nerve growth factor receptor, desmin, and actin isoforms (Figs. 7.25 and 7.26).

Amelanotic Melanoma Versus Other Epithelioid Malignancies

In reference to the expression of melanocytic markers in metastatic tumors, Plaza and colleagues have demonstrated...
Metastatic Melanoma Versus Malignant Glioma

In the CNS, metastatic amelanotic melanoma may closely imitate the microscopic appearance of a high-grade malignant glioma. This resemblance is further complicated by the common reactivity seen in both lesions for S100 protein, GFAP, and the potential for a small minority of melanomas to express GFAP as well. Hence, other melanocytic markers such as HMB-45, antityrosinase, and MART-1 are crucial to making this diagnostic distinction. All of those markers should be absent in pure glial neoplasms.

Melanoma Versus Soft Tissue Sarcomas

The ability of melanoma to simulate the appearance of various soft tissue sarcomas is also well documented. Among others, these include gastrointestinal stromal tumors (GISTs), epithelioid angiosarcoma, rhabdoid tumors, osteosarcomas, and PNETs. The detailed immunophenotypic properties of those lesions are provided elsewhere in this book. However, most sarcomas do not react for gp100-related melanocytic markers or tyrosinase.

A notable exception to that statement is represented by clear cell sarcoma of soft tissue (“melanoma of soft parts”), which clearly exhibits true melanocytic differentiation. Although the immunohistologic profile of that tumor and melanoma are superimposable, clear cell sarcoma regularly shows a t(12;22) chromosomal translocation apposing the
Immunohistology of Melanocytic Neoplasms

with all the histologic features of MPNSTs—dense fascicles of spindle cells with numerous mitotic figures, hemangiopericytoid vascular pattern, and prominent endothelial cells—but arising in congenital nevi and showing strong and diffuse S100 expression; as mentioned before, these two features are associated with melanoma.

Melanoma Versus Cutaneous Granular Cell Tumor

Melanoma is one of the lesions in the skin that may assume a granular cell composition along with cutaneous granular cell tumor (CGCT), basal cell carcinoma, angiosarcoma, and leiomyosarcoma. Gleason and Nascimento225 have compared S100 protein–positive CGCTs and melanomas regarding their immunoreactivity for S100 protein, MART-1, HMB-45, and MITF. Of these markers, HMB-45 was the best discriminator, followed closely by MART-1. On the other hand, MITF was commonly seen in both CGCT and melanoma.216 Calretinin and inhibin represent additional determinants that are selective for CGCT in this specific setting.

EWS and ATFI or a t(2;22) translocation that apposes the EWS and CREB genes.214–216

The most difficult diagnostic distinction to make in this context is that of sarcomatoid melanoma versus superficial malignant peripheral nerve sheath tumor (SMPNST). In many ways, the two are nearly identical.217 Obviously, a history of previous melanoma would be important information to have in such a case, but if that is unavailable, a diagnosis of SMPNST would be favored in lesions associated with a large nerve or preexisting neurofibroma, or when they occur in patients with neurofibromatosis.218 As mentioned earlier in reference to small cell lesions, a useful IHC clue in the distinction between spindle cell melanoma and SMPNST concerns the intensity and level of cellular labeling for S100 protein. If the neoplastic cells are only focally positive or negative for that marker, a diagnosis of SMPNST is favored. Both of these neoplastic categories share potential reactivity for nestin, p16 (cINK4a protein), CD10, CD99, Bcl-2 protein, and Wilms tumor 1 (WT-1) protein.219–224 Furthermore, to illustrate the close relationship between these two lesions of neuroectodermal derivation, we have observed tumors

![Image: Epithelioid fibrous histiocytoma resembling melanoma. (A and B) Hematoxylin and eosin images of epithelioid cells with irregular nuclei. (C) Expression of factor XIIIa. (D) Expression of CD68 by many tumor cells. ([C and D] Antifactor XIIIa and anti-CD68 with diaminobenzidine and hematoxylin.)](image-url)
entrance into the vertical-growth phase of melanoma; CD26/dipeptidyl-aminopeptidase IV, a membrane-bound protease that assists tumor cell invasion; osteopontin, an adhesive matricellular glycoprotein that upregulates metalloproteinase production; NM23, a metastasis-suppressor gene product; Cdc42 and CXCR4 proteins, moieties that influence cellular motility; E-cadherin, a protein concerned with cell-matrix interaction; and cyclin-D1, cyclin-D3, Trk-A, and p16INK4-α (CDKN2A), gene products (cell-cycle regulators). These markers have been reported to be associated with decreased survival: decreased p16, increased p21; increased

**Prognostic Markers and Targeted Therapy for Melanoma**

IHC has been proposed as an adjunct technique for evaluating primary and metastatic melanomas regarding prognosis. Among the markers studied are p53 protein, a promoter of programmed cell death; Ki-67, a proliferating cell nuclear antigen, and Ki-S5, cell-cycle–related indicators of proliferation; heat shock proteins, markers of replicating or “activated” cells; Bcl-2 protein, an inhibitor of apoptosis; VLA-4 and α-v/β-3 integrins, intercellular adhesion molecules that correlate with
angiogenesis is correlated with an adverse prognosis.\textsuperscript{241,242} Furthermore, anti–D2-40 has been used to detect lymphatic space invasion, and it correlates with positivity in the SLN and with decreased disease-free survival.\textsuperscript{243} Further analysis is necessary to determine whether any of these markers should be used along with the current histopathologic features included in the AJCC: Breslow thickness, ulceration, and mitotic count.

Probably the most exciting application of IHC is to help detect mutations suitable for targeted therapy. The first target was c-kit, a member of the protein tyrosine kinases (PTKs). This protein, also essential in acute myelogenous leukemia and GISTs, is mutated or overexpressed in a number of melanomas, particularly in acral lentiginous and mucosal melanomas.\textsuperscript{244} Thus some of these patients have responded to antikit therapies such as imatinib (Gleevec). IHC may be used to try to detect those melanoma cases that overexpress c-kit but are lacking the mutations commonly analyzed.\textsuperscript{245} Other targets currently being studied are AKT, MEK/MAPKK, MAPK, BRAF, and mTOR. Of these, BRAF inhibitors have already demonstrated therapeutic effectiveness. Recently, an antibody that detects the BRAF V600E mutation may help in the selection of patients eligible for targeted therapy.\textsuperscript{246}

\textbf{Summary}

The many faces of malignant melanoma pose a serious diagnostic dilemma to the pathologist, especially when presenting as an undifferentiated tumor. IHC can be used as a diagnostic aid in primary melanoma versus benign melanocytic lesions to report prognostic factors and, in the near future, to report potential theranostic parameters.

\begin{itemize}
  \item A complete reference list can be found online at ExpertConsult.com.
\end{itemize}

\section*{Selected References}


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