Comparison of Five Antibodies as Markers in the Diagnosis of Melanoma in Cytologic Preparations

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Abstract

We determined the sensitivity and specificity of 3 novel antibodies (microphthalmia transcription factor [Mitf], Melan-A, and tyrosinase) as markers for melanoma in cytologic preparations and compared the results with those of commonly used markers (S-100 protein [S-100] and HMB-45). We stained 72 cell blocks from 40 patients with melanoma and 32 with nonmelanocytic malignant neoplasms with antibodies against S-100, HMB-45, Mitf, Melan-A, and tyrosinase. Histologic correlation was available in more than 95% of cases. Nuclear staining for Mitf and cytoplasmic staining for S-100, HMB-45, Melan-A, and tyrosinase in more than 10% of tumor cells was considered positive. All 3 novel markers demonstrated sensitivity superior to S-100 and HMB-45. Mitf, Melan-A, and tyrosinase were less specific. Sensitivity and specificity for the combination Mitf+/Melan-A+ were 95% and 100%, respectively, whereas they were 80% and 100%, respectively, for S-100+/HMB-45+. Mitf, Melan-A, and tyrosinase are sensitive markers for epithelioid melanoma. Mitf and Melan-A seem more specific than S-100 and tyrosinase. An antibody panel consisting of Mitf and Melan-A is superior to a panel of S-100 and HMB-45 in the diagnosis of melanoma in cytologic specimens.

The cytologic diagnosis of metastatic melanoma can be challenging. Melanoma often manifests with a diverse cytologic appearance that may include a dyshesive single cell pattern or a cohesive cellular arrangement. The cell shape varies from epithelioid to spindled, or a mixture of epithelioid and spindle cell patterns might be seen. Furthermore, the cytologic features of melanoma often are shared with other poorly differentiated malignant neoplasms, including carcinomas, lymphomas, and sarcomas. In addition, metastatic melanoma can be found anywhere in the body and may manifest with a myriad of clinical signs and symptoms. Nevertheless, it is important to differentiate melanoma from nonmelanocytic malignant neoplasms because prognosis and therapy differ radically among these entities.

Immunocytochemical studies often are used as an aid in the diagnosis of melanoma. The most frequently used melanocytic markers in clinical practice are S-100 protein and HMB-45. Monoclonal antibody to S-100 protein, a calcium binding F-hand protein originally isolated from the brain, is a sensitive marker that reacts with more than 90% of melanomas. However, this protein also is present in adipocytes, chondrocytes, Schwann cells, and myoepithelial cells. Tumors derived from these tissues usually retain immunoreactivity to S-100 protein. Thus, S-100 protein reacts with a broad range of benign and malignant neoplasms, therefore limiting its specificity as a melanocytic marker. In addition, certain epithelial neoplasms such as mammary carcinoma may be positive for S-100 protein. Monoclonal antibody against HMB-45 antigen recognizes melanosome-specific gp100. Although it is quite specific for melanocytic neoplasms, HMB-45 is less sensitive than S-100 protein for identifying melanoma. Some melanomas,
particularly the spindle cell and desmoplastic variants, fail to react with HMB-45. In addition, HMB-45 has been shown to react with other neural crest–derived tumors and occasionally with adenocarcinomas and other neoplasms.

Several new monoclonal antibodies raised against melanocytic differentiation antigens have become available for routine diagnostic use. These markers, which include microphthalmia transcription factor (Mitf; clone D5), tyrosinase (clone T311), and Melan-A (or MART-1; clone A103), are thought to be specific for melanocytic lesions. Mitf is a nuclear transcription factor critical for melanocyte development in the embryo and for survival of these melanocytes postnatally. Tyrosinase is a key enzyme involved in the early stages of melanin production and is present in cells of melanocytic lineage. Melan-A protein is a product of the MART-1 gene that is recognized by autologous cytotoxic T cells. It is a cytoplasmic protein that is expressed in mature melanocytes; however, its biologic function is unknown.

In a previous study, Dorvault et al demonstrated that Mitf was a sensitive and specific marker for melanomas in cytologic specimens and might be superior to the presently used melanocytic markers, S-100 protein and HMB-45. The purpose of the present study was to compare the diagnostic usefulness of the other novel melanoma markers, namely, tyrosinase and Melan-A with Mitf and with S-100 protein and HMB-45 as markers for melanoma in cytologic preparations.

Materials and Methods

We retrieved 72 formalin-fixed, paraffin-embedded cell blocks from 40 patients with melanoma (2 cases of spindle cell melanoma) and 32 patients with nonmelanocytic malignant neoplasms (carcinoma, 25; mesothelioma, 4; lymphoma, 2; islet cell tumor, 1) from the files of the Departments of Pathology at the University of Alabama at Birmingham and New York University Medical Center, New York, NY. The primary sites of the carcinomas were as follows: ovary, 7; lung, 6; kidney, 4; breast, 3; colorectal, 3; liver, 1; and uterine cervix, 1. These cases were retained from a previous study. Corresponding histologic material was available for 69 cases. Three cases of melanoma were from patients with disseminated metastatic disease. Among the melanomas, specimen sources were fine-needle aspiration biopsy of the following: lymph nodes, 15; lungs, 9; liver, 6; subcutaneous nodules, 6; orbital mass, 1; and pancreas, 1; and from pleural and peritoneal fluid, 1 each. Among the nonmelanocytic malignant neoplasms, the specimen sources were fine-needle aspiration biopsy of the following: kidney, 6; liver, 6; lung, 2; and pancreas, 1; and from pleural fluid, 10; and peritoneal fluid, 7.

Immunocytochemical analysis was performed using standard protocols. Briefly, 5-µm sections were cut, placed on electrostatically charged glass slides, and deparaffinized. Staining for Mitf was performed using the monoclonal antibody to Mitf (D5, undiluted, provided by D.E.F.) obtained from tissue culture supernatant. For Mitf, heat-induced epitope retrieval was accomplished by microwave heating for 10 minutes in a 0.01-mol/L concentration of citrate buffer, pH 6. Immunostaining then was accomplished manually by using a modified avidin-biotin peroxidase technique. Staining for the remaining primary antibodies was performed using the Ventana ES automated immunohistochemistry system and the Ventana DAB Detection Kit (Ventana Medical Systems, Tucson, AZ). The primary antibody used included antibodies to S-100 protein (clone S-100, Ventana Medical Systems), HMB-45 antigen (clone gp100, Ventana Medical Systems), tyrosinase (clone T311, Neomarkers, Fremont, CA), and Melan-A (clone A013, Neomarkers). All 4 antibodies were prediluted and incubated with the tissue section for 1 hour at room temperature. Counterstaining was performed with hematoxylin. Appropriate positive and negative controls were used; the latter was achieved by omitting the primary antibodies. All cases were examined independently and blindly by 3 observers (Mitf reviewed by C.C.D., K.N.W., and D.C.C.; S-100 protein and HMB-45 by C.C.D., H.Y., and D.C.C.; tyrosinase and Melan-A by M.V.S., H.Y., and D.C.C.). Nuclear staining with Mitf in more than 10% of tumor cell nuclei was considered positive, whereas the presence of cytoplasmic staining in more than 10% of the tumor cells was considered positive staining with S-100 protein, HMB-45, tyrosinase, and Melan-A.

Results

Results are summarized in Table 1. Positive nuclear staining with antibodies directed against Mitf was present in all 40 (100%) melanoma cases. In 36 (90%) of these cases, the staining was intense and present in more than 50% of the tumor cells. Weak cytoplasmic staining with anti-Mitf was noted occasionally in histiocytes. Positive cytoplasmic staining with anti-tyrosinase and anti–Melan-A was noted in 39 (98%) and 38 (95%) of melanoma cases, respectively. Staining was noted in more than 50% of the tumor cells in 33 and 36 cases with Melan-A and tyrosinase, respectively. Positive staining with anti–S-100 protein and anti–HMB-45 was identified in 35 (88%) and in 37 cases (92%), respectively. Both spindle cell melanomas in our study marked with S-100 protein, HMB-45, Mitf, and Melan-A. Only 1 of 2 spindle cell melanomas stained positively with tyrosinase. All melanomas were immunoreactive with at least 2 of the 5
markers. Immunostaining results for HMB-45, Mitf, Melan-A, and tyrosinase were discordant in 4 (10%) cases. Results for these cases were as follows: HMB-45−, Mitf+, Melan-A+, and tyrosinase+, 2 cases; HMB-45−, Mitf+, Melan-A−, and tyrosinase+, 1 case; and HMB-45+, Mitf+, Melan-A−, and tyrosinase−, 1 case.

Focal nuclear staining for Mitf was identified in 1 (3%) of 32 cases of nonmelanocytic malignant neoplasms. This false-negative case was a metastatic breast adenocarcinoma involving the pleural cavity. However, the staining was weak and present in about 10% of the tumor cells. One nonmelanocytic malignant neoplasm that was a renal cell carcinoma stained focally positive for Melan-A. Tyrosinase staining was seen in 5 nonmelanocytic neoplasms that included 2 renal cell carcinomas, 1 lung carcinoma, 1 mesothelioma, and 1 colon carcinoma. All demonstrated less intense staining compared with that of the melanomas. Cytoplasmic staining for S-100 protein was identified in 10 (31%) nonmelanocytic malignant neoplasms that included 4 ovarian adenocarcinomas, 3 adenocarcinomas of the lung, 1 mesothelioma, 1 colonic adenocarcinoma, and 1 mammary adenocarcinoma. Positive staining with anti HMB-45 antigen was noted in 1 (3%) nonmelanocytic malignant neoplasm, a renal cell carcinoma. Except for 1 renal cell carcinoma that stained for both HMB-45 and Melan-A, none of the nonmelanocytic malignant neoplasms stained with more than 1 of the 5 markers.

The sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio for S-100, HMB-45, Mitf, Melan-A, and tyrosinase for melanoma are summarized in Table 2. Mitf, Melan-A, and tyrosinase as markers for melanoma demonstrated a higher sensitivity compared with that for HMB-45. However, only Melan-A and Mitf had a specificity comparable to that of HMB-45. The likelihood that a tumor that stained positive with Mitf was a melanoma was 90:1, and the likelihood with Melan-A was 75:1.

The sensitivity and specificity of the immunoprofile Melan-A+/Mitf+ were 95% and 100%, respectively. The likelihood that a tumor with the former profile was a melanoma was 84:1. The results were better than the results for the S-100+/HMB-45+ immunoprofile in which the sensitivity was 80%; the specificity, 100%; and the likelihood ratio, 59:1.
Discussion

The 2 antibodies used most frequently in the clinical setting to confirm the cytologic diagnosis of melanoma are those directed against epitopes on S-100 protein and HMB-45. S-100 protein is a sensitive marker for melanomas, including amelanotic and spindle cell variants. However, anti–S-100 protein often reacts with a host of nonmelanocytic tumors that may share cytomorphologic features with malignant melanomas.8,9,11,24 In the present study, S-100 protein, which was a polyclonal antibody, was detectable in almost one third of nonmelanocytic neoplasms, resulting in a specificity of 70% for diagnosing melanomas. We also observed a relatively low sensitivity (88%) of S-100 protein in the recognition of melanomas. However, the reason for this observation is unclear.

HMB-45 is a more specific marker for melanoma than S-100 protein. Our experience supports this contention; the specificity of HMB-45 for detecting melanoma was 96.9% and, thus, considerably higher than that of S-100 protein. However, HMB-45 is detectable in only 50% to 75% of all melanomas.15,25 Therefore, it is less sensitive as a marker for melanoma than S-100 protein. In addition, spindle cell and desmoplastic melanomas tend to be nonreactive with anti–HMB-45.16,17

Anti–Melan-A (A103), antityrosinase (T311), and anti-Mitf (D5) are 3 recently discovered monoclonal antibodies raised against melanocyte-specific antigens. The gene that encodes Melan-A (or MART-1) encodes a melanocytic antigen that is expressed in skin, retina, and melanocytic cell lines but not in other normal tissues.21,26 Two monoclonal antibodies, A103 and M2-7C10, have been raised against the

Table 2
Comparison of the Sensitivity, Specificity, Positive and Negative Predictive Values, and Likelihood Ratio of S-100, HMB-45, Mitf (D5), Melan-A (A103), and Tyrosinase (T311) as Markers for Diagnosing Melanomas

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Mitf, microphthalmia transcription factor.
*Except for likelihood ratios, values are given as percentages. Clones A103 and T311 from Neomarkers, Fremont, CA.
Melan-A protein. In the present study, we used the antibody A103 partly because of its more frequent use in practice and, thus, more frequent evaluation in the literature between the 2 antibodies and partly because of its known ability to stain more cells than M2-7C10. In our study, A103 was a sensitive and specific marker for melanoma. The only nonmelanocytic malignant neoplasm that was positive for A103 was a metastatic renal cell carcinoma; the staining was focal and less intense than that for melanoma. The mechanisms modulating this immunoreactivity are unknown. Other investigators have not reported the presence of any similar staining in nonmelanocytic, non–steroid-producing neoplasms.

Several investigators have demonstrated that A103 reacts with adrenocortical adenomas and carcinomas as well as testicular Leydig cell tumors and ovarian Sertoli-Leydig tumors. The most likely explanation for positive staining of steroid-producing cells with A103 is cross-reactivity with a similar epitope on another protein since no immunoreactivity to steroid-producing tumors has been reported using another Melan-A antibody, M2-7C10.

Tyrosinase, a copper-containing enzyme in the melanosomal membranes, is essential for the production of melanin. T311 is a monoclonal antibody that recognizes tyrosinase. Using either cytologic preparations or tissue sections, staining with antityrosinase was noted in 85% to 92% of malignant melanomas analyzed. Our experience with antityrosinase in recognizing melanomas paralleled that of others. Of 40 malignant melanomas, 39 demonstrated positive staining with antityrosinase. However, our observation of antityrosinase with nonmelanocytic neoplasms was quite different from that previously reported; 16% (5/32) of such neoplasms demonstrated focal staining with antityrosinase. Kaufmann et al did not report any staining of antityrosinase in any of the 146 nonmelanocytic neoplasms examined. In a more recent study, 2 adenocarcinomas, 1 of lung and 1 of colonic origin, demonstrated weak focal reactivity in the mucin and adjacent connective tissue. The possibility that immunoreactive stromal cells were interpreted as melanocytic cells could not be excluded completely.

The microphthalmia gene (Mitf) is essential to the development and survival of melanocytes. Biochemical studies revealed that Mitf was able to transactivate several gene promoters, including those that regulated the pigmentation enzyme genes tyrosinase, TRP 1, and TRP 2. We and others have demonstrated that monoclonal antibody to Mitf (D5) was a sensitive marker for epithelioid melanomas; 88% to 100% of melanomas were positive for the anti-Mitf. It also is a specific marker for epithelioid melanoma. King et al and Miettinen et al found no significant immunoreactivity in 81 and 192 nonmelanocytic neoplasms, respectively, when immunostained with anti-Mitf. We observed 1 case of metastatic breast carcinoma involving the pleural fluid that showed weak nuclear staining with anti-Mitf in about 10% of the cells. It is noteworthy that King et al also reported that 2 nonmelanocytic tumors showed cytoplasmic but not nuclear staining with Mitf. Both were breast carcinomas. The significance of nuclear and/or cytoplasmic staining with Mitf in breast carcinoma is uncertain. Many breast carcinomas secrete factors, including cathepsin K, that are involved in bone resorption. It was shown that cathepsin K messenger RNA and protein were deficient in Mitf mutant osteoclasts, and overexpression of wild-type Mitf protein dramatically up-regulated expression of endogenous cathepsin K in cultured human osteoclasts. These findings suggested that cathepsin K expression was regulated by Mitf. In a recent study that analyzed Mitf expression in 386 nonmelanocytic tumors, the authors reported positive Mitf staining in 32 (8.3%) of these tumors. These findings contradict our observations and those of others, which may be attributed to the difference in the duration of incubation with primary antibody, overnight vs 1 hour. Similar to HMB-45, both Melan-A and Mitf stain angiomyolipomas positively.40-53

One diagnostic challenge is to differentiate spindle cell and desmoplastic melanomas from other spindle cell lesions. The former frequently exhibits positive staining with S-100 protein but tends to be negative for HMB-45. However, as previously noted, positive S-100 protein immunostaining is of limited value because many nonmelanocytic spindle cell lesions, including Schwannomas, neurofibromas, and malignant peripheral nerve sheath tumors, also may be immunoreactive. Our series included 2 melanomas with spindle cell features; they were positive for both Mitf and Melan-A, but only 1 was positive for tyrosinase. A recent study showed that antityrosinase (T311) did not react with any of the 60 nonmelanocytic spindle cell lesions examined. However, only 1 of 3 spindle cell melanomas and 1 of 8 desmoplastic melanomas were immunoreactive with antityrosinase. Similarly, Melan-A also lacks sensitivity in the recognition of spindle cell melanocyes; the proportion of spindle cell and desmoplastic melanomas that were immunoreactive with Melan-A ranged from 15% to 33%. The literature on the immunoreactivity of Mitf in melanocytic and nonmelanocytic spindle cell lesions remains confusing. King et al reported that all 9 spindle cell melanomas demonstrated positive D5 staining. In a more recent study, Koch et al reported that 55% (11/20) of spindle cell and desmoplastic melanomas were immunoreactive for Mitf, but none of the 12 malignant peripheral nerve sheath tumors and only 2 of 10 neurofibromas were positive. These authors commented that the sensitivity and specificity of Mitf for spindle cell and desmoplastic melanomas equaled or exceeded that of HMB-45. Other investigators, however, were unable to reproduce these observations. In 2 studies, one third to one half of nonmelanocytic spindle cell
tumors and only 30% of spindle cell and desmoplastic melanomas were reactive for Mitf.\textsuperscript{50,55} Miettinen et al\textsuperscript{43} reported that only 1 (3%) of 30 desmoplastic melanomas demonstrated Mitf reactivity. Based on these observations collectively, Mitf seems to be neither a sensitive nor a specific marker for spindle cell and desmoplastic melanomas. However, because of these conflicting results, further study is necessary to resolve this issue.

The standard of practice in diagnosing melanoma is to use a panel of antibodies consisting primarily of S-100 protein and HMB-45. Based on our experience and that of others, it is logical to conclude that a new panel consisting of Melan-A (A103) and Mitf (D5) would be superior to the existing one for diagnosing epithelioid melanomas. For spindle cell and desmoplastic melanomas, however, S-100 protein and HMB-45 should remain within the antibody panel since S-100 protein is the most sensitive marker for these entities and evidence is lacking that either Melan-A or Mitf are better markers than HMB-45 for recognizing spindle cell melanocytic lesions.

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