Immunophenotypic Differences Between Uveal and Cutaneous Melanomas

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Objective: To determine the immunophenotypic differences between uveal and cutaneous melanomas, employing standard melanoma markers as well as p75 neurotrophin receptor (p75NTR) and microphthalmia transcription factor (MITF).

Design: Fifteen uveal melanomas (5 spindle, 5 epithelioid, and 5 mixed uveal subtypes) were immunolabeled with a panel of antibodies that included S100, tyrosinase, melan-A, HMB-45 and HMB-50 combination, MITF, and p75NTR. The results were tabulated on the basis of intensity and pervasiveness of the labeling and compared with a prior study on cutaneous spindle and epithelioid melanomas.

Results: In contrast to its strong labeling of cutaneous melanomas, S100 immunolabeling of uveal melanomas was weak and variable. p75NTR, known to differentiate spindle from epithelioid melanomas of the skin, did not immunolabel uveal melanomas. HMB-45, HMB-50, tyrosinase, melan-A, and MITF immunolabeled all uveal melanomas strongly, irrespective of the histologic subtype, but not cutaneous melanomas. Microphthalmia transcription factor was especially clear in its labeling of uveal melanomas.

Conclusions: Although cutaneous and uveal melanomas share many molecular markers in common, there are differences between the 2 types of melanoma. First, the level of expression of S100 differs between cutaneous and uveal melanomas. Second, while cutaneous melanomas can be further subdivided into spindle and epithelioid types based on their immunophenotype, the uveal melanomas cannot.

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IN 1931, CALLENDER proposed a classification of uveal melanomas based on 2 major cell types, spindle and epithelioid. This classification has been demonstrated to be a valuable indicator of prognosis. Patients with spindle melanoma cells exhibit a better clinical course than those with epithelioid cell types. Over the years, numerous modifications of the Callender classification have been published, as thoroughly reviewed by McLean.1 While tumor size, mitotic activity, tumor-infiltrating lymphocytes, age, and tumor vascular pattern2 have prognostic value, the Callender classification by cell type as modified by the Armed Forces Institute of Pathology remains one of the most reliable prognostic determinants.3 More recently, Moshari and McLean4 reported that the mean length of the longest nucleoli measured on silver-stained sections has a predictive value as reliable as cell type. A similar tendency of nucleolar size was detected by electron microscopy in 1972.5

Cutaneous melanomas can also be classified into spindle and epithelioid cell types. Furthermore, studies have revealed distinct differences in the immunophenotype between spindle and epithelioid melanomas, especially when cells are labeled by the antibody to the p75 neurotrophin receptor (p75NTR).6,7 With the exception of S100, standard melanoma markers, including HMB-45, HMB-50, tyrosinase, and melan-A, are strongly expressed in epithelioid cutaneous but are poorly expressed in spindle cutaneous melanomas.8-11 In contrast, certain Schwann cell markers, particularly the p75NTR, are strongly expressed in spindle cutaneous but not in epithelioid melanomas.6 Microphthalmia transcription factor (MITF) is also preferentially expressed in epithelioid but not spindle subsets.12

Prior immunohistochemical studies of uveal melanomas have involved S100,13-19 HMB-45,20,21 melan-A,19,22 and tyrosinase.22 There have been reports suggesting differences between uveal and cutaneous melanomas based on S100 labeling,15,16 but to our knowledge, there has been no systemic side-by-side comparison using a large panel of antibodies.
We sought to determine whether the differences in immunophenotype between spindle and epithelioid cells noted in cutaneous melanomas6,7 were also applicable to uveal melanomas. Our hope was that such studies could be helpful in the cellular classification and perhaps have prognostic value in uveal melanomas.

We examined the immunophenotype of 5 spindle, 5 epithelioid, and 5 mixed uveal melanomas. Detailed analyses of immunolabeling were performed based on signal intensity and pervasiveness. We compared the results with our recent report of the immunophenotype of spindle and epithelioid cutaneous melanomas. A comparison was made for the expression of S100, melan-A, HMB-45 and HMB-50, tyrosinase, MITF, and p75NTR in the 3 categories of uveal melanomas. This is the first report, to our knowledge, of p75NTR expression in uveal melanomas.

RESULTS

A total of 15 uveal melanomas, including 5 spindle, 5 epithelioid, and 5 mixed types, were immunolabeled with a panel of antibodies that included p75NTR, S100, HMB-45, HMB-50, tyrosinase, melan-A, and MITF. The mean±SEM values of the intensity and of the pervasiveness are shown with our previous results from the immunophenotypic differences in expression patterns between epithelioid and spindle melanomas of the skin6 (Table and Figure 2). The cutaneous melanoma results for all markers except MITF are presented in Figure 2 and the Table for comparison purposes only.

Nontumor ocular tissue from tumor-containing eyes served as internal controls. We also labeled 6 normal eye specimens (with no ocular pathology) to establish normal immunolabeling patterns. For MITF, the positive internal controls included the retinal pigment epithelium and the variable labeling of stromal cells of the ciliary body and choroid. For melan-A, variable but distinct staining of the stromal cells of the iris, ciliary body, and choroid. For melan-A, variable but distinct staining of the stromal cells of the iris, ciliary body, and choroid. For melan-A, variable but distinct staining of the stromal cells of the iris, ciliary body, and choroid.

We examined the tissues in the paraffin sections using a 20× objective and indirect immunoperoxidase staining with DAB chromogen (Vector Laboratories, Inc, Burlingame, Calif). The immunolabeled specimens were interpreted according to the intensity and the pervasiveness of the immunocytochemical staining. The intensity was ranked on a scale of 0 to 3 as follows: 0=absence of labeling; 1=focal or less than 10% of cells; 2=patchy or 10% to 75% of cells; and 3=diffuse or more than 75% of cells. Internal positive controls are discussed in the “Results” section. Agreements of scores were independently rated by at least 2 authors. Four of the authors had been involved in the cutaneous melanoma study with which this set of data has been compared. Agreement of scores was reached in all cases. Data are given as mean±SEM. Results were graphed for comparison purposes and placed side by side with corresponding results of spindle and epithelioid cutaneous melanoma as reported previously.

PHOTOGRAPHY

For Figure 1, images of labeled slides were directly collected using a CoolSnap digital camera (R. S. Photometrics, Tucson, Ariz) attached to a Leitz Orthoplan (Leica Microsystems Inc, Bannockburn, Ill) microscope (magnification to the charge-coupled device chip >27.5). Images were saved as 1392×1040 24 bit RGB TIFF (tagged image file format) files. The figure was assembled in Adobe Photoshop 5 (Adobe Systems Inc, San Jose, Calif) with minimal contrast and brightness enhancement.

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Five-micrometer formalin-fixed, paraffin-embedded sections were obtained from the archived blocks, dehydrated, rehydrated, and treated with 3% hydrogen peroxidase. For S100 labeling, nonspecific binding was blocked by incubating slides with normal goat serum for 10 minutes. Following heat-induced epitope retrieval in 10 mM of sodium citrate at a pH of 6.0, slides were immunolabeled with the following antibodies for 40 minutes at room temperature: p75NTR antibody, 1:200 (mouse monoclonal MS-394-Pi; Neomarkers, Freemont Calif); S100, 1:8000 (Z311; Dako, Carpinteria Calif); MITF, 1:25 (a gift from David Fisher, MD, PhD, Dana Farber Cancer Institute, Boston, Mass). After several rinses with phosphate-buffered isotonic sodium chloride solution (PBS), slides were incubated with biotinylated secondary antibodies for 25 minutes. The slides were then rinsed in PBS followed by incubation with avidin-biotin complex (Vector Elite, Burlingame) for 25 minutes at room temperature. Binding was visualized using 3,3′diaminobenzidine as a chromogen, with nickel chloride enhancement and a methyl green counterstain. For negative controls, we used substituted diluted normal rabbit serum for the primary antibody. Some of the uveal melanoma specimens were immunolabeled concurrently with some of the cutaneous melanoma specimens, although the results of the cutaneous melanomas have been reported separately.

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receptor labeling, the peripheral nerves of the iris and ciliary body as well as the Müller glial cells of the retina were used as the internal positive controls.

p75NTR was absent in all of the choroidal melanoma specimens, which was in striking contrast to its labeling of spindle melanomas in the skin. S100 labeled 3 of 5 spindle melanomas with an intensity of 0.6±0.24 and a pervasiveness of 0.8±0.37. In the epithelioid melanomas, 4 of 5 specimens were labeled with an intensity of 1.2±0.37 and a pervasiveness of 1.4±0.4. In the mixed melanomas, 5 of 5 specimens were labeled with an intensity of 1.4±0.24 and a pervasiveness of 2.1±0.18. HMB-45 AND HMB-50 labeled 5 of 5 specimens in the spindle melanomas with an intensity of 2.8±0.2 and 2.7±0.2 for pervasiveness. For the epithelioid melanomas, 5 of 3 specimens were labeled with an intensity of 2.5±0.22 and a pervasiveness of 2.8±0.2. In the mixed melanomas, 5 of 5 specimens were labeled with an intensity of 3.0 and a pervasiveness of 2.6±0.24.

Figure 1. Photomicrographs of a spindle uveal melanoma illustrating immunophenotypic labeling patterns. Note the complete absence of p75NTR (neurotrophin receptor) immunolabeling and the relatively low level of S100 immunolabeling. In contrast, HMB-45, tyrosinase, melan-A, and MITF (microphthalmia transcription factor) all show extensive immunoreactivity of the tumor (original magnification ×27.5). H&E indicates hematoxylin-eosin.

Relative Immunophenotypic Expression Patterns of Cutaneous and Uveal Melanomas*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cutaneous Spindle</th>
<th>Cutaneous Epithelioid</th>
<th>Uveal Spindle</th>
<th>Uveal Epithelioid</th>
<th>Uveal Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intensity</td>
<td>Pervasiveness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p75 NTR</td>
<td>2.9 (0.06)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>S100</td>
<td>3.0 (0)</td>
<td>2.6 (0.20)</td>
<td>0.6 (0.24)</td>
<td>1.2 (0.37)</td>
<td>1.2 (0.20)</td>
</tr>
<tr>
<td>HMB-45/50</td>
<td>0.9 (0.31)</td>
<td>2.7 (0.15)</td>
<td>2.8 (0.20)</td>
<td>2.5 (0.22)</td>
<td>2.5 (0.37)</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>0.7 (0.27)</td>
<td>2.8 (0.12)</td>
<td>2.4 (0.24)</td>
<td>2.6 (0.24)</td>
<td>2.6 (0.22)</td>
</tr>
<tr>
<td>Melan A</td>
<td>1.0 (0.32)</td>
<td>2.9 (0.10)</td>
<td>2.8 (0.20)</td>
<td>2.8 (0.20)</td>
<td>2.8 (0.24)</td>
</tr>
<tr>
<td>MITF</td>
<td>3.0 (0.0)</td>
<td>3.0 (0.0)</td>
<td>3.0 (0.0)</td>
<td>3.0 (0.0)</td>
<td>3.0 (0.0)</td>
</tr>
</tbody>
</table>

*Data are given as mean (SEM). p75NTR indicates p75 neurotrophin receptor; MITF, microphthalmia transcription factor; and ellipses, not applicable.
Tyrosinase labeled 5 of 5 spindle melanomas with an intensity of 2.4±0.24 and a pervasiveness of 3.0. In the epithelioid melanomas, 5 of 5 specimens were labeled with an intensity of 2.6±0.24 and a pervasiveness of 2.6±0.24. In the mixed melanomas, 5 of 5 specimens were labeled with an intensity of 2.3±0.2 and a pervasiveness of 2.2±0.37. In spindle melanomas, melan-A labeled 5 of 5 specimens with an intensity of 2.8±0.2 and pervasiveness of 3. In the epithelioid melanomas, 5 of 5 specimens were labeled with an intensity of 2.8±0.2 and a pervasiveness of 3.0. In the mixed melanomas, 5 of 5 specimens were labeled with an intensity and pervasiveness score of 3.0. Microphthalmia transcription factor labeled 5 of 5 specimens for each of the 3 types of choroidal melanomas, spindle (n=5), epithelioid (n=5), and mixed (n=5), with an average value of 3.0 for both intensity and pervasiveness. Of particular interest was the clear specificity of this marker owing to the intense nuclear localization of the signal (Figure 1). Patterns of immunoactivity for each antibody are shown in Figure 1 for a spindle uveal melanoma.

**COMMENT**

This study was undertaken to compare the immunophenotypic expression patterns between melanomas of the skin and of the uvea. We had initially set out to determine whether p75NTR, which can distinguish spindle from epithelioid skin melanomas,6,7 would also be helpful in distinguishing spindle from epithelioid populations within uveal melanomas. Such a marker would have potential prognostic value in uveal melanomas. However, the absence of p75NTR immunolabeling in all of the uveal melanomas despite the presence of all internal positive controls is a striking feature that distinguishes cutaneous from uveal melanomas. Thus, the p75NTR, a marker that is useful in distinguishing spindle from epithelioid melanomas in the skin, is not helpful in identifying the spindle cells of uveal melanomas. It is possible that labeling with p75NTR reflects desmoplasia, which is a common feature in spindle cutaneous melanomas but is rarely, if ever, present in uveal melanomas.

Another interesting difference between cutaneous and uveal melanomas was apparent with S100 immunolabeling. S100 immunoreactivity was relatively low when measured by intensity and pervasiveness in the uveal melanomas; conversely, in the skin melanomas, the reactivity was high. The difference could be due to different S100 isoforms. The low immunoreactivity to the S100 antibody in uveal compared with skin melanomas is in accord with prior detailed studies by Kan-Mitchell et al15,16 and others who have reported decreased S100 com-

![Figure 2. Immunophenotypic results from uveal melanomas compared with results of cutaneous melanoma data obtained from a previous study.6 A shows staining intensity while B shows pervasiveness of staining. The MITF results reflect the uveal melanoma data only. Note the complete absence of p75NTR labeling in any of the melanomas, and the high level of MITF staining for both intensity and pervasiveness.](https://archophth.jamanetwork.com/)
pared with HMB-45 immunolabeling in primary\textsuperscript{18,19} and in metastatic\textsuperscript{20} uveal melanomas.\textsuperscript{19,20}

The remaining markers, HMB-45, HMB-50, tyrosinase, melan-A, and MITF also showed differences in expression between uveal and cutaneous melanomas in that they were expressed at high levels whether the uveal melanoma was of the spindle, epithelioid, or mixed type. In contrast, in skin melanomas, these same antibodies are expressed at high levels only in the epithelioid type and expressed poorly in the spindle subsets.\textsuperscript{6} Figure 2 and the Table compare immunolabeling patterns between skin and uveal melanomas that were performed in our laboratory; however, it should be noted that the comparison is a historical rather than a side by side comparison. Although Figure 2 and the Table do not present data on MITF-labeling of cutaneous melanomas, MITF is also poorly expressed in cutaneous spindle melanomas.\textsuperscript{12} Our MITF expression patterns confirm an earlier preliminary study by Sharara et al,\textsuperscript{21} which found that MITF does not distinguish uveal from cutaneous melanomas.

Due to the differences in immunolabeling patterns between skin and uveal melanomas, the current panel of antibodies does not have the same diagnostic power for uveal melanomas as it does for cutaneous melanomas to delineate spindle from epithelioid cell types. However, this study also demonstrates that the markers melan-A, tyrosinase, HMB-45, HMB-50, and MITF are all superior markers to S100 for the identification of uveal melanomas.

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