S100 Immunophenotypes of Uveal Melanomas

June Kan-Mitchell,* Narsing Rao,† Daniel M. Albert,‡ Linda J. Van Eldik,§ and Clive R. Taylor*

To determine whether ocular melanomas are immunophenotypically identical to cutaneous melanomas, 34 primary and metastatic choroidal melanomas representing all major histotypes defined by the Callender’s classification, plus one melanoma of the iris and one conjunctival melanoma, were subjected to a panel of immunostains designed to distinguish anaplastic biopsies of cutaneous melanomas from carcinomas and lymphomas. All ocular melanomas were found to express the intermediate filament vimentin but not keratin, and all but 2 were melanotic by immunostaining. Thirty-three of 34 (97%) choroidal melanomas were strongly stained with a rabbit polyclonal antibody (P-S100) developed against the S100 protein family. In contrast, none of 14 spindle cell primary lesions was stained with a monoclonal antibody (MAB-079) specific for both S100α and S100β, the best-characterized S100 polypeptides. Furthermore, only 2 of 5 epithelioid and 3 of 10 mixed-cell-type melanomas were weakly reactive. Overall, 14.7% (5 of 29) were stained. In comparison, MAB079 stained 85% of all cutaneous melanomas. Five metastases of choroidal melanomas (spindle B, epithelioid, and mixed cell types) from different organ sites also were stained by P-S100 but not by MAB079. These findings were corroborated by immunostaining with another monoclonal antibody (MAB4D4) specific for S100β. Differential staining by the polyclonal but not the monoclonal antibodies suggests the possible presence of a variant S100 polypeptide(s) in choroidal melanomas. Since S100α, S100β, and related proteins appear to be physiologically important, additional studies of these S100 proteins may shed light on the etiology or pathology of choroidal melanomas. Invest Ophthalmol Vis Sci 31:1492–1496, 1990

The term S100 refers to a family of small, structurally related, calcium-binding proteins that originally were isolated by their solubility in saturated ammonium sulfate at neutral pH. Although previously believed to be a homogeneous protein of the nervous system, the S100 protein fraction was subsequently determined to be a mixture of polypeptides present in a large variety of normal and neoplastic cells. Two of the S100 proteins have been structurally characterized. S100α and S100β are polypeptides of 93 and 91 amino acids, respectively, and share 54 identical amino acids. In fact, these proteins were found to be highly conserved among mammals, suggesting important physiologic roles. It is noteworthy, too, that they can form homologous (αα or ββ) or heterologous (αβ) dimers in vitro. Despite the high degree of homology in their primary structures, S100α and S100β are distributed differentially among tissues and species. Because the synthesis of each polypeptide appear to be independently regulated, it is possible that S100 polypeptides may have tissue-specific or developmental stage-specific functions.

Despite its broad distribution in tissues, S100 immunoreactivity has proven to be important for the diagnosis of malignant melanomas. In particular, a panel of immunostains reactive to S100, intermediate filaments, and melanosomes is used in surgical pathology as an aid to distinguish biopsied specimens of anaplastic melanoma from that of carcinomas and lymphomas. Absence of staining for S100 has been used as evidence against the diagnosis of melanoma.

With antisera generated against the complete S100 protein fraction, S100 immunoreactivity was noted in only 81% of primary lesions of choroidal melanomas, whereas 100% of cutaneous melanomas and melanocytic lesions were reactive. More recently, with the use of monoclonal antibodies specific for both S100α and S100β or for S100β alone, the levels of these two S100 polypeptides were found to vary significantly among human neoplasms. In the current study, we examined the expression of S100 polypeptides in the different histotypes of primary and metastatic uveal melanomas by comparing the
staining of monoclonal antibodies to that of the polyclonal antiserum. Only 17% of all four histotypes of primary choroidal melanomas expressed low levels of $\text{S100}_\alpha$ and $\text{S100}_\beta$, as evidenced by staining with monoclonal antibodies. However, all reacted strongly with the polyclonal antiserum. Moreover, systemic metastases of choroidal melanoma biopsied years after enucleation retained this distinctive $\text{S100}$ staining profile. In contrast, 85–95% of primary and metastatic cutaneous melanomas were stained with the monoclonal and the polyclonal antibodies, respectively.

**Materials and Methods**

**Case Selection**

Paraffin blocks of 31 primary and 5 metastatic ocular melanomas and 4 primary and 6 metastatic of cutaneous melanomas were obtained from the files of the Doheny Eye Institute (Los Angeles, CA), the Massachusetts Eye and Ear Infirmary (Boston, MA), the University of California at Davis, the University of Southern California School of Medicine, and Bowman Gray School of Medicine, Wake Forest University (Winston-Salem, NC). All tumors were formalin-fixed and routinely processed between 1983 and 1989. Tumor histotypes of the primary choroidal melanoma lesions were determined according to the modification of the Callender classification.17

**Antibodies**

Immunohistochemical analysis was performed with a battery of commercially available polyclonal and monoclonal antibodies applicable to formalin-fixed, paraffin-embedded tissues. Polyclonal rabbit antiserum to $\text{S100}$ (Dako, Santa Barbara, CA), raised against the bovine $\text{S100}$ protein fraction, were reactive to $\text{S100}_\alpha$ and $\text{S100}_\beta$ as well as to the other, less-well-characterized $\text{S100}$ polypeptides; they were used at 1:1000 dilution. The mouse monoclonal antibody MAB 07913 (Chemicon, El Segundo, CA), which recognizes an epitope common to both $\text{S100}_\alpha$ and $\text{S100}_\beta$, was used at a dilution of 1:300. HMB.4518 (Enzo, New York, NY) specific for melanosomes, MU 074-UC specific for vimentin (Biogenex, Dublin, CA) and anti-cytokeratin monoclonal antibodies, AE1 and AE3, specific for keratin polypeptides of 40, 50, 56.5, 58, and 65–67 kD (Hybritech, San Diego, CA) all were used at dilutions of 1:100.

The production and characterization of the highly specific monoclonal antibody (4D4) to $\text{S100}_\beta$ has been described.19 Briefly, spleen cells from Balb/c mice that had been immunized with purified bovine $\text{S100}_\beta$ were fused with mouse myeloma cells (P3X63-Ag8.653). The antibody-producing hybridoma was cloned by limiting dilution. The antibody is specific for the $\text{S100}_\beta$ polypeptide, as determined by enzyme-linked immunosorbent assay, radioimmunoassay, and immunoblot analysis, and shows little or no reactivity with the $\text{S100}_\alpha$ polypeptide. MAB4B4 was used at a dilution of 1:50.

**Immunohistochemistry**

After deparaffinization and rehydration, the 5-μm tissue sections were placed in 0.6% hydrogen peroxide for 30 min. Slides to be stained for keratin were subsequently placed in a 0.05% protease (type XXIII; Sigma, St. Louis, MO) and incubated at 37°C for 5 min. All slides then were incubated sequentially for 30 min with 5% normal horse serum, primary antibody, biotinylated secondary antibody (Vector, Burlingame, CA), and an avidin–biotin–peroxidase complex (Vector). Peroxidase activity was determined by adding aminoethylcarbazole at 0.4 mg/ml (Sigma) in 0.1 M sodium acetate buffer, pH 5, containing 0.015% hydrogen peroxide, for 5 min. The sections were counterstained briefly with Mayer’s hematoxylin (Sigma) and mounted under a coverslip with Aquamount (Sigma). Red precipitates denoted binding by the primary antibody, and this staining was readily distinguishable from melanin granules in the melanoma cells. Immunoreactivity was scored on an arbitrary scale of 1 to 4+, with 1+ denoting minimal but definite staining above background; 2+, light staining; 3+, strong staining; and 4+, maximal staining. Each stained section was evaluated independently by two investigators. There was consensus on the assigned intensities in all cases studied.

**Statistical Analysis**

For comparison of proportions of sections stained by each type of antibody in these paired samples, we used the chi-squared analysis with one degree of freedom, with Yates’s correction for continuity.

**Results**

Thirty-six primary and metastatic ocular melanomas were subjected to a panel of mouse monoclonal antibodies (MAB079, HMB45, MU074, AE1/AE2) designed to distinguish anaplastic biopsies of cutaneous melanomas from carcinomas and lymphomas. In addition, 22 of these cases also were tested with the rabbit polyclonal antiserum (P-S100) reactive to the complete $\text{S100}$ protein fraction, a standard antiserum used in diagnostic pathology. Twenty-one of 22 specimens (95%) stained positively with P-S100. In addition, all four major histotypes of
Table 1. Immunophenotypes of ocular melanomas

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Number stained / Number tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-S100</td>
</tr>
<tr>
<td>Choroidal melanoma</td>
<td></td>
</tr>
<tr>
<td>Primaries</td>
<td></td>
</tr>
<tr>
<td>Spindle A</td>
<td>2/2</td>
</tr>
<tr>
<td>Spindle B</td>
<td>5/5</td>
</tr>
<tr>
<td>Epithelioid</td>
<td>2/2</td>
</tr>
<tr>
<td>Mixed</td>
<td>6/6</td>
</tr>
<tr>
<td>Metastases</td>
<td></td>
</tr>
<tr>
<td>Melanoma of the iris</td>
<td>4/5</td>
</tr>
<tr>
<td>Conjunctival melanoma</td>
<td>1/1</td>
</tr>
<tr>
<td>Cutaneous melanoma</td>
<td>50/50</td>
</tr>
</tbody>
</table>

Only 6 of the 22 (27%) were stained with the monoclonal antibody MAB-079. The data are summarized in Table 1. Chi-squared (one degree of freedom) for the difference in these proportions of staining was 13.0, \( P < 0.001 \). As with their cutaneous counterparts, both uveal and conjunctival melanomas expressed vimentin, the intermediate filament of mesenchymal cells; none of the tumors was reactive with anti-cytokeratin antibodies. Furthermore, all but one spindle A type tumor were melanotic, as evidenced by positive staining with the anti-melanosome monoclonal antibody HMB-45.

The most striking result was the absence of staining in all spindle cell type melanomas by the monoclonal antibody, MAB-079. This antibody recognizes an epitope found only on S100a and S100/5 in the S100 protein fraction. In one of the spindle B lesions that was judged negative for reactivity, there was weak focal staining of the larger melanoma cells (less than 3% of all tumor cells). On the other hand, two of the five epithelioid (40%) and three of the ten mixed cell type (30%) choroidal melanomas were reactive with MAB079. Nonetheless, except for one mixed cell type melanoma that was stained 2+, the majority were stained only weakly. In contrast, 85–90% of more than 50 cases of cutaneous melanomas studied to date were stained. Furthermore, the staining was usually quite intense, between 2+ and 4+.

The absence of S100α and S100β immunoreactivity was confirmed with a second monoclonal antibody, MAB4D4, that recognizes another epitope found only on S100β. Two cases from each histotype that stained with P-S100 were studied. The data are summarized in Table 2. Here again, the spindle cell type melanomas were nonreactive. Although the epithelioid melanomas were positive also with MAB4D4, the intensity of staining in one specimen was less than that observed with the polyclonal antibodies. Under these conditions, MAB4B4 stained only six of ten cutaneous melanoma samples that were reactive with both P-S100 (Table 2) and MAB079 (data not shown).

To determine whether metastases of choroidal melanomas were similarly devoid of S100α and S100β immunoreactivity, five lesions biopsied from different organ sites and three matching primary tumors were stained for S100 proteins (Table 3). All metastases, regardless of their histotypes, organ sites, and time elapsed since enucleation, were reactive to S100α and S100β.
Table 3. Immunohistochemical distribution of the different S100 proteins in primary and metastatic choroidal melanomas

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histotype, site</th>
<th>MAB-079</th>
<th>MAB4D4</th>
<th>P-S100</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Spindle B, primary</td>
<td>0</td>
<td>0</td>
<td>3+*</td>
</tr>
<tr>
<td>2</td>
<td>Mixed, spinal metastasis (4)</td>
<td>0</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>3</td>
<td>Epithelioid, primary</td>
<td>0</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>4</td>
<td>Epithelioid, lymph node metastasis (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>5</td>
<td>Mixed, liver metastasis (11)</td>
<td>0</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>6</td>
<td>Mixed, rib/muscle metastasis</td>
<td>0</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>7</td>
<td>Spindle B, brain metastasis (27)</td>
<td>0</td>
<td>0</td>
<td>2+</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the number of years postenucleation when biopsy was performed.

* Intensity of staining of the melanoma cells in each case.

P-S100 but not to either MAB-079 or MAB4D4. Thus, they retained the distinctive S100 immunophenotype characteristics of primary lesions of choroidal melanomas. It is worthy of note that most were biopsied at least 1 yr postenucleation; in patient 5, biopsy was performed 27 yr postenucleation. Because only a small number of patients was studied, we cannot extrapolate from these results the immunophenotypes of metastases of uveal melanoma in general. Our data, however, showed that this S100 immunophenotype was stably expressed in some metastases. Incidentally, the “primary” lesion from patient 1 was a recurrence of a melanoma of the iris 19 yr after excision.20

**Discussion**

S100 immunoreactivity has been demonstrated previously in 16 intraocular melanomas with a polyclonal antiserum to the S100 protein fraction.13 Because the presence of S100 has proven useful for the diagnosis of secondary melanomas,4 immunohistochemical distribution of individual S100 proteins may provide additional insight into the biology and etiology of this tumor. For this reason, we compared the S100 immunophenotypes of primary and metastases of choroidal melanomas of the four major histotypes with monoclonal antibodies specific for the two major components of S100, S100α, and S100β.14,15

Our data are in complete accord with the previous findings.13 The commercial rabbit polyclonal antibody to S100 stained all 31 primary lesions of uveal melanomas (29 melanomas of the choroid, 1 of the iris, and 1 of the conjunctiva). Four of 5 systemic metastases of choroidal melanoma also were reactive. Furthermore, a difference in S100 immunoreactivity among the histotypes was not discerned with this reagent.

In contrast, when the monoclonal antibody MAB-079 was used to identify S100α and S100β in these sections, only 17% (5 of 29) of the primary tumors were reactive. This lack of reactivity within the tumor cells is in sharp contrast to the intense staining of the optic nerve. Thus, this epitope was well preserved in the same section, in a tissue adjacent to the tumor cells. Although the epitope identified by MAB-079 is stable to routine tissue-processing procedures in clinical pathology laboratories, including the five from which these specimens were identified, the presence of S100β in selected sections was determined with another monoclonal antibody, MAB4D4. Essentially identical results were obtained with this antibody specific for a different epitope on S100β.

Discrepancies between immunostaining with monoclonal and polyclonal antibodies to S100 proteins have been described for many tissue antigens. Indeed, other monoclonal antibodies (S1-61-64 and S2-20) to common epitopes of S100α and S100β, shown to identify novel sites of S-100 immunoreactivity21 in a variety of nonmelanocytic tissues, are more sensitive than polyclonal reagents. Recently, primary mucosal melanomas also were found to be less frequently stained by MAB-079 (42%) than were cutaneous melanomas (85%).22 The common factor between the rare, mucosal melanomas and choroidal melanomas that accounts for this distinctive S100 immunophenotype is still a matter of conjecture.

Absence of staining by the monoclonal antibodies in choroidal melanomas may indicate that S100α and S100β are missing or that the ocular S100 proteins are molecular variants in not expressing the epitopes defined by these reagents. Since the epitopes have not been mapped on the S100 molecules, the significance of their absence remains to be determined. The fact that these melanomas were indeed stained by the polyclonal antiserum to the S100 fraction may reflect a nonspecific staining by this antiserum. However, it is also possible that the reactivity reflects the presence of yet uncharacterized S100-like proteins. Indeed, S100α, S100β, and a variety of
structurally related proteins and cDNA structures appear to physiologically important. Molecular characterization of the S100 protein family expressed by choroidal melanomas may identify novel functional proteins important to its differentiation, development, or pathologic state. In particular, this information may be useful in elucidating the etiologic relationship among human melanomas. To date, antigens described in cutaneous melanomas are usually also present in choroidal melanomas, although these diseases exhibit different clinobiologic characteristics.

**Key words:** S100 protein, S100α, S100β, choroidal melanoma, immunohistochemistry

### Acknowledgments

The authors are grateful to Dr. Alan M. Roth at the University of California at Davis for providing some of the choroidal melanoma sections and to Dr. Wain L. White at Bowman Gray School of Medicine for providing cutaneous melanoma tissues. They would like to thank Dr. Malcolm S. Mitchell for his assistance in the statistical analysis of our data and to acknowledge the excellent technical assistance of Ms. Lillian L. Young.

### References