Detection of Cytoplasmic S-100 Protein in Primary and Metastatic Intraocular Melanomas

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The authors sought S-100 protein in sections cut from formalin- or glutaraldehyde-fixed, paraffin-embedded tumors from the eye and other sites by an immunoperoxidase technique, using rabbit antihuman S-100 protein antiserum, swine antirabbit serum, a peroxidase-antiperoxidase preparation, and amino-ethylcarbazole as developer. Tissue from 26 intraocular tumors was examined, the pathologic diagnosis being unknown to the investigators at that time that the tests were performed. Thirteen of 16 malignant melanomas of the choroid contained S-100 protein (81%), while only one of seven retinoblastomas contained S-100 protein (14%). One cutaneous malignant melanoma metastatic to the eye contained S-100 protein, while none of three metastatic nonmelanocytic, nonneural tumors contained the protein. These results are similar to our findings with nonocular tumors, in which 56 of 56 cutaneous melanomas contained S-100 protein (100%) and only 1 of 51 nonneural, nonmelanocytic tumors contained S-100 protein (2%). S-100 protein may be useful as a marker for ocular malignant melanomas. Invest Ophthalmol Vis Sci 24:1153–1155, 1983

S-100 protein is an acidic protein of unknown function named from its solubility in saturated ammonium sulfate at neutral pH. It has been demonstrated in glial cells in the brain,¹,² in the enteric nervous system,³ in Schwann cells of the peripheral nervous system, and in satellite cells of peripheral ganglia. More recently there have been claims that S-100 protein may occur in other tissues, including chondrocytes,⁴ interdigitating reticulum cells in lymph nodes,⁵ and Langerhans cells in the epidermis. It exhibits a strong serologic cross-reactivity among a variety of different species. S-100 protein persists after formalin fixation and dehydration, and may be sought in conventionally fixed and embedded tissue.

We demonstrated S-100 protein in melanoma cells using both complement fixation and immunofluorescence assays⁹ and immunoperoxidase technique.¹⁰ The expression of S-100 protein is apparently not correlated with the degree of synthesis of melanin, which makes it suitable for identification of hypomelanotic or amelanotic tumors. We have investigated the extent to which S-100 protein is demonstrated in melanomas and other intraocular tumors. The presence of S-100 protein in ocular melanomas could serve as a tumor marker to aid in the diagnosis of amelanotic or otherwise atypical tumors, and for the identification of tumor extensions and metastases.

Materials and Methods. Tumor tissues from 17 patients with various ocular tumors were obtained from the files of the Pathology Laboratory of the Jules Stein Eye Institute, UCLA School of Medicine. Tissue sections (5 μm) were cut from these paraffin-embedded tissues and used in the immunoperoxidase assay. Additional slides were obtained from ten patients with various intraocular tumors who had attended the Tennent Institute of Ophthalmology, The Western Infirmary, Glasgow. These tissues had been routinely fixed in glutaraldehyde. The intraocular tumors included primary intraocular choroidal malignant melanomas, retinoblastomas, and tumors metastatic to the eye.

All slides were coded prior to being read and the histologic diagnosis was not known to the individuals performing the assays and reading the results (Drs. Wen and Cochran). Diagnoses were provided only after completion of the S-100 protein studies.

Immunoperoxidase technique. The slides were deparaffinized by exposure to xylene for 10 min and washed for 3 min in 100% ethyl-alcohol three times. Slides then were washed in each of the following solutions for 2 min: 95% ethyl-alcohol, 75% ethyl-alcohol, and distilled water. Endogenous peroxidase in the tissues were quenched by exposure to 3% hydrogen peroxide for 20 min. The slides were washed in running tap water for 1 min, dipped in distilled water, and rinsed three times in a tris-saline solution. The slides were exposed in 5% egg albumin for 20 min and rinsed in tris-saline. Rabbit antiserum to bovine S-100 protein was applied for 1 hr at dilutions ranging from 1:50 to 1:200. Slides were washed three times with tris-buffer. Swine antirabbit serum diluted 1:20 (Dako Inc., Santa Barbara, CA) was applied for 30 min and the slides then were rinsed three times in tris-buffer. A peroxidase anti-peroxidase preparation diluted 1:100 (Dako Inc., Santa Barbara, CA), was applied for 30 min and the slides were washed in tris-buffer for 5 min. The slides then were immersed in substrate solution for 10 min. Substrate solution consists of 6 ml of amino-ethyl carbazole (AEC) developer plus 50 ml of a 0.02 moomonoammonium acetate buffer, plus 0.4 ml of a 3% solution of hydrogen peroxide. The AEC developer is prepared by dissolving 167 mgs of 3-amino 9-ethyl carbazolone and 100 ml of dimethyl sulfoxide. The slides were cleansed in running tap water, dipped in distilled water,
Table 1. S-100 protein in intraocular and nonocular tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Tested No.</th>
<th>Positive No.</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choroidal malignant melanomas</td>
<td>16</td>
<td>13</td>
<td>81%</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>7</td>
<td>1</td>
<td>14%</td>
</tr>
<tr>
<td>Metastatic tumors to the eye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonmelanocytic, nonneural</td>
<td>3</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Cutaneous melanoma</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nonocular tumors10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant melanomas</td>
<td>56</td>
<td>56</td>
<td>100%</td>
</tr>
<tr>
<td>Melanocytic nevi</td>
<td>33</td>
<td>33</td>
<td>100%</td>
</tr>
<tr>
<td>Nonneural, nonmelanocytic</td>
<td>51</td>
<td>1</td>
<td>2%</td>
</tr>
</tbody>
</table>

Counterstained with hematoxylin for 3 min, washed again in tap water, and finally dipped in a saturated aqueous solution of sodium bicarbonate. The slides were finally rinsed in distilled water and a cover glass was applied.

As a negative control, slides were treated with normal rabbit serum at dilutions of 1:20 to 1:100, rather than with rabbit antiserum to bovine S-100 protein. A positive control was provided by exposing slides containing known reactive melanoma and cutaneous nerves to both antibodies and developer. The optic nerve and smaller transcleral nerve twiglets served as additional internal positive controls. Absorptions of the anti-S-100 protein removed all the activity against melanoma and nerve.

Staining is remarkably durable, but for uniformity the preparations were read within 24 hrs. The advantage of aminocarbazole is that it produces a red color that is readily distinguishable from the range of browns represented by the melanos.

Antiserum to bovine S-100 protein was prepared against antigen purified as described previously. The serologic specificity of the antiserum was shown by double diffusion in agar and by quantitative complement fixation analysis employing soluble protein extracts of a variety of tissues and antigen. Further evidence of specificity is provided by the extensive immunoperoxidase studies of a wide range of tumorous and normal tissues described in a recent publication.

Results. The results obtained with the tissues from Los Angeles and Glasgow showed no significant differences and have been combined for Table 1 and our subsequent consideration of the results.

We tested 16 intraocular malignant melanomas and found 13 to contain S-100 protein (81%). To the present, we have not examined any intraocular nevi. We examined seven retinoblastomas of varying degrees of differentiation and found evidence of S-100 protein in one (14%). The one S-100 protein-containing retinoblastoma was a poorly differentiated tumor. Of special interest were three nonmelanocytic tumors that had metastasized to the eye from extraocular primary sites. None of these stained positively with the anti-S-100 protein serum. These represented a considerable variety of origins: one metastatic lung carcinoma, one disseminated malignant lymphoma, and one adenocarcinoma metastatic from a bowel primary.

As a counterpoint to the above, we include our current results with nonocular tumors. Thus far, all cutaneous melanomas tested have contained S-100 protein (56/56 or 100%), as have all melanocytic nevi (33/33 or 100%). The overwhelming majority of nonneural, nonmelanocytic tumors are devoid of S-100 protein (1/51 or 2%).

Discussion. The majority of intraocular choroidal malignant melanomas contained S-100 protein. This fact supports the previously reported finding of S-100 protein in 100% of nonocular malignant melanomas. In tumors that stained positively, more than 80% of tumor cells contained S-100 protein in their cytoplasm, though the intensity of coloration of the reaction product, and hence the S-100 protein content varied widely from cell to cell. The expression of S-100 protein was not correlated with tumor cell morphology, being present equally in epithelioid and spindle-shaped cells. The fact that three intraocular melanomas did not contain S-100 protein could not be explained on the basis of tumor characteristics such as size, location, or cell type. Neither could it be explained on the basis of differences in specimen fixation or assay technique.

The functional significance of S-100 protein is unknown. Identification of this protein, however, can serve as a potentially useful tumor marker. As a practical application of the technique, S-100 protein is of aid in distinguishing amelanotic or hypomelanotic ocular melanoma from carcinoma metastatic to the eye. Applied in this manner, S-100 protein identification appeared in our studies to be a very useful adjunct to the traditional diagnostic approaches. Moreover, S-100 protein is likely to be useful in the search for tumor extensions and microscopic foci of metastatic disease.

Nonocular melanocytic nevi consistently have been shown to contain S-100 protein. We have not yet investigated the presence of S-100 protein in ocular nevi. If our experience with nonocular tumors is borne out, it is not likely that the presence of S-100 protein will be a marker for malignancy or will be of assistance in separating nevi from malignant melanomas.

Our observation that one of seven retinoblastomas
contained S-100 protein is of interest and clearly needs to be studied further in a larger number of such tumors. Since glial cells contain S-100 protein, and both retinal neurones and glial cells apparently arise initially from a common stem cell, it is perhaps not surprising that the cells of an occasional retinoblastoma tumor also will be positive for S-100 protein. The presence of S-100 protein in both melanoma and retinoblastoma, however, should not present a problem in tumor diagnosis. These two tumors not only have different histologic characteristics but also present in different clinical settings. Our own recent studies and those of Nakajima et al. indicate that S-100 protein-like immunoreactivity may be detected in some unusual tumors. Many of these have an embryologic relationship with neural tissues (granular cell myoblastoma, ganglioneuroma, paraganglioma, and carcinoid tumors), however, in others (chordoma, histicytosis X, pleomorphic adenoma of the salivary gland, and chondrosarcoma) the explanation for the presence of S-100 protein is unclear. While it is necessary to know that these exceptions exist it is unlikely that, given access to all relevant clinical and conventional histologic information, any of these conditions would present as an ophthalmologic problem.

The study of S-100 protein promises to provide additional information regarding the basic biology of intraocular melanomas, as well as being a potentially useful tumor marker. Nontumorous cells containing S-100 protein also are present in the intact retina and are under study. The identification and characteristics of such cells may contribute to knowledge of retinal anatomy and embryology.

**Key words:** S-100 protein, malignant melanoma, intraocular tumors, tumor marker, immunoperoxidase assay

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**References**