Transactions of the Illinois Academy of Science
(1986), Volume 79, 3 and 4, pp. 263-269

S-100 PROTEIN IN THE DIFFERENTIAL DIAGNOSIS OF METASTATIC AMELANOTIC MELANOMA FROM UNDIFFERENTIATED CARCINOMA

Ali-Reza Armin, M.D.
Gayle L. Winters, M.D.
Roberto Gradini, M.D.
James V. Robinson, HTL(ASCP)
Loyola University Medical Center
Department of Pathology
2160 S. First Avenue
Maywood, IL 60153

ABSTRACT

Thirty-one cases of metastatic poorly differentiated and undifferentiated carcinomas from various primary sites, one case of known Kaposi's sarcoma, and four cases of metastatic malignant amelanotic melanoma were stained by immunoperoxidase method for S-100 protein. Only cases of metastatic malignant melanoma showed marked positivity. Cases of infiltrating breast carcinoma showed borderline positivity. Metastases from all other sites studied were S-100 negative. These results indicate that S-100 staining may be helpful in differentiating metastatic malignant amelanotic melanomas from other primary tumors.
Key words: S-100 Protein, Metastatic Amelanotic Melanoma, Undifferentiated Carcinoma.

INTRODUCTION

Malignant melanoma is rapidly becoming one of the more common malignancies in the United States, causing an average of 5,500 deaths annually. [1] Although most malignant melanomas arise de novo, it is well known they can arise in congenital melanocytic nevi[2] and in dysplastic nevus syndrome.[3].

The diagnosis of metastatic malignant melanoma in the presence of a known skin or mucous membrane primary does not usually create a major diagnostic problem. However, we have encountered a number of cases in which the diagnosis of malignant melanoma was made on a metastatic lesion in the absence of a well documented primary lesion. The diagnostic difficulty is compounded if the metastatic lesion is amelanotic. These metastases are presumably derived from a "mole"

removed years earlier or a lesion that has regressed leaving little or no scar or pigmentation.

To differentiate metastases of malignant amelanotic melanoma from those of an undifferentiated carcinoma, we utilized immunoperoxidase staining by S-100 protein which is positive in melanocytic cells but negative in most tumors of endodermal origin.

MATERIALS AND METHODS

Thirty cases of metastatic undifferentiated carcinomas and one case of Kaposi's sarcoma with known primary as well as four cases of metastatic amelanotic malignant melanoma were studied.

Representative paraffin blocks from the 35 cases were sectioned at 4 microns. The resulting slides, containing the paraffin sections, were heated for 30 minutes at 60°C and then allowed to cool to room temperature.

Deparaffinization of the sections was accomplished using 3 changes of limonene and the sections were rehydrated using decreasing grades (100%, 95%, and 80%) of ethanol, prior to rinsing in distilled water.

Endogenous peroxidase activity was blocked using freshly prepared 3% hydrogen peroxide, for 7-1/2 minutes. The slides were then immersed in phosphate buffered saline (PBS) for 5 minutes.

To accomplish the remainder of the immunoperoxidase staining technique, the Vector Laboratories Rabbit IgG ABC staining kit was employed. The primary antibody (rabbit antibovine S-100 protein) was obtained from DAKO Corporation. The working dilutions of the ABC kit reagents were prepared as instructed in the accompanying literature and the S-100 antibody was used at a working dilution in PBS of 1:200.

Incubation times were: normal goat serum, 20 minutes; primary antibody, 30 minutes; biotinylated antibody, 30 minutes; and avidinbiotinylated horseradish peroxidase complex, 45 minutes.

Loci of peroxidase complex deposition were identified using 0.01% diaminobenzidine and 0.03% hydrogen peroxide in PBS. Incubation time was 5 minutes. The slides were then washed in running tap water for 5 minutes prior to counterstaining with Modified Harris Hematoxylin, decolorizing in acid alcohol, and bluing in ammonia water. The slides were then dehydrated using 95% and 100% ethanol, cleared in 3 changes of limonene and 1 change of xylene, and coverslipped with Permount.

RESULTS

Four cases of electron microscopically proven metastatic malignant melanoma showed marked (+ + +) positivity for S-100 protein. One of the cases had extensive metastasis to the breast, which illustrates the contrast between positive melanoma cells and negative ductal epithelial cell (Figure 1). This consisted of intensely positive immunoreactivity in the cytoplasm of at least 90% of the tumor cells.

Six cases of primary infiltrating carcinoma of the breast were found to have borderline positivity due to staining of myoepithelial cells; however, metastases stained negatively (Figure 2).

Twenty-four cases of metastasis from undifferentiated or poorly differentiated carcinomas and one case of metastasis from Kaposi's sarcoma (Table 1) were uniformly negative for S-100 protein. (Figure 3).

Table 1. Immunoperoxidase Staining by S-100 Protein

Number of Cases	Primary Tumor	S-100 Protein
3	Adenocarcinoma, Lung	−
3	Squamous Cell Carcinoma, Lung	−
2	Adenosquamous Carcinoma, Lung	−
2	Squamous Cell Carcinoma, Esophagus	−
1	Undifferentiated Carcinoma, Esophagus	−
6	Adenocarcinoma, Stomach	−
1	Clear Cell Carcinoma, Kidney	−
1	Transitional Cell Carcinoma, Renal Pelvis	−
1	Squamous Cell Carcinoma, Vagina	−
1	Carcinosarcoma, Uterus, (MMMT, HT)	−
2	Squamous Cell Carcinoma, Floor of Mouth	−
1	Kaposi Sarcoma	−
3	Infiltrating Lobular Carcinoma, Breast	+/− −
3	Infiltrating Ductal Carcinoma, Breast	+/− −
4	Malignant Melanoma	+ + +

DISCUSSION

S-100 protein is one of the first neural specific proteins extracted from brain tissue.[1] This protein is also present in the peripheral nervous system, satellite cells of the adrenal medulla and spinal ganglia, pituitary and pineal glands.[5-8]

More recent studies have shown that S-100 protein is not restricted to the nervous system but is widely distributed in many tissues including melanocytes where its presence can be explained by the neural crest origin of these cells.[9,10] However, reports of S-100 positivity in interdigitating reticulum cells of a lymph node,[11] Langerhans cells of the epidermis, and human T-lymphocytes[12] raise interesting areas for further investigation.

The results of this study show a method which clearly separates metastases from malignant amelanotic melanomas from other primary tumors. Although primary breast carcinomas resulted in occasional S-100 positivity, this was due to uptake by myoepithelial cells. No positivity was observed in metastasis. Occasional positive cells in lymph nodes is due to uptake by reticular cells.

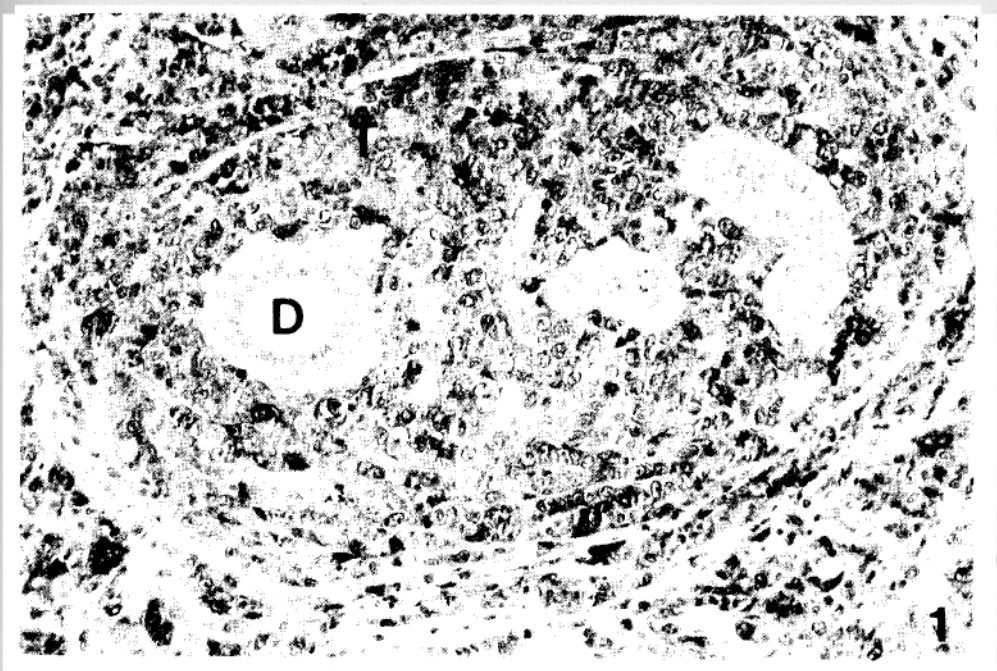
Differentiating metastatic undifferentiated lesions can pose a difficult diagnostic problem particularly if the primary site is not obvious. Various special studies including histochemical and immunoperoxidase staining as well as electron microscopy are available to help delineate the source of primary tumor. We have found immunoperoxidase staining with S-100 protein to be a valuable diagnostic aid in differentiating metastatic amelanotic melanomas from metastatic lesions with other primary sites.

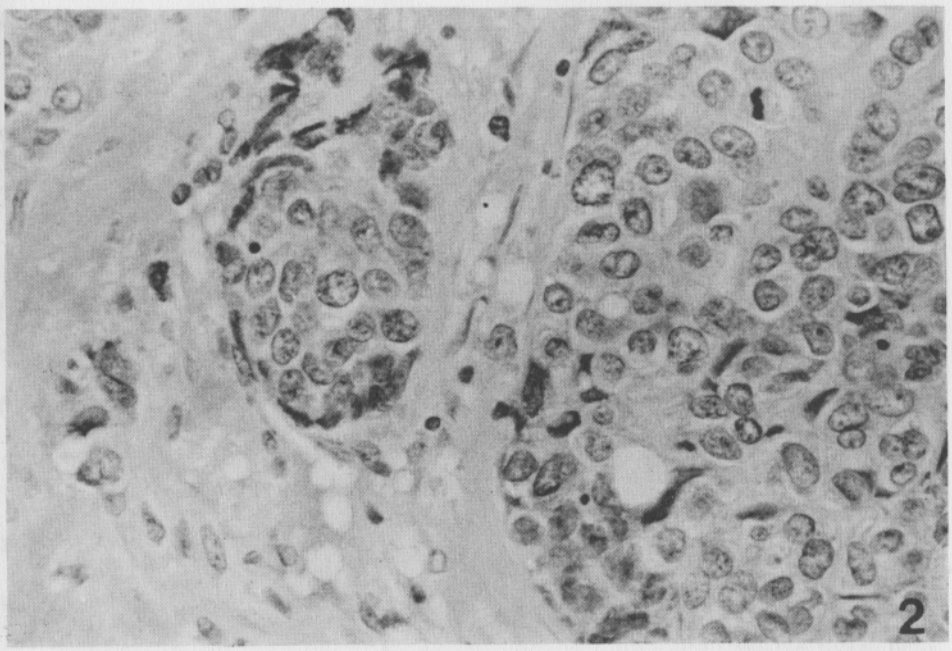
REFERENCES

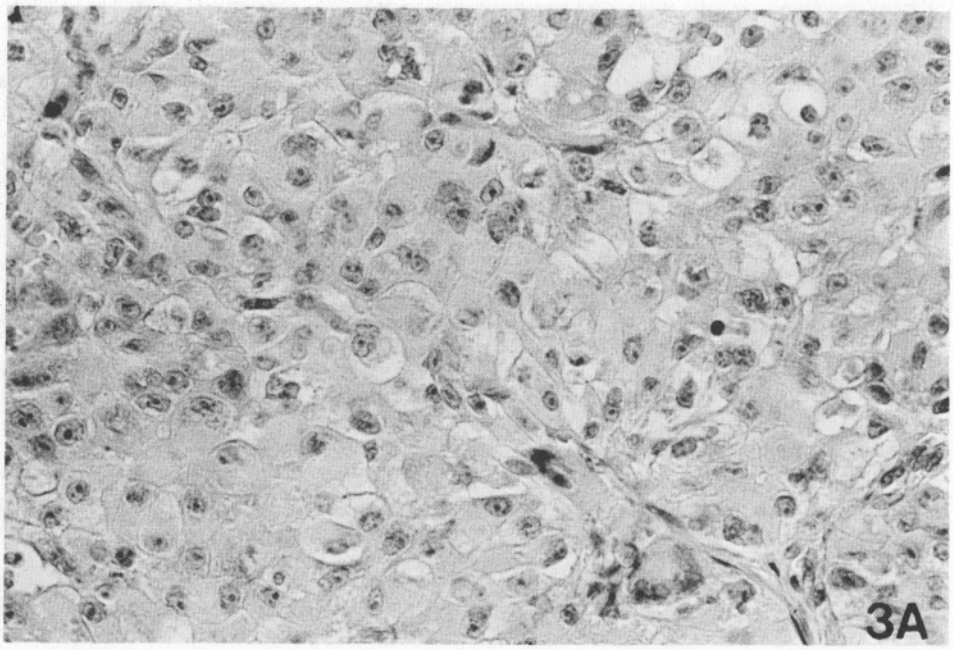
Silverberg, E., Cancer Statistics. CA 1985; 35: 19-35.
Kopf, A.W., Bart R.S., Hennessey P. Congenital nevocytic nevi and malignant melanomas. J. Am Acad Dermatol 1979; 1:123-130.
Clark, W.H Jr., Reimer R.R., Greene, M., et al. Origin of familial malignant melanomas from heritable melanocytic lesions.
Moore, B.W., McCregor, D., Chromatographic and electrophoretic fractionation of soluble proteins of brain and liver. J. Biol. Chem. 1965; 240: 1647-1653.
Cocchia, D. Immunocytochemical localization of S-100 protein in the brain of adult rat. Cell Tissue Res 1981; 214: 529-540.
Cocchia, D., Michetti, F. S-100 antigen in satellite cells of the adrenal medulla and the superior cervical ganglion of the rat. Cell Tissue Res 1981; 215: 103-112.
Cocchia, D., Miani, N. Immunocytochemical localization of the brain specific S-100 protein in the pituitary gland of adult rat. J. Neurocytol 1980; 9:771-782.
Nakajima, T., Yamaguchi, H., Takahashi, K. S-100 protein in folliculostellate cells of the rat pituitary anterior lobe. Brain Res. 1980; 191: 523-531.
Gaynor, R., Irie, R., Morton, D. et al. S-100 protein is present in cultured human malignant melanomas. Nature 1980; 286:400-401.
Gaynor, R., Irie, R., Morton, D. et al. S-100 protein: A marker for human malignant melanomas. Lancet 1981; 1:869-871.
Takahashi, D., Yamaguchi, H., Ishizeki, J. et al. Immunohistochemical and immunoelectron microscopic localization of S-100 protein in the interdigitating cells of human lymph node. Virchows Arch (Cell Path) 1981; 37:125-135.
Takahashi, D., Toshiaki, I., Ohtsuki, Y. et al. S-100 protein positive Human T-Lymphocyte. Am J Clin Pathol 1985; 83: 69-72.

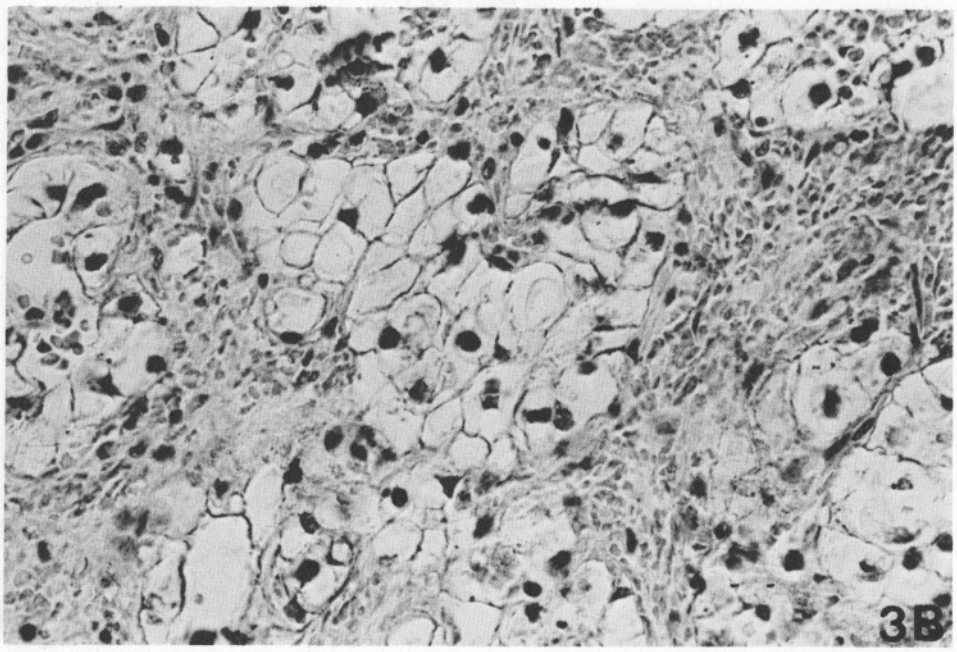
LEGENDS

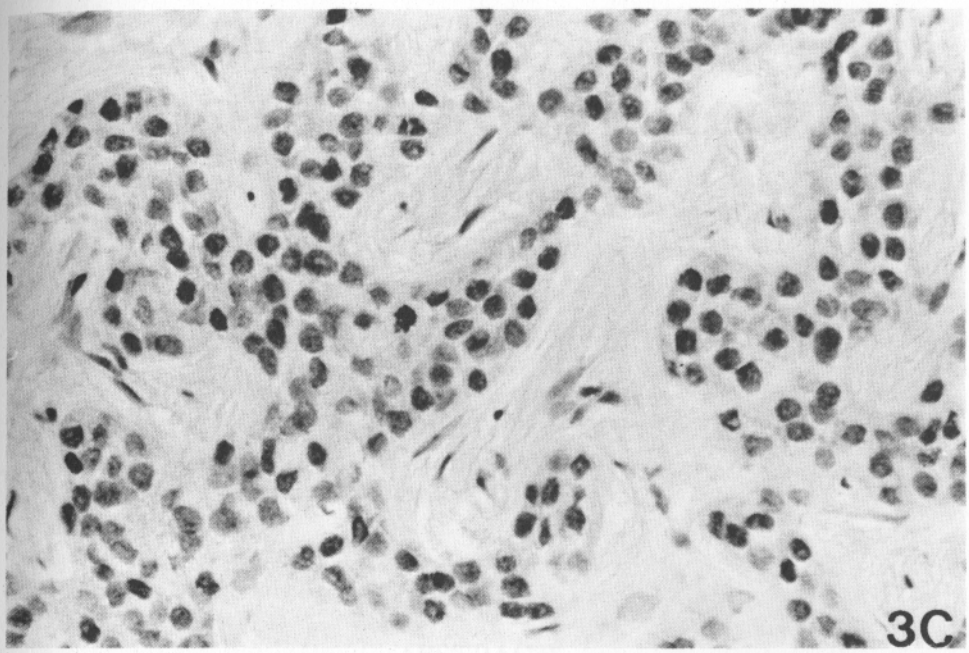
Fig. 1. Metastatic amelanotic melanoma in breast showing marked S-100 positivity of tumor cells (T) as compared to negative ductal epithelial cells (D). (P.A.P. Staining x200)
Fig. 2. Infiltrating ductal carcinoma of breast metastatic to lymph node. Only occasional reticulin cells of the lymph node are S-100 positive. (P.A.P. Staining x200)
Fig. 3. S-100 negative metastasis from non-melanoma primary sites. (P.A.P. Staining x200)
(A) Squamous cell carcinoma, lung.
(B) Clear cell carcinoma, kidney.
(C) Adenocarcinoma, stomach.
(D) Kaposi's sarcoma.

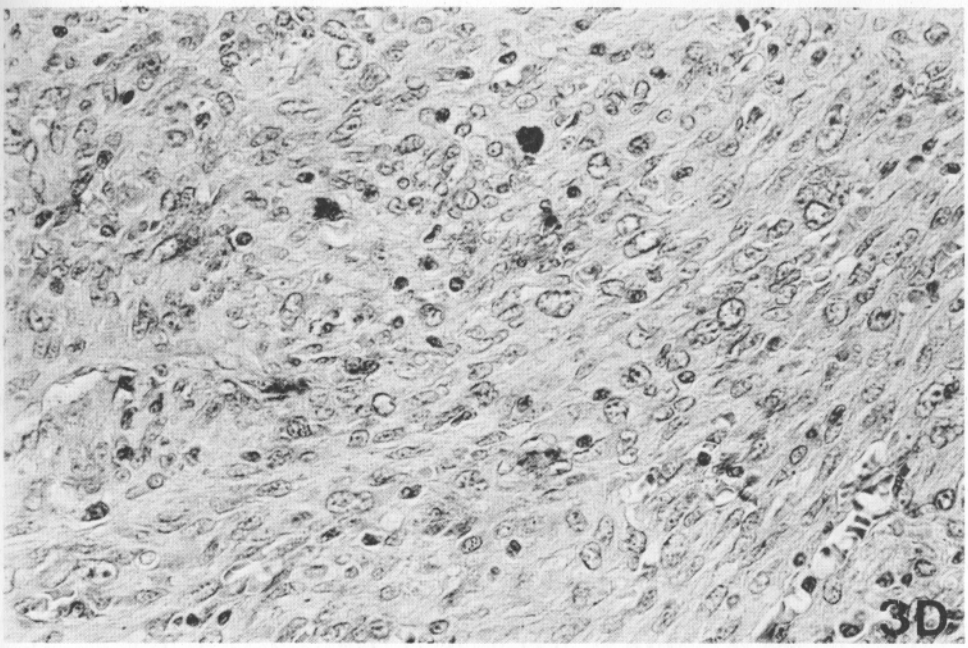

D
1


2


3A


3B


3C


3D