Protein S-100 immunostaining as a diagnostic tool for DMBA-induced melanotic lesions

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Summary. In the present study we demonstrate immunohistochemically the presence of S-100 protein in numerous melanotic and amelanotic lesions (benign, premalignant and malignant) induced in albino guinea-pigs on topical application of the carcinogen. Positive staining was also present in all their amelanotic metastases. We reiterate the value of this stain in the diagnosis of melanomas, in particular amelanotic melanomas.

Key words: Melanocytic carcinogenesis, DMBA, Protein S-100

Introduction

In the last decade immunohistochemical procedures have been developed which have revolutionized surgical pathology: enzymes have been introduced as antibody-marking substances and have provided the pathologist with a useful auxiliary means in the diagnosis of different pathological entities. These markers have been studied in depth by numerous authors. S-100 protein was first isolated by Moore in 1965 and its immunoreactivity has been noted with cells of glial origin (Isobe et al., 1984); with peripheral nerve fibres, Schwann cells and mature chondrocytes (Nakajima et al., 1982; Stefansson et al., 1982); and with Langerhans epidermal cells (Springall et al., 1983; Takahashi et al., 1984). Protein S-100 was also observed in a limited number of different types of tumours of neuroectodermal origin (Stefansson et al., 1982). In previous studies various authors (Gaynor et al., 1981; Cochran et al., 1982; Nakajima et al., 1982; Springall et al., 1983; Wen et al., 1983) have noted its positivity in almost all melanomas, primary and metastatic, and in junctional compound, intradermal, blue and Spitz's nevi. This type of staining, which is obtained from tissue embedded in paraffin after fixing in 10% formal, has proved specially useful in the recognition of anaplastic melanomas, and in addition is positive in 100% of amelanotic melanomas.

Materials and methods

In our experimental model we used 40 two-month-old male Dunkin-Hartley albino guinea-pigs. Each was treated twice a week for 13 consecutive months with 0.3 ml of a 1% solution of 7,12-dimethyl-benzanthracene (DMBA) in acetone which was applied to a previously shaved 5 x 5 cm area of dorsal flank. All the guinea-pigs were sacrificed in small homogeneous groups during the 17 months after stopping the application of the carcinogen.

Samples of cutaneous lesions and of metastases were taken for light microscopical study and processed in the usual way. Multiple sections were made and stained with Hematoxylin-eosin and Masson-Fontana technique. The indirect immunoperoxidase staining technique using the anti-S-100 protein serum as primary antibody was applied at a 1:200 dilution for 30 minutes, followed by swine anti-rabbit immunoglobulin antiserum (DAKO) at a 1:20 dilution. The sections were washed in PBS and incubated with a rabbit peroxidase anti-peroxidase complex (DAKO) at a 1:30 dilution for 30 minutes, and 3-amino-ethyl carbazole (AEC) was used as chromogen.

A series of controls was used in order to be able to assess the specificity of the immunostaining method and the silver stain technique. The controls were processed in identical fashion and were stained simultaneously and systematically with the rest of the material. In each immunostaining a positive control and a negative control were used. At the same time, whenever possible, an attempt was made to evaluate the presence of structure which were constantly reactive to the immunostain, e.g. chondrocytes.


**Results**

In 100% of the animals we observed countless pigmented lesions immediately under the basal layer of the epidermis which were difficult to classify. As they were small and made up of accumulations of melanophages, we named them «melanic spots». 87.5% of the animals presented numerous lesions of melanocytic origin (1,114 lesions). The majority of these were benign, with 674 areas of melanocytic hyperplasia and 164 of nevus type, which, because of their similarity to nevi in humans, we about its histogenesis, classification, treatrnent and eventual prevention are are strongly positive

Among these were to be (Fig. 4), kidney or adrenal gland (Table 1).

In normal tissues immunoreactivity to the S-100 protein was observed in Schwann cells, in the chondrocytes, in pulmonary bronchioles and in the cutaneous normal melanocytes. In areas with melanocytic lesions induced by the carcinogen we observed that with the Hematoxylin-eosin technique the pigment was of variable presentation in the «melanic spots», areas of hyperplasia, nevi, melanocytic dysplasia and melanomas: abundant melanin pigment was observed in 16 cases and in the remainder it was sparse in the neoplastic cells, though among these were to be seen accumulations of melanin-laden melanophages. This technique was completely negative in all the metastases (Table 1).

With the Masson-Fontana technique the sections were strongly positive in all cases of «melanic spots» and epidermal melanocytic hyperplasia. Its positivity was variable in the nevi and in areas of melanocytic dysplasia. In the melanomas 4 cases were strongly positive, 7 moderately so, 22 lightly positive, and 13 negative. This technique proved negative in 100% of the metastases (Table 1).

With the S-100 protein technique the immunoreactivity was negative in all lesions denominated «melanic spots», 75% of areas with epidermal melanocytic hyperplasia were positive to a greater or lesser degree (Fig. 1) and the remaining 25% were negative. All the nevi were positive to the S-100 Protein to a varying extent: intradermal nevi were strongly positive (Fig. 2), compound and junctional nevi, and blue nevi were moderately positive. The epidermal melanocytic dysplasias were positive to a lesser degree in all areas. The 46 cases of melanoma were reactive to the S-100 protein technique, to a greater or lesser extent (Fig. 3), as were all the metastases, whether in lymph-nodes, lung (Fig. 4), kidney or adrenal gland (Table 1).

**Discussion**

Melanoma remains an enigmatic disease. Questions about its histogenesis, classification, treatment and eventual prevention are still unresolved (Pawlowski and Lea, 1983). To diagnose melanoma is still extremely difficult because of the many factors which affect the course of the illness: age, sex, location and type of melanoma (McGovern, 1982). Melanoma is noted for presenting late-developing metastases and, in certain cases, tumoral dissemination and death in spite of having presented a favourable morphological prognosis in the primary tumour. Traditionally, the silver staining Masson-Fontana technique has been accepted as the most specific for the diagnosis of these lesions.

In the present experimental work with guinea-pigs it was observed that a third of the primary lesions, viewed macroscopically, did not show any melanic pigmentation, and that, with the hematoxylin-eosin stain, in half of the cases melanic pigmentation either was not to be seen or the pigment was observed in small granules in the cellular periphery, seeming to thicken or reinforce the membrane. Using the Masson-Fontana technique, the pigment was seen in only 52.3% of the lesions. This contrasts strongly with the results obtained with the immunohistochemical technique of S-100 protein, in which the immunoreactivity, although variable, was present in benign, premalignant and malignant lesions: it was observed in 75% of melanocytic hyperplasias and in 100% of nevi analyzed, though with greatly varying cellular staining and number of immunostained cells. Similar results have been obtained by Cochran et al. (1982) and by Wen et al. (1983), who saw 100% positivity in 33 human nevi analyzed by each one of the groups, by Pérez-Bacete and Llombart (1986), who saw 95% in 19 cases and by Springall et al. (1983), who saw 83.3% positivity in the six cases analyzed.

100% of the melanomas in the present study, a third of which were amelanotic, and their metastases, which were all amelanotic, were reactive to S-100 protein albeit with great variation in the pattern of immunostaining. This is in agreement with the observations of Cochran et al. (1982) in their analysis of 56 cases, and with those of Wen et al. (1983) (33 cases), and Pérez-Bacete and Llombart (1986) (23 cases). However, Rode and Dhillon (1984) describe 85% positivity in the 14 cases analyzed by them.

**Table 1. Distributions of staining technique in induced melanotic lesions**

<table>
<thead>
<tr>
<th></th>
<th>Hematoxylin-Eosin</th>
<th>Masson-Fontana</th>
<th>S-100 Protein</th>
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<tbody>
<tr>
<td>Melanotic spots</td>
<td>+++</td>
<td>+++</td>
<td>- to +</td>
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<tr>
<td>Hyperplasias</td>
<td>-to +</td>
<td>+ to +</td>
<td>+ to + +</td>
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<tr>
<td>Nevi</td>
<td>+ + +</td>
<td>+ to + + +</td>
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<tr>
<td>Dysplasias</td>
<td>- to +</td>
<td>+ to + + +</td>
<td>+ to + +</td>
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<tr>
<td>Melanomas</td>
<td>- to +</td>
<td>- to + + +</td>
<td>+ to + + +</td>
</tr>
<tr>
<td>Metastases</td>
<td>-</td>
<td>-</td>
<td>+ to + + +</td>
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(- negative; + lightly positive; ++ moderately positive; +++ strongly positive)
**S-100 protein in induced melanotic lesions**

**Fig. 1.** Melanocytic Hyperplasia: Detail of epidermal melanocytes stained by S-100 protein. × 300

**Fig. 2.** Intradermal nevus cells showing positive stain by S-100 protein. × 300

**Fig. 3.** Malignant melanoma: the cytoplasm of the melanocytes is stained by the S-100 protein. × 300

**Fig. 4.** Lung metastasis: detection of S-100 protein in amelanotic cells. × 500
We believe that, for diagnosis, these facts confirm the enormous practical value of the immunohistochemical techniques with monoclonal antibodies, not only in melanic lesions induced experimentally but also, in human lesions, especially in amelanotic melanomas.

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References


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