Reticulin immunostaining revisited

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ABSTRACT

Introduction: Reticulin staining has been suggested as an inexpensive tool in the differential diagnosis of melanoma versus benign nevi. In the present study, reticulin immunostaining patterns in malignant melanomas, benign intradermal nevi, and blue nevi were observed. The concordance in evaluation of the pattern between observers was also done. **Materials and Methods:** A retrospective search was performed in the computer database of the Ackerman Academy of Dermatopathology for "melanoma," "melanocytic nevus," and "blue nevus". Fifty-six melanomas (30 of nodular subtype and 26 of superficial spreading subtype), 54 benign compound nevi, and 27 blue nevi were selected for the study. Patterns of reticulin staining in the dermis and the basement membrane in these melanocytic lesions were evaluated and the concordance between the two groups of authors was assessed. Statistical evaluation was performed with the Statistica® 10 program, Tulsa, OK. Concordance of the pattern evaluation was evaluated using Cohen's kappa coefficient. **Results:** Melanomas show a variable basement membrane pattern some of which show flat, thin and smooth pattern. Benign nevi almost never showed this flat pattern at the basement membrane zone. In the dermis, melanomas showed reticulin fibers surrounding groups of melanocytic cells while nevi predominantly had reticulin fibers around individual cells. There was greater agreement in evaluating the dermal component compared to the basement membrane pattern. **Conclusion:** The dermal reticulin staining pattern may be of some value in the diagnosis of melanocytic lesions, but poor concordance in evaluation of the basement membrane zone pattern limits its usefulness.

Key words: Benign nevi, blue nevi, melanoma, reticulin, reticulin stain

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INTRODUCTION

Reticulin staining has been suggested as an inexpensive tool in the differential diagnosis of melanoma versus benign nevi.[1-3] The pattern of reticulin staining in benign nevi has been reported to include an intact basal membrane (BM) as well as reticulin fibers surrounding individual cells. In melanoma, thinning or disruption of the BM is seen, as well as reticulin fibers predominantly around melanocytic nests rather than individual cells.[1,3] Recently, a reticulin immunostain was suggested as a means of distinguishing between nevi and metastatic melanoma in lymph nodes.[4] In the present study, we observed reticulin immunostaining patterns in malignant melanomas, benign intradermal nevi, and blue nevi. We also evaluated the concordance in evaluation of the pattern between observers.

MATERIALS AND METHODS

A retrospective search was performed in the computer database of the Ackerman Academy of Dermatopathology for "melanoma," "melanocytic nevus," and "blue nevus". Fifty-six melanomas (30 of nodular subtype and 26 of superficial spreading subtype), 54 benign compound nevi, and 27 blue nevi were selected for the study. Diagnosis was established using HE staining; melanocytic markers (S100, MelanA, Mitf were used on several occasions). The specimens were re-evaluated by the senior author and the diagnosis was confirmed. The paraffin embedded tissue was cut at 2 microns and stained with reticulin (DAKO Reticulin stain kit, Carpinteria, CA, USA). The dermal pattern of reticulin fibers and BM characteristics were evaluated independenly by two groups

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of authors. The following criteria were used for evaluation of the BM (thick and spiky BM, thin and flattened BM, mixed pattern, difficult to evaluate) and for dermal reticulin fibers (fibers predominantly around melanocytic nests, fibers predominantly around individual cells, mixed pattern, and difficult to evaluate).

Statistical evaluation was performed with the Statistica® 10 program, Tulsa, OK. Concordance of the pattern evaluation was evaluated using Cohen's kappa coefficient. The interpretation of the results was as follows: <0, poor agreement; 0.01–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and 0.81–0.99, almost perfect agreement.[5]

RESULTS

The results of the evaluation by two appraisers are presented in table 1. Melanomas showed a variable BM pattern. The thin and smooth BM pattern was more frequently seen in nodular melanomas [Figure 1]. Benign nevi usually presented with the thick and spiky BM [Figure 2] or mixed BM pattern, but there was poor agreement between investigators in the assessment of BM pattern stained by reticulin (kappa = −0.14). The most consistent finding was that benign nevi almost never had a flattened BM [Table 1].

No melanomas showed the pattern of reticulin fibers surrounding only individual cells [Figures 1 and 3]. On the contrary, nevi predominantly had reticulin fibers around individual cells [Figure 2 and 4] or showed a mixed pattern, with reticulin fibers surrounding larger nests in the upper part of the nevus and around individual cells in the base of the lesion. Assessment of the dermal reticulin pattern showed moderate agreement between investigators (kappa = 0.5).

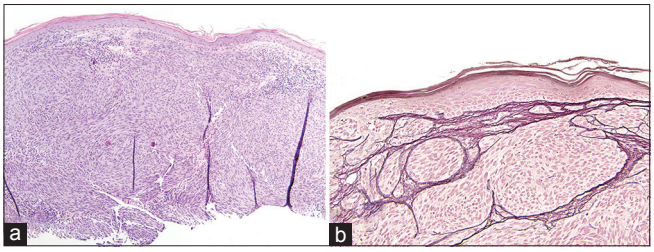

Figure 1: (a) Nodular melanoma, H and E, ×10. (b) Nodular melanoma exhibiting thin and smooth BM and fibers highlighting large confluent melanoma nests in the dermis, reticulin stain ×100

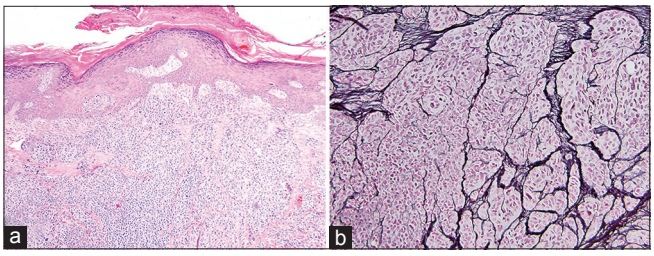

Figure 3: (a) Nodular melanoma, H and E, ×10. (b) Reticulin stain pattern surrounding large nests of nodular melanoma, reticulin stain ×100

DISCUSSION

Melanoma–stroma interactions may play an important role in the tumor's ability to grow and metastasize.[6] Melanoma cells participate in the synthesis of some BM proteins as well as collagen.[7] Elastic fibers have been shown to be displaced in melanoma in contrast to benign nevi.[8] The frequency of the various patterns of reticulin staining between melanocytic nevi and melanoma has been studied previously,[1,9] but is not widely known or used. The main goal of this study was to evaluate the usefulness of reticulin staining in this setting as well as the degree of concordance between investigators evaluating the pattern.

Leviatan and Steiglender have shown that the number of reticulin fibers is decreased in melanomas, but increased in the surrounding dermis.[9] Briggs compared melanomas, banal nevi, and Spitz nevi, and suggested that the presence of an intact

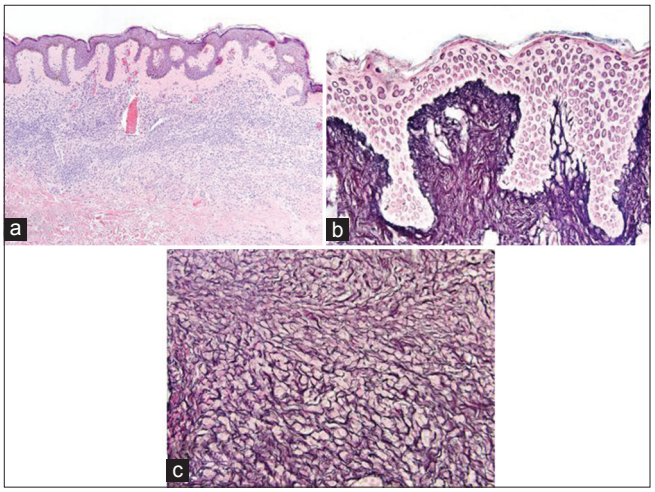

Figure 2: (a) Intradermal nevus, H and E, ×10. (b) Intradermal nevus exhibiting thick and spiky BM, reticulin stain ×100. (c) Reticulin pattern stain surrounding individual melanocytes in the dermis, reticulin stain ×100

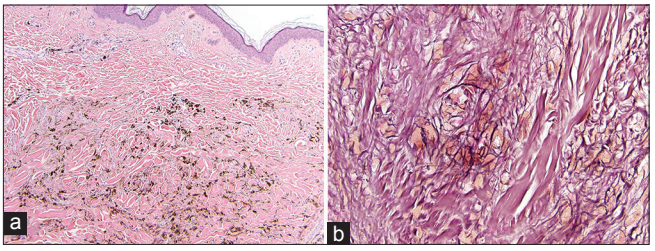

Figure 4: (a) Blue nevus, H and E, ×20. (b) Reticulin fibers surrounding individual cells in blue nevus, reticulin stain ×40

Table 1: Evaluations of the BM and dermal reticulin pattern by two appraisers

Patterns of reticulin staining	Nodular MM *N* (%)		SS-MM *N* (%)		Compound nevi *N* (%)		Blue nevi *N* (%)		Concordance
BM patterns	1	2	1	2	1	2	1	2	−0.14
Thinned flattened BM	4 (13.3)	8 (26.7)	9 (34.6)	6 (23.1)	0	0	1 (3.7)	0	
Thick spiky BM	17 (56.7)	4 (13.3)	9 (34.6)	1 (3.9)	54 (100)	14 (25.9)	26 (96.3)	4 (14.8)	
Dermal patterns									
Mixed pattern of BM	2 (6.7)	10 (33.3)	5 (19.33)	16 (61.5)	0	38 (70.4)	0	23 (85.2)	
Non-defined BM pattern	7 (23.3)	8 (26.7)	3 (11.5)	3 (11.5)	0	2 (3.7)	0	0	
Dermal reticulin fibers pattern									0.5
Reticulin fibers purely around nests	29 (96.7)	23 (76.7)	19 (73.1)	14 (53.8)	0	2 (3.7)	0	0	
Reticulin fibers around individual cells	0	0	0	0	27 (50.0)	12 (22.2)	15 (55.6)	25 (92.6)	
Mixed pattern of dermal reticulin fibers	1 (3.3)	7 (23.3)	7 (26.9)	10 (38.5)	27 (50.0)	38 (70.4)	0	1 (3.7)	
Nondefined dermal pattern	0	0	0	2 (7.7)	0	2 (3.7)	12 (44.4)	1 (3.7)	

BM: Basal membrane, M: Melanoma

BM and reticulin fibers surrounding individual melanocytes are strong indicators of a benign lesion.[1] Histopathological subtypes of melanoma and nevi were not specified in the study. A Pubmed search failed to reveal any previous studies that addressed interobserver concordance in the assessment of reticulin staining patterns, and we found no prior studies that included blue nevi.

The thick and spiky BM pattern was observed in both benign nevi and nodular melanomas. In contrast, melanomas with a prominent junctional component "flattened" the BM pattern creating a thin smooth pattern of reticulin staining. In contrast to the Briggs' study we rarely identified the thin and smooth BM pattern in benign compound nevi despite the presence of a junctional component. The reticulin stain demonstrates a maturation pattern in benign nevi, as the fibers surround larger nests in the papillary dermis and individual cells in the reticular dermis, whereas in melanomas the fibers surround large bulky nests throughout the tumor. Our concordance data indicate a greater agreement in evaluating the dermal component compared with the BM component, suggesting that the latter may be of limited value in actual practice. In contrast, the dermal staining pattern may be of some value in the diagnosis of melanocytic lesions. However, it is the authors' belief that there is limited benefit in performing the reticulin stain as the dermal staining patterns can already be appreciated on H and E. The relatively small number of biopsies is a limitation of the present study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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