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MITF Accurately Highlights Epidermal Melanocytes in Atypical Intraepidermal Melanocytic Proliferations

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Abstract

Atypical intraepidermal melanocytic proliferations (AIMP) have random cytologic atypia and other histologic features that are concerning for malignancy and often require immunohistochemistry to differentiate from melanoma in situ. Immunostaining with S100, Melan-A, and MITF was performed for forty-nine morphologically well-characterized AIMP lesions. The percentage of cells in the basal layer of the epidermis that were identified as melanocytes by immunohistochemistry was compared to the percentage observed by morphology on hematoxylin and eosin staining, which is the gold standard stain for identifying cytologic atypia within an AIMP. Melan-A estimated the highest percentage of melanocytes and S100 the fewest in 47 of the 49 lesions examined. The estimated percentage of melanocytes was 23.3% (95% CI: 18.6, 28.1; $p < 0.001$) higher for Melan-A compared to hematoxylin and eosin staining. Melanocyte estimates were similar for H&E and MITF ($p = 0.15$) while S100 estimated 21.8% (95% CI: –27.2, –16.4; $p < 0.001$) fewer melanocytes than H&E. Melan-A staining produces higher estimates of epidermal melanocytes than S100 and MITF, which may increase the likelihood of diagnosing melanoma in situ. In contrast, melanoma in situ may be underdiagnosed with the use of S100, which results in lower estimates of melanocytes than the other two immunostains. Therefore, the best immunohistochemical marker for epidermal melanocytes is MITF.

Keywords/Phrases

Atypical Intraepidermal Melanocytic Proliferation (AIMP); Immunohistochemistry; Melan-A; Microphthalmia-Associated Transcription Factor (MITF); S100; Melanoma in situ

Introduction

The presence of cytologic atypia or pagetoid scatter within a melanocytic proliferation overlying sun-damaged skin can be highly concerning for evolving melanoma in situ (MIS). In order to distinguish benign lesions from those that are malignant, immunohistochemical stains are often used to look for a lentiginous proliferation of melanocytes along the dermal-

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epidermal junction, which is suggestive of the radial growth phase of melanoma. In cases where immunohistochemical staining and the histologic features of the lesion are insufficient for a definitive diagnosis of cancer, a descriptive diagnosis of atypical intraepidermal melanocytic proliferation (AIMP) is reported, which informs the clinician that the lesion has certain features that are concerning for evolving melanoma and that it should be widely excised. The most commonly used immunohistochemical stains for distinguishing between AIMP and MIS are S100, Melan-A, and MITF. However, each immunostain has its own advantages and limitations.

The S100 antigen is expressed in melanocytes making it an excellent marker for metastatic melanoma. However, the diagnostic utility of this immunostain is limited by broad expression of its target antigen in other cell types of neural crest origin and its failure to stain up to 7% of melanomas.¹⁻⁶ Melan-A (and its closely related antigen MART-1) is a commonly used alternative to S100 because of its perceived high sensitivity and specificity for melanocytes. Recent studies, though, have suggested that Melan-A overestimates the number of melanocytes in solar lentigenes and MIS, and stains keratinocytes in the setting of inflammation.⁷⁻¹⁴ Microphthalmia-associated transcription factor (MITF) is a newer melanocytic marker that may allow for more accurate estimation of the number of melanocytes within the epidermis.¹⁴ It is a nuclear antigen that encounters less interference from cytoplasmic melanin pigment on routine immunohistochemical staining using DAB chromogen and may have the advantage of providing valuable prognostic information for the treating clinician as higher levels of this protein are found in metastatic melanoma and are associated with a decreased response to chemotherapeutics.¹⁵

In this study, we evaluated the percentage of cells stained by S100, Melan-A, and MITF for a cohort of AIMP lesions. Each immunostain was compared to the percentage of epidermal melanocytes estimated to be present by conventional hematoxylin and eosin staining. We hypothesized that there would be discrepancies in the number of epidermal melanocytes estimated by each antibody. The question of staining variability in AIMP lesions has not previously been evaluated and is especially germane to pathologists reviewing these cases as the extent of epidermal staining may influence the decision to call a lesion benign or malignant.

Materials and Methods

Atypical Intraepidermal Melanocytic Proliferation

The descriptive diagnosis of atypical intraepidermal melanocytic proliferation is specifically used for melanocytic lesions that have random cytologic atypia with one or more of the following histologic features: upward migration of melanocytes within the epidermis and melanocyte contiguity. The diagnosis of melanoma in situ was avoided in these cases because these lesions failed to demonstrate uniform atypia and a continuous proliferation of melanocytes along the dermal-epidermal junction on routine hematoxylin and eosin (H&E) staining. Morphologic evaluation on H&E sectioning is the standard technique for identifying cytologic atypia and near confluent growth along the dermal-epidermal junction, which characterize AIMP. In this study we chose morphologically well-characterized AIMP lesions and compared the detection of various melanocytic markers with the number of morphologically recognizable melanocytes on H&E, which was used as a reference or “gold standard” for melanocyte detection in AIMP.

Cases

Tissue samples (N=49) of morphologically well characterized AIMP biopsied between 2005 and 2010 were retrieved from the archives of the Department of Pathology and Laboratory

Medicine at the Hospital of the University of Pennsylvania. Slides were pulled for these cases and reviewed to confirm the diagnosis in each case and tissue blocks were cut for immunostaining.

Immunostaining

All tissue was fixed in phosphate-buffered neutral formaldehyde (10%) for 12–36 hours and embedded in paraffin. Four-micron thick sections were mounted on Probe-on slides and used for immunohistochemical staining. Antibodies against S100 (polyclonal, 1:3000, Dako, Carpinteria, CA, USA), MITF (clones D5 and C5, 1:200; NeoMarkers, Fremont, CA, USA), and Melan-A (clone A103, 1:25, Dako, Carpinteria, CA, USA) were applied after antigen retrieval and a standard avidin–biotin complex method was used on an autostainer (Techmate 1000, Ventana/Biotek Medical Systems). Slides were microwaved for 4 minutes twice in 1× HIER buffer (Lab Vision, Fremont, CA, USA) for Melan-A and boiled in 1× EDTA buffer (Lab Vision, Fremont, CA, USA) for 20 minutes for MITF. Immunoreactivity was evaluated under the light microscope and was considered specific when cytoplasmic for S100 and Melan-A, and nuclear for MITF. For practical reasons cases were considered positive only when unequivocal specific reactivity was found in a minimum of 5% of the tumor cells. Epithelioid melanomas known to express the above markers were used as positive controls. A section of each block was incubated with normal mouse serum (prediluted, Vantana, AR, USA) for our negative controls.

Assessment and Statistical Analysis

Three pathologists independently examined each of the slides and were blinded to the results of their colleagues. Each reviewer estimated the percentage of cells within the basal layer of the epidermis that were melanocytes at 200–400X magnification. All percentages reported in this paper refer to the basal layer of the epidermis and were calculated in lesional areas that were identified by the reviewer to have the highest density of melanocytes. For each specimen, we calculated the difference between the percentage of melanocytes estimated by immunostaining and the percentage estimated by morphology on H&E. The mean difference was reported for each melanocyte stain (vs. H&E) and the statistical significance of this calculation was evaluated using a Wilcoxon signed rank test after standard diagnostics revealed that not all of the immunostains had a normal distribution of values. All statistical analyses were performed using the computer program Stata 11 (StataCorp, College Station, TX).

Results

This study examined 49 AIMP lesions collected between 2005 and 2010 by the Department of Pathology at the Hospital of the University of Pennsylvania. Thirty-one of the lesions were biopsied from women and eighteen were biopsied from men. The mean patient age was 53 years old (range: 14–94). Case lesions were taken from many different parts of the body including the head and neck (n=13), abdomen/chest/back (n=12), extremities (n=11), and genitalia (n=13). Insufficient tissue existed for two of the cases to perform all three immunohistochemical stains. Melan-A was selected for one of these cases and MITF and S100 for the other in order to balance the use of the different melanocyte stains.

Melan-A estimated the highest proportion of melanocytes and S100 the fewest in 47 of the 49 lesions (Figure 1, Figure 2, Figure 3). The estimated percentage of melanocytes was 23.3% (95% CI: 18.3, 28.3; $p<0.001$) higher for Melan-A compared to hematoxylin and eosin staining. Melanocyte estimates were similar for H&E and MITF ($p=0.15$) while S100 estimated 21.8% (95% CI: –27.5, –16.1; $p<0.001$) fewer melanocytes than H&E (Table 1).

Discussion

Atypical intraepidermal melanocytic proliferations are frequently encountered in clinical practice and can be difficult to distinguish from melanoma in situ.¹⁶ Immunohistochemical staining is an important diagnostic tool utilized by pathologists to help them make this distinction. However, certain markers and their variable staining may affect these diagnoses by misrepresenting the number of melanocytes present. Our study follows up on recently published data showing that Melan-A overestimates the number of melanocytes within sun-damaged skin.¹³ We sought to determine if these findings for Melan-A were also true for AIMP lesions by comparing this antibody to other commonly used immunostains for melanocytic tumors along with traditional H&E staining.

Melan-A appeared to stain the greatest number of cells in 47 of the 49 specimens examined and the estimated percentage of epidermal melanocytes by Melan-A was 23.3% higher than the percentage observed on H&E. These findings suggest that Melan-A overestimates the number of melanocytes in AIMP compared to S100, MITF, and H&E. This finding is consistent with previous studies examining the number of cells stained in pigmented actinic keratoses, solar lentigenes, and MIS by several of the most commonly used melanocyte stains in clinical practice.^{8,13} Previous studies have hypothesized that Melan-A is taken up by a small percentage of keratinocytes in addition to its target cell, the melanocyte.⁹ This could partially explain the increased amount of staining observed with Melan-A. However, we believe the distribution of the immunostain target antigen within cells may provide an alternative explanation for our observations. Both Melan-A and S100 are cytoplasmic proteins. Melanocytes have long dendritic processes of cytoplasm that encircle nearby keratinocytes to help facilitate the transfer of melanin pigment to these cells (Figure 4). Consequently, the staining of antigen within these cytoplasmic extensions may encircle neighboring keratinocytes and thus artificially increase the perceived number of melanocytes. In addition, we have also recently observed Melan-A reactivity in some schwannomas in our practice (data not shown). The melanocytic specificity of Melan-A is also questioned in those cases.

MITF staining of AIMP lesions estimated a similar number of melanocytes as morphologic evaluation on H&E. In addition to its high concordance with morphologic assessment in identifying melanocytes, the MITF immunostain may also be the easiest to interpret amongst the three because of its clean nuclear expression and minimal interference from cytoplasmic melanin pigment. Furthermore, because of its nuclear location, it would not stain dendritic processes of melanocytes, which could “pseudostain” the adjacent keratinocytes as in the case of Melan-A staining. Therefore, it may be the most helpful and reliable melanocytic marker in the epidermis for these melanocytic lesions (Figure 4). MITF along with the other immunohistochemical stains also has the advantage over H&E of helping pathologists to identify melanocyte pagetoid scatter within the epidermis, which can be a concerning histologic feature for melanoma in situ.

S100 is also predominantly a cytoplasmic antigen, and is well known for its high sensitivity in melanocytes. Our finding of significantly fewer S100 positive melanocytes in the epidermis than those counted on H&E stains or those detected by MITF is interesting (Figure 2, Figure 3). S100 negative melanocytes and melanomas are well documented.¹⁷ The decreased staining with S100 in the epidermal melanocytes may reflect reduced expression of this antigen within AIMP lesions. Thus, this finding suggests that epidermal melanocytes in AIMP express MITF, a master transcription factor required in early melanogenic differentiation, but not S100. The other possibility for this finding would be poor affinity of the S100 polyclonal antibody for its target antigen.¹⁷ However, this is unlikely since this antibody is one of the most commonly used in-vitro diagnostic (IVD)

antibodies. It has been used in our laboratory for a long time and such decreased affinity or sensitivity has not been observed in other types of specimens and lesions. The biologic significance of these MITF positive, S100 negative melanocytes/premelanocytes in AIMP remains to be clarified. In any case, caution should be taken when using S100 to interpret AIMP as this immunohistochemical stain may inappropriately reduce the number of melanocytes seen in these lesions leading to difficulty in reaching the correct diagnosis.

Overall, our findings confirm the results of similar recent studies showing that Melan-A/MART-1 may overestimate melanocytic staining in intraepidermal melanocytic lesions and that MITF may be the most reliable marker in this setting. This study is the first to extend these claims to AIMPs and highlights the potential concern that staining these lesions with Melan-A may cause pathologists to overdiagnose melanoma in-situ. Additionally, S100 staining in our study was far lower than the other two immunostains and H&E, which suggests that this stain is unreliable in AIMPs.

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Figure 1. Representative Photos of Immunostaining in AIMP Lesions

Representative photos of immunohistochemical staining at high and low magnification for three representative cases. The intensity of staining was highest for Melan-A and lowest for S100. MITF staining was of intermediate intensity compared to the other two antibodies.

Figure 2. Percentage of Melanocytes in the Basal Layer of the Epidermis

Figure 2 shows the distribution of percentages for each of the three immunohistochemical stains and H&E staining. Melan-A estimated a higher percentage of melanocytes than S100 and MITF. S100 consistently stained fewer cells in the basal layer of the epidermis than the other two immunostains.

Figure 3. The Estimated Percentage of Melanocytes in the Basal Layer of the Epidermis, Immunohistochemistry vs. H&E

The percentage of melanocytes in the basal layer of the epidermis was estimated by immunohistochemistry (Melan-A, MITF, S100). These percentages were then compared to the percentage observed on H&E and the difference in estimates is represented in the above graph. Figure 3 illustrates that Melan-A consistently estimates a higher percentage of melanocytes than the other two immunostains and H&E. S100 stained the fewest cells in 47 of the 49 cases reviewed.

Figure 4. “Pseudostaining” in the Epidermis with Melan-A

Figure 4 is an illustration of immunostaining with Melan-A (top panel) and MITF (lower panel). Basal keratinocytes are surrounded by melanocyte dendritic processes stained by Melan-A, which may be misinterpreted as an increased number of melanocytes (“pseudostaining”). MITF only stains the nucleus of melanocytes and is therefore often easier to interpret than Melan-A under the microscope.

Table 1
Mean Difference of the Estimated Percentage of Melanocytes by Immunostaining Compared to H&E

The percentage of stained cells in the basal layer of the epidermis identified by immunohistochemistry was compared to H&E for all 49 specimens and the mean difference for each immunostain is listed in Table 1. Positive percentages indicate that a particular immunostain estimated a higher number of melanocytes than H&E with the reverse being true for negative percentages. P-values were calculated by performing a Wilcoxon signed rank test comparing the percentage of melanocytes estimated by each immunostain to the percentage estimated on H&E for all 49 specimens.

Immunostain	Mean Difference (Immunostain vs. H&E)	95% CI	P value
Melan-A	23.3%	18.3,28.3	<0.001
MITF	1.5%	-3.6,6.7	0.27
S100	-21.8%	-27.5, -16.1	<0.001
H&E	N/A	N/A	N/A