Original Research

Preferentially Expressed Antigen in Melanoma (PRAME) Staining on Eyelid Skin: Comparison with SOX-10 Immunohistochemistry to Establish a Normal Baseline for Evaluating Periocular Melanoma Specimens

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The management of periocular melanoma is fraught with difficulty because of the desire to conserve tissue while at the same time ensuring complete removal of neoplastic cells. Chronically sun damaged skin of the face and eyelids is associated with melanocytic hyperplasia that can complicate the histologic interpretation of clear margins. The SRY-Box Transcription Factor 10 (SOX-10) gene is relatively specific for melanocytes within the epidermis but does not differentiate between benign and malignant melanocytes. Preferentially expressed antigen in melanoma (PRAME) is a relatively new marker that may be useful in differentiating benign from malignant melanocytic proliferations. Our aim was to determine baseline staining for PRAME compared to SOX-10 on eyelid skin that does not contain melanoma or junctional melanocytic proliferation to guide margin assessment. We performed a retrospective review of histopathologic specimens of the eyelid. The most recent fifty specimens that did not include a diagnosis of melanoma or a junctional melanocytic proliferation with ≥ 1 mm of normal epidermis were included (n = 50). The first 1 mm of epidermis with a relatively flat surface from the left margin of the skin sample was assessed, and the mean number of cells in the 1 mm window that stained positive for PRAME and SOX-10 was counted. There were on average 28.06 more SOX-10-stained cells than PRAME-stained cells (95% CI 24.83 - 31.29, p < 0.0001). Although SOX-10 staining intensity varied considerably in normal eyelid tissue, PRAME staining was minimal and did not exceed 3 cells and may be more likely with increased age. When PRAME staining is sustained at a rate higher than 3/mm, melanoma in situ should be considered and additional analysis may be warranted. Expression of PRAME detected by immunohistochemical staining can aid in the diagnosis of melanoma arising in a precursor nevus, primary acquired melanosis of the conjunctiva, and conjunctival melanoma.
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Key Words: *Melanoma in situ; lentigo maligna; immunohistochemistry; eyelid; Preferentially expressed Antigen in Melanoma (PRAME); margin; excision; SOX-10; junctional melanocytic proliferation; melanocyte; immunohistochemistry*

INTRODUCTION

The utility of immunohistochemical (IHC) studies in assisting in the differential diagnosis of melanoma in situ from histologic mimics such as pigmented actinic keratosis, solar lentigo, and precancerous conjunctival lesions is well established.[1,2] The SRY-Box Transcription Factor 10 (SOX-10) gene is involved with neural crest cells and is relatively specific for melanocytes within the epidermis but does not differentiate between benign and malignant melanocytes. IHC staining for preferentially expressed antigen in melanoma (PRAME) is a relatively new marker to the diagnostic armamentarium that has only recently become commercially available. PRAME staining is useful in differentiating benign from malignant melanocytic proliferations, but false positive and false negative staining occurs.[3,4,5] Eyelid skin has a high density of melanocytes and is often exposed to extensive actinic damage.6 Sun damage can be characterized by attributes such as thinning skin, solar elastosis, and telangiectatic blood vessels, and is more prevalent in elderly populations. Margins of resection for melanoma in situ on the eyelid are constrained by cosmetic and functional considerations. Ophthalmic pathologists and dermatopathologists need to be able to evaluate margins with confidence and understanding normal baseline staining for PRAME in eyelid skin without clinical or histologic melanocytic neoplasia will help clarify expected staining patterns.

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METHODS

We performed a retrospective review of histopathologic specimens of the eyelid that did not include a diagnosis of melanoma or a junctional melanocytic proliferation. The most recent fifty specimens with 1 mm or more of uninvolved epidermis were identified and reviewed to ensure the presence of histologically normal epidermis upon routine microscopic examination of hematoxylin and eosin-stained sections (n = 50). The specimens were processed into slides that were de-identified. PRAME (Biocare Medical) and SOX-10 (StatLab) stains were then performed according to the manufacturer protocol, using full staining of nuclei with the EnVision FLEX HRP Magenta Substrate Chromogen System (Dako Omnis). The first 1 mm from the left margin of the skin sample with a relatively flat surface was assessed, and the number of cells that stained positive for PRAME and Sox-10 was counted. Cutaneous appendages were not included. Mean staining and standard deviation were calculated using Microsoft Excel. The mean staining of PRAME and SOX-10 were compared via unpaired t-test using GraphPad Prism software.

RESULTS

SOX-10 immunostaining of melanocytes revealed considerable variation in melanocytes (mean 28.34; standard dev = 11.49) (**Figures 1a, 1b**).

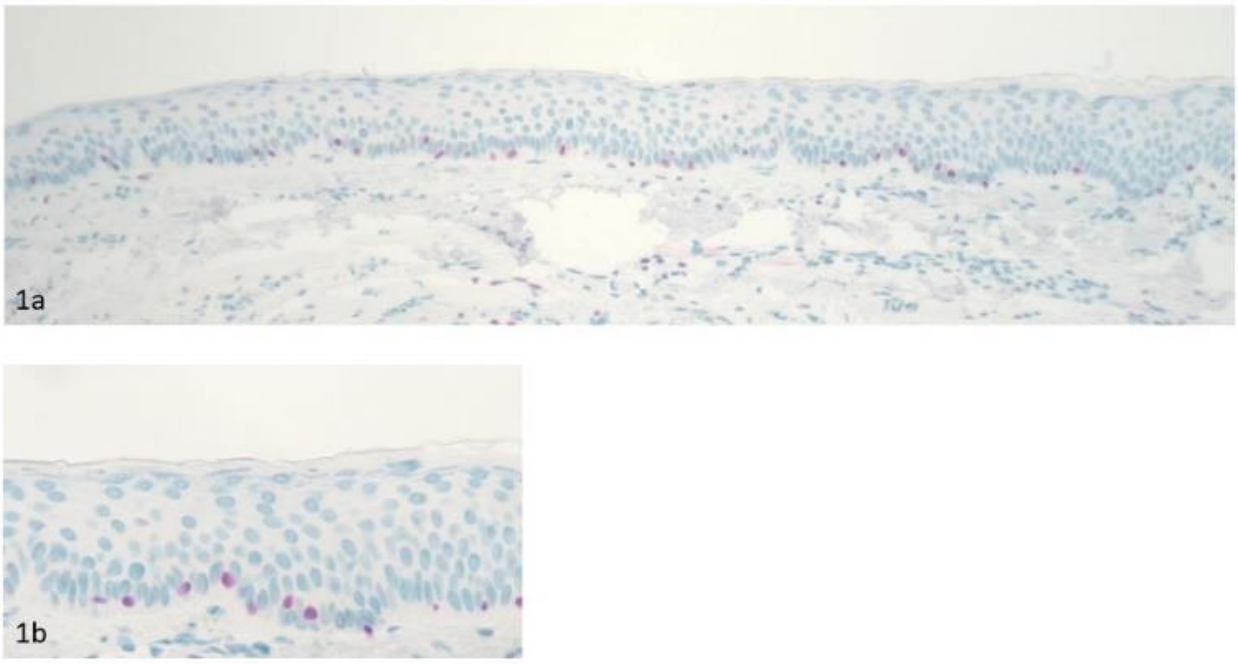

Figure 1. SOX-10 staining Immunohistochemical staining for SOX-10 decorates single melanocytes along the dermal epidermal junction in a fairly uniform manner. **1a**) Original magnification 200x, **1b**) Original magnification 400x.

PRAME staining was absent or negligible in normal specimens, never exceeding 3 (mean 0.28; standard dev = 0.81) (**Figures 2a, 2b**).

There were on average 28.06 more SOX-10-stained cells than PRAME-stained (95% CI 24.83-31.29, $p < 0.0001$) (**Table 1**).

The average age of patients exhibiting PRAME staining was 72.83 years (standard dev = 18.84), whereas the average age of patients who did not exhibit PRAME staining was 66.41 years (standard dev = 14.31) The mean age difference of patients who exhibited PRAME staining versus those who did not was 6.42 years, but this was not found to be statistically significant ($p = 0.3252$, 95% CI -6.57 - 19.42).

Incidentally, twenty-four out of the fifty specimens were excisional specimens for basal cell carcinoma. Other observations included strong staining with PRAME of all sebaceous lobules, staining of 25% of the basal cell carcinomas, and staining of a hidrocystoma (**Figures 3a-3d**).

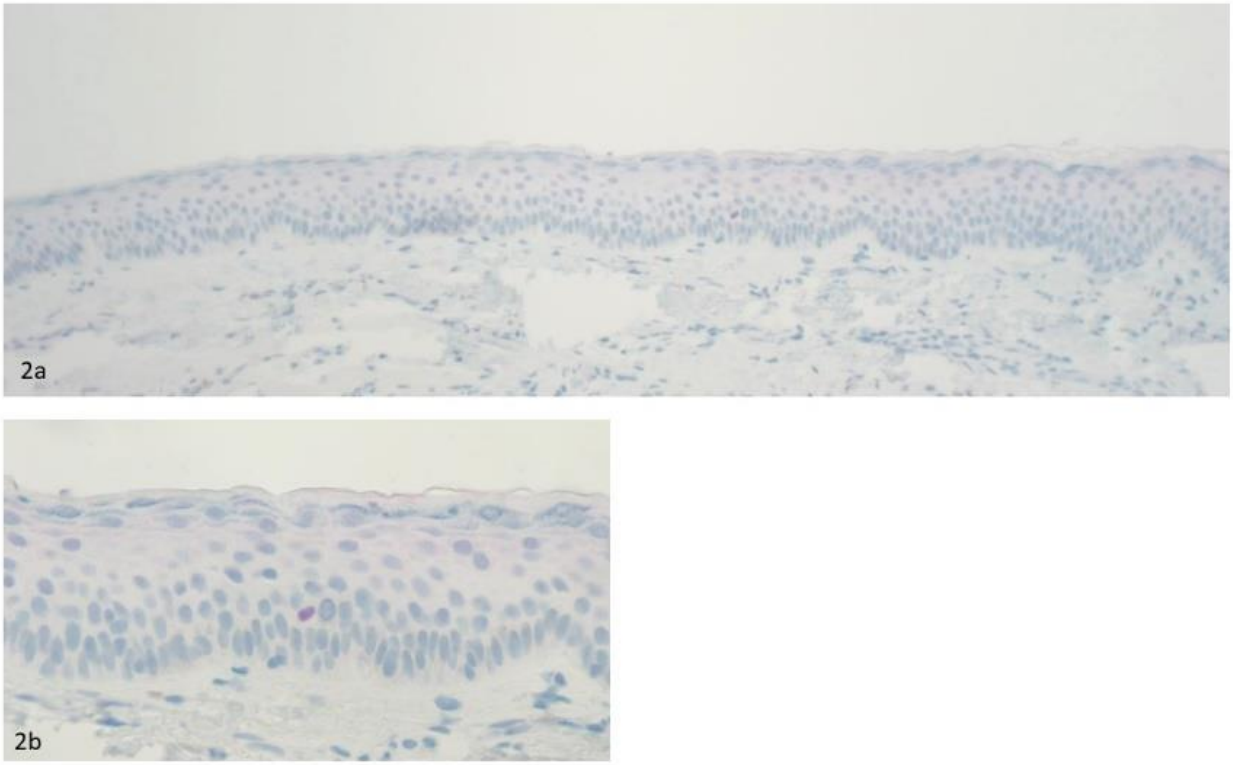

Figure 2. PRAME staining Immunohistochemical staining for PRAME decorates only a single melanocyte **2a**) Original magnification 200x, **2b**) Original magnification 400x.

Table 1. PRAME and SOX-10 Staining in a 1 mm window.

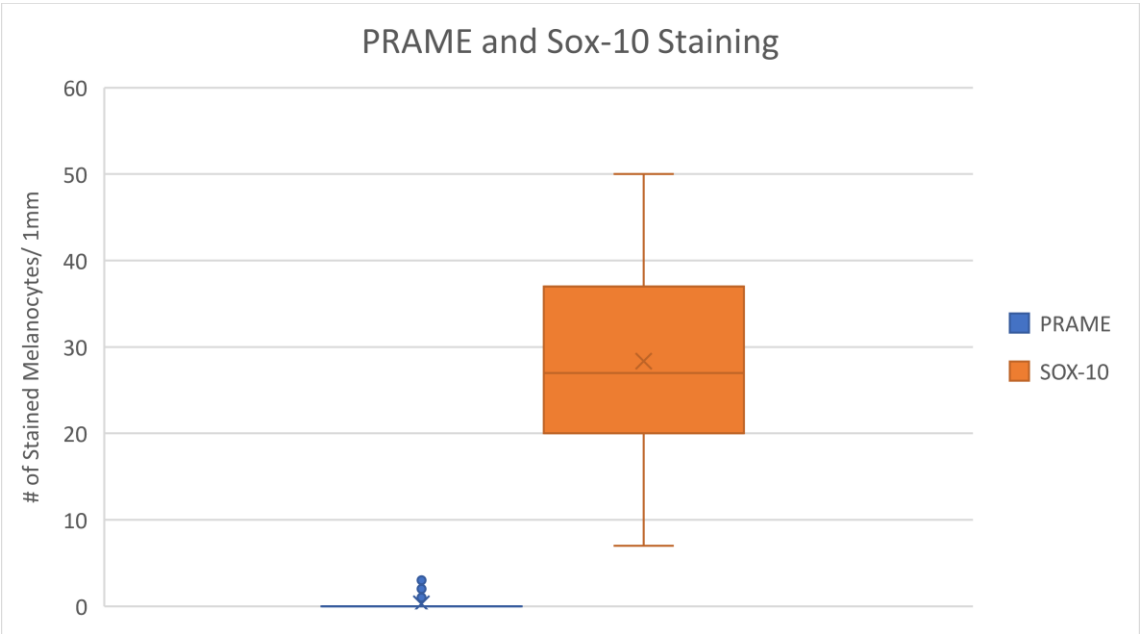

Table Legend. PRAME staining is represented in blue with mean = 0.28 cells, standard dev = 0.81 cells. SOX-10 staining is represented in orange with a mean = 28.34 cells, standard dev = 11.49. There were on average 28.06 more SOX-10-stained cells than PRAME-stained cells (95% CI 24.83 - 31.29, $p < 0.0001$).

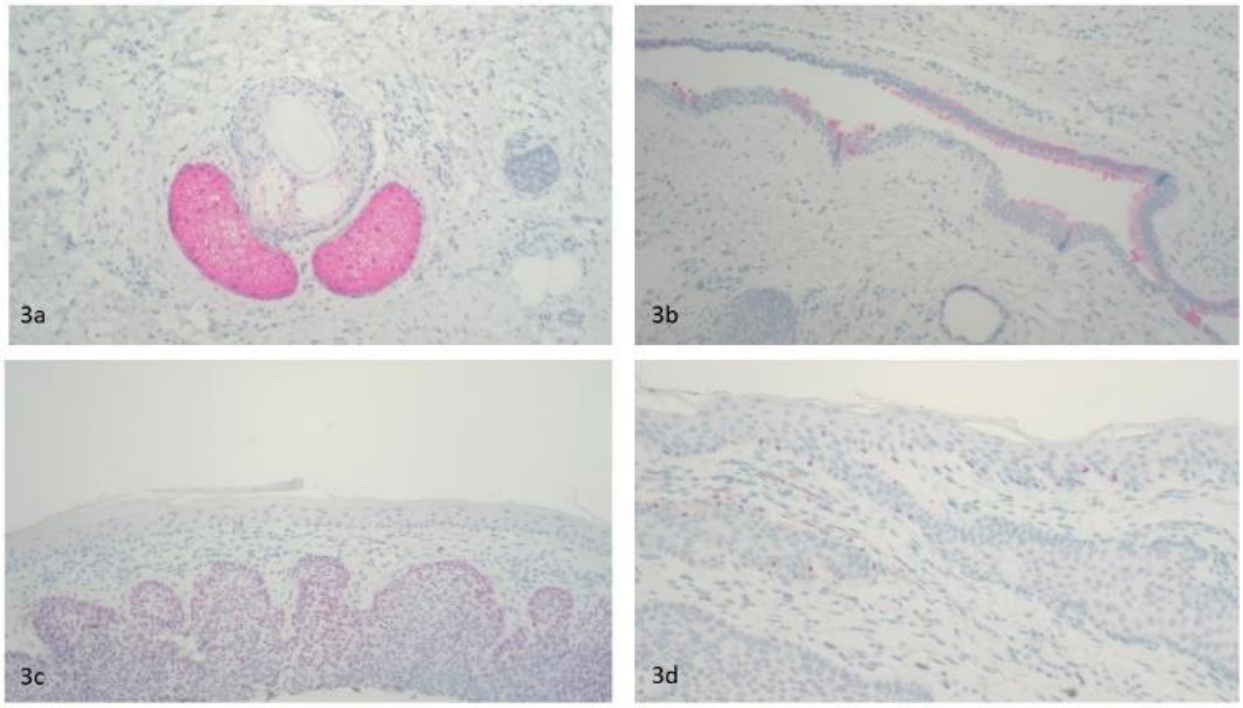

Figure 3. Incidental PRAME Findings PRAME staining can be seen in **3a**) sebaceous lobules **3b**) basal cell carcinoma **3c**) hidrocystoma **3d**) or in occasional clusters of unknown significance. (Original magnification 200x)

DISCUSSION

Understanding the staining characteristics of clinically normal (although potentially actinically damaged) skin without other obvious melanocytic pathology is important to avoid over diagnosing melanoma in situ. SOX-10 has been shown to be a specific and helpful stain for identifying melanocytes.[7] PRAME staining can be very helpful in identifying melanoma.[3] We have shown that occasional staining of melanocytes with PRAME can occur in the absence of melanocytic neoplasia on clinical and routine histologic inspection, but that this staining is minimal. The occasional clusters of PRAME-positive cells in the absence of melanocytic neoplasia arose in a generally older population with sun damaged skin. However, the difference in age was found to be statistically insignificant, likely due to a type II error due to small sample size. The mechanism involved with PRAME staining of sebaceous lobules, basal cell carcinoma, and occasional adnexal neoplasms such as hidrocystoma are not clear. However, it has been shown that almost half of non-melanomatous skin cancers such as basal cell carcinomas exhibit low-intensity PRAME expression.[8] Basal cell carcinomas are the most likely non-melanomatous skin cancers to exhibit low-intensity PRAME expression, with > 85% of lesions showing some degree of staining.8 Although SOX-10 staining intensity varied considerably in normal eyelid tissue, PRAME staining was minimal.

A study assessing PRAME staining of melanoma in situ reveals a range of 16 - 173 cells/mm, with a median of 63 and mean of 75.13 (SD 45.60).[9] Olds et al. have demonstrated that solar lentigo and non-lesional sun-damaged skin had 10 or fewer PRAME-positive cells but that melanoma in situ had at least 16 PRAME-positive cells; suggesting that 10 cells in a 1 millimeter window may be an acceptable threshold of positivity.[9] When PRAME staining is prominent and sustained at a rate higher than 3 per mm, careful analysis is warranted. This is especially true in the context of a re-excision specimen for melanoma in situ known to be PRAME positive. At this time, we consider positive staining for PRAME at more than 5 single melanocytes with complete nuclear staining in any 1 mm window at the edge of a biopsy scar suspicious for persistent disease since this study revealed a maximum of 3 single melanocytes with complete nuclear staining in skin without melanoma or junctional melanocytic proliferation detected on routine sections. A small study of ten excisions for melanomas in situ using PRAME and SOX-10 IHC revealed that the melanocytes of melanoma in situ were immunoreactive for PRAME although the melanocytes of adjacent tumor-negative skin were not.[3] Gradecki et al report that about 6.5% of melanoma in situ can be PRAME negative by IHC.[10] A pigmented lesion assay utilizing PRAME and LINC00518 has a sensitivity and specificity of 95 and 91%,

also suggesting that the incidence of PRAME negative melanoma is likely low.[11] Associated findings on routine hematoxylin and eosin stained sections such as irregular nests of melanocytes, pagetoid spread, spread down adnexal structures, and the presence of inflammation all need to be considered. Although our study looked at single melanocytes, when studying nevi and melanoma arising in a nevus, the percentage of cells staining becomes important.[12]

Limitations of our study include that PRAME can sometimes stain atypical lesions other than melanoma, such as basal cell carcinoma, albeit at low levels.[8,13] Also, the relatively small sample size and the subjective process of deciding which flat 1 mm of epidermis to use to count the number of PRAME-positive cells increases chance of bias.

The way in which PRAME staining can assist in diagnosis is still growing. PRAME expression has been associated with poor prognosis, and is important in assessing metastatic risk of uveal melanoma as part of a 12-gene prognostic assay.8 PRAME can help differentiate benign conjunctival or episcleral melanocytic proliferations from melanoma precursors.[14,15] PRAME is also being explored as immunotherapy for several tumors including melanoma, and is now in clinical trials.8 Immunohistochemical marker studies continue to help with diagnosis in histologically ambiguous situations, but careful clinical-pathological correlation remains the key to accurate and confident diagnosis.

CONFLICT OF INTEREST

We have no conflicts of interest to declare. There was no funding for this research. The work is original and has not been submitted elsewhere.

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