PRAME Expression in Melanocytic Proliferations in Special Sites

Joseph L. Maniaci, MD; Shyam S. Raghavan, MD

PRACTICE **POINTS**

- Special-site nevi are benign melanocytic proliferations at special anatomic sites. Although cytologic atypia and architectural distortion may be present, they are centrally located and should not be present at the borders of the lesion.
- Strong expression of the preferentially expressed antigen in melanoma (PRAME) via immunohistochemistry provides a reliable indicator for benignity in differentiating a special-site nevus from a malignant melanoma occurring at a special site.

The subset of nevi occurring at special sites (eg, acral skin, anogenital region, breast, ear, flexural surfaces) have normal histologic variations that preclude the use of routinely used diagnostic criteria for malignancy. Suggested criteria for differentiating malignant special-site lesions from benign lesions have been described, but there is an unmet need for a validated test aiding in the delineation of benign and malignant lesions at special sites. Preferentially expressed antigen of melanoma (PRAME) expression has been characterized as a relatively specific marker of melanoma, but not within the specific population of special-site lesions. This study aimed to determine if PRAME may serve as a specific marker of melanoma within the population of special-sites lesions.

Cutis. 2024;113:43-47.

The assessment and diagnosis of melanocytic lesions can present a formidable challenge to even a seasoned pathologist, which is especially true when dealing with the subset of nevi occurring at special sites—where baseline variations inherent to particular locations on the body can preclude the use of features routinely used to diagnose malignancy elsewhere. These so-called special-site nevi previously have been described in the literature along with suggested criteria for differentiating malignant lesions from their benign counterparts.[1] Locations generally considered to be special sites include the acral skin, anogenital region, breast, ear, and flexural regions.[1,2]

When evaluating non–special-site melanocytic lesions, general characteristics associated with a malignant diagnosis include confluence or pagetoid spread of melanocytes, nuclear pleomorphism, cytologic atypia, and irregular architecture[3]; however, these features can be compatible with a benign diagnosis in special-site nevi depending on their extent and the site in question. Although they can be atypical, special-site nevi tend to have the bulk of their architectural distortion and cytologic atypia in the center of the lesion as opposed to the edges.[1] If a given lesion is from a special site but lacks this reassuring feature, special care should be taken to rule out malignancy.

Preferentially expressed antigen in melanoma (PRAME) is an antigen first identified in tumor-reactive T-cell populations in patients with malignant melanoma. It is the product of an oncogene that frequently is overexpressed in melanomas, lung squamous cell carcinomas, sarcomas, and acute leukemias.[4] It functions as an antagonist of the retinoic acid signaling pathway, which normally serves to induce further cell differentiation, senescence, or apoptosis.[5] PRAME inhibits retinoid

Dr. Maniaci is from the Department of Pathology, University of North Carolina, Chapel Hill. Dr. Raghavan is from the Department of Pathology, University of Virginia, Charlottesville.

The authors report no conflict of interest.

Correspondence: Joseph L. Maniaci, MD, UNC Department of Pathology, Campus Box #7525, 300 Brinkhous-Bullitt Bldg, Chapel Hill, NC 27599-7525 (joseph.maniaci@unchealth.unc.edu).

doi:10.12788/cutis.0923

signaling by forming a complex with both the ligand-bound retinoic acid holoreceptor and the polycomb protein EZH2, which blocks retinoid-dependent gene expression by encouraging chromatin condensation at the RARβ promoter site[5]; therefore, expressing PRAME allows lesional cells a substantial growth advantage.

PRAME expression has been extensively characterized in non–special-site nevi and has filled the need for a rather specific marker of melanoma.[6-10] Although PRAME has been studied in acral nevi,[11] the expression pattern in nevi of special sites has yet to be elucidated. Herein, we present a dataset characterizing PRAME expression in these challenging lesions.

Methods

We performed a retrospective case review at the University of Virginia (Charlottesville, Virginia) and collected a panel of 36 special-site nevi that previously were diagnosed as benign by a trained dermatopathologist from January 2020 through December 2022. Special-site nevi were identified using a natural language filter for the following terms: *acral*, *palm*, *sole*, *ear*, *auricular*, *lip*, *axilla*, *armpit*, *breast*, *groin*, *labia*, *vulva*, *umbilicus*, and *penis*. This study was approved by the University of Virginia institutional review board.

The original hematoxylin and eosin slides used for primary diagnosis were re-examined to verify the prior diagnosis of benign nevus at a special site. We performed a detailed microscopic examination of all benign nevi in our cohort to determine the frequency of various characteristics at each special site. Sections were prepared from the formalin-fixed and paraffin-embedded tissue blocks and stained with a commercial PRAME antibody (#219650 [Abcam] at a 1:50 dilution) and counterstain. A trained dermatopathologist (S.S.R.) examined the stained sections and recorded the percentage of tumor cells with nuclear PRAME staining. We reported our results using previously established criteria for scoring PRAME immunohistochemistry[7]: 0 for no expression, 1+ for 1% to 25% expression, 2+ for 26% to 50% expression, 3+ for 51% to 75% expression, and 4+ for diffuse or 76% to 100% expression. Only strong clonal expression within a population of cells was graded.

Data handling and statistical testing were performed using the R Project for Statistical Computing (https://www.r-project.org/). Significance testing was performed using the Fisher exact test. Plot construction was performed using ggplot2 (https://ggplot2.tidyverse.org/).

TABLE 1. Study and Control Cohort Lesion Sites

Lesion site	Nevus (study)	MIS/IM (control)	Total
Acral	1	11	12
Anogenital	3	3	6
Breast	16	1	17
Ear	1	6	7
Flexural	15	4	19
Total	36	25	61

Abbreviations: IM, invasive melanoma; MIS, melanoma in situ.

Results

Our study cohort included 36 special-site nevi, and the control cohort comprised 25 melanoma in situ (MIS) or invasive melanoma (IM) lesions occurring at special sites. Table 1 provides a breakdown of the study and control

TABLE 2. Special-Site Nevi Histopathologic Characteristics

Characteristic	Acral	Anogenital	Breast	Ear	Flexural	All sites
Discohesive nests	0	1	2	0	2	5
Enlarged nests	1	2	5	0	9	17
Inflammatory infiltrate	0	2	4	0	2	8
Lentiginous component	0	3	13	0	8	24
Lamellar fibroplasia	0	2	11	0	8	21
Mild cytologic atypia	0	2	4	0	2	8
Suprabasal scatter	1	1	2	0	0	4
All nevi	1	3	16	1	15	36

cohorts by lesion site. Table 2 details the results of our microscopic examination, describing frequency of various characteristics of special-site nevi stratified by site.

Of the 36 special-site nevi in our cohort, 20 (56%) had no staining (0) for PRAME, 11 (31%) demonstrated 1+ PRAME expression, 3 (8%) demonstrated 2+ PRAME expression, and 2 (6%) demonstrated 3+ PRAME expression. No nevi showed 4+ expression. In the control cohort, 24 of 25 (96%) MIS and IM showed 3+ or 4+ expression, with 21 (84%) demonstrating diffuse/4+ expression. One control case (4%) demonstrated 0 PRAME expression. These data are summarized in Table 3 and Figure 1. There is a significant difference in diffuse (4+) PRAME expression between special-site nevi and MIS/IM occurring at special sites ($P=1.039\times10^{-12}$).

Based on our cohort, a positivity threshold of 3+ for PRAME expression for the diagnosis of melanoma in a special-site lesion would have a sensitivity of 96% and a specificity of 94%, while a positivity threshold of 4+ for PRAME expression would have a sensitivity of 84% and a specificity of 100%. Figures 2 through 4 show photomicrographs of a special-site nevus of the breast, which appropriately does not stain for PRAME; Figures 5 and 6 show an MIS at a special site that appropriately stains for PRAME.

TABLE 3. PRAME Expression Score Distribution

PRAME score[a]	Nevus (study)	MIS/IM (control)	Total
0	20	1	21
1+	11	0	11
2+	3	0	3
3+	2	3	5
4+	0	21	21
Total	36	25	61

Abbreviations: IM, invasive melanoma; MIS, melanoma in situ; PRAME, preferentially expressed antigen of melanoma.

[a]0=no expression; 1+=1%-25% expression; 2+=26%–50% expression; 3+=51%–75% expression; 4+=diffuse or 76%–100% expression.

Comment

The distinction between benign and malignant pigmented lesions at special sites presents a fair challenge for pathologists due to the larger degree of leniency for architectural distortion and cytologic atypia in benign lesions at these sites. The presence of architectural distortion or cytologic atypia at the lesion's edge makes rendering a benign diagnosis especially difficult, and the need for a validated immunohistochemical stain is apparent. In our cohort, strong clonal PRAME expression provided a reliable immunohistochemical marker, allowing for the distinction of malignant lesions from benign nevi at special sites. Diffuse faint PRAME expression was present in several benign nevi within our cohort, and these lesions were considered negative (0) in our analysis.

Given the described test characteristics, we support the implementation of PRAME immunohistochemistry with a positivity threshold of 4+ expression as an ancillary test supporting the diagnosis of IM or MIS in special sites, which would allow clinicians to leverage the high specificity of 4+ PRAME expression to distinguish an IM or MIS from a benign nevus occurring at a special site. We do not recommend the use of 4+ PRAME expression as a screening test for melanoma or MIS among special-site nevi due to its comparatively low sensitivity; however, no one marker is always reliable, and we recommend continued clinicopathologic correlation for all cases. Although PRAME can assist in the delineation of malignant lesions from benign ones, microscopic examination of hematoxylin and eosin–stained section remains the gold standard for diagnosing malignant melanoma and MIS.

Although our case series included nevi and MIS/IM from all special sites, we were limited in the number of

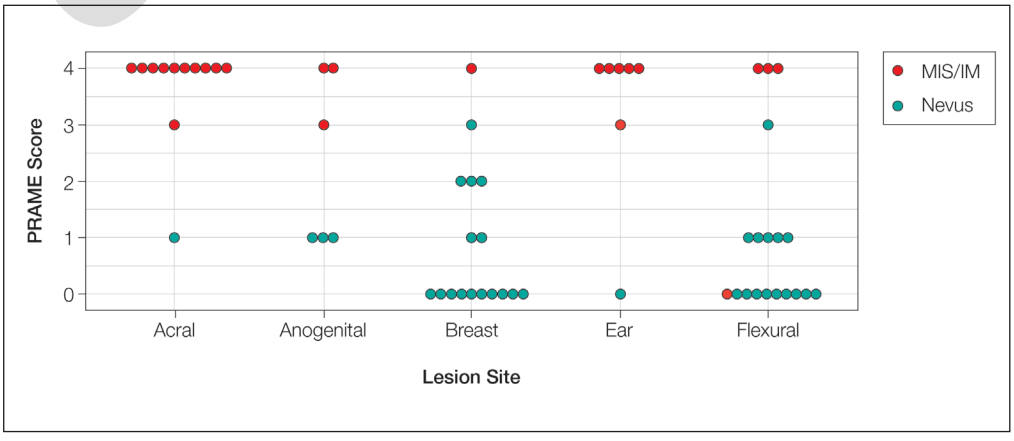

FIGURE 1. Preferentially expressed antigen of melanoma (PRAME) expression score by special-site lesion type (0=no expression; 1+=1%–25% expression; 2+=26%–50% expression; 3+=51%–75% expression; 4+=diffuse or 76%–100% expression). IM indicates invasive melanoma; MIS, melanoma in situ.

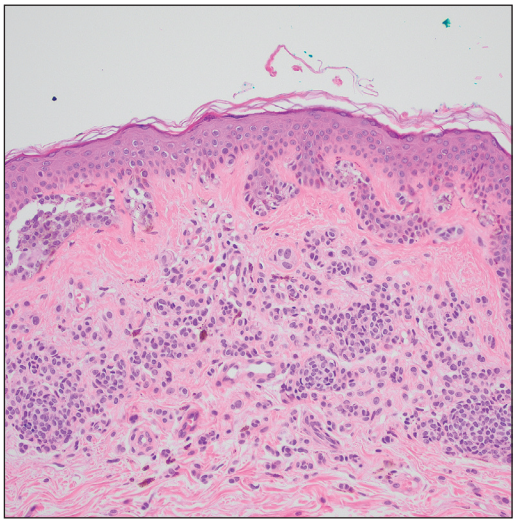

FIGURE 2. Special-site nevus histopathology showing a compound nevus with mild melanocyte cytologic atypia and architectural distortion at center of lesion (H&E, original magnification ×200).

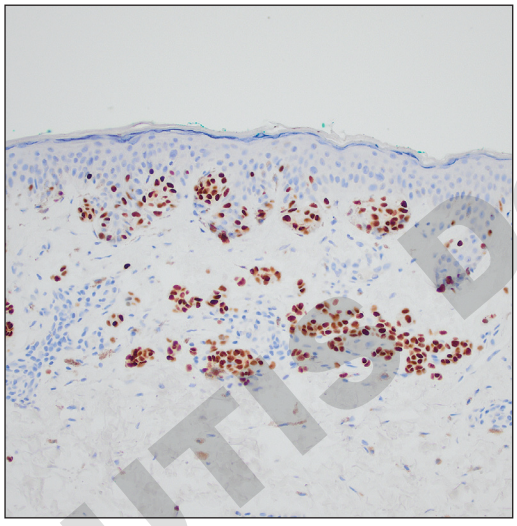

FIGURE 3. Special-site nevus histopathology with SOX10 stain highlighting the melanocytic proliferation (original magnification ×200).

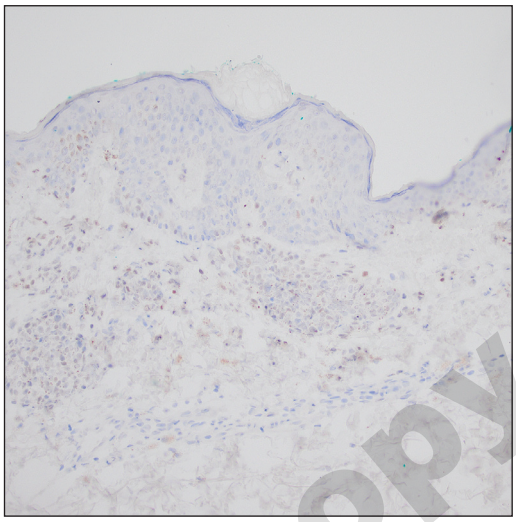

FIGURE 4. Special-site nevus histopathology stained positive for preferentially expressed antigen of melanoma (PRAME)(original magnification ×200). PRAME immunohistochemical stain is negative in the melanocytes previously highlighted by SOX10, supporting the benign diagnosis.

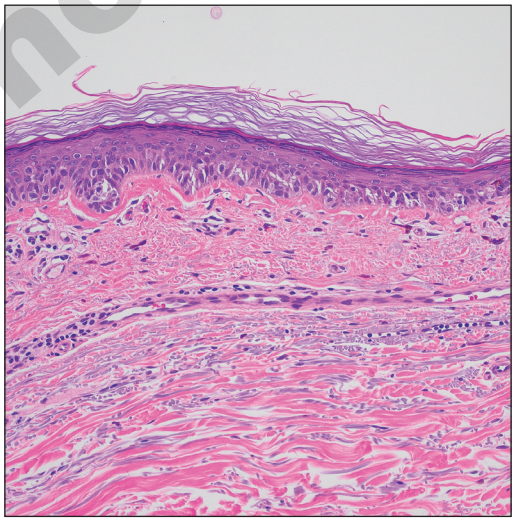

FIGURE 5. Melanoma in situ histopathology showed a highly atypical melanocytic proliferation at the base of the epidermis that does not cross the dermoepidermal junction, supporting an in-situ diagnosis (H&E, original magnification ×200).

acrogenital and ear nevi included due to a relative paucity of biopsied benign nevi from these locations at the University of Virginia. Additionally, although the magnitude of the difference in PRAME expression between the study and control groups is sufficient to demonstrate statistical significance, the overall strength of our argument would be increased with a larger study group. We were limited by the number of cases available at our institution, which did not utilize PRAME during the initial diagnosis of the case; including these cases in the study group would have undermined the integrity of our argument because the differentiation of benign vs malignant initially was made using PRAME immunohistochemistry.

Conclusion

Due to their atypical features, special-site nevi can be challenging to assess. In this study, we showed that PRAME expression can be a reliable marker to distinguish benign from malignant lesions. Our results showed that 100% of benign special-site nevi demonstrated 3+ expression or less, with 56% (20/36) demonstrating no expression at all. The presence of diffuse PRAME expression (4+ PRAME staining) appears to be a specific indicator of a malignant lesion, but results should always be interpreted with respect to the patient's clinical history

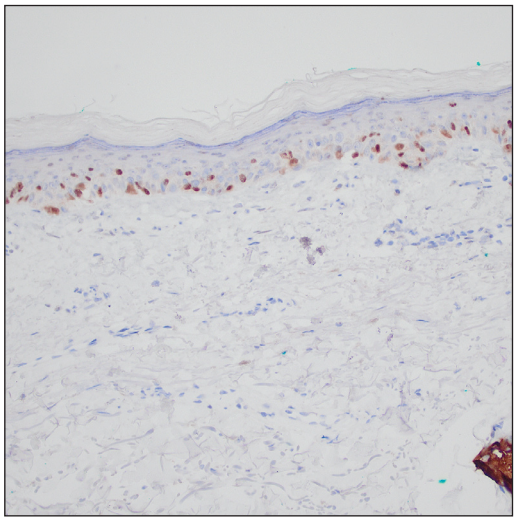

FIGURE 6. Melanoma in situ histopathology stained positive for preferentially expressed antigen of melanoma (PRAME), which highlights the malignant melanocytes in the epidermis, supporting the diagnosis of melanoma in situ (original magnification ×200).

and the lesion's histomorphologic features. Further study of a larger sample size would allow refinement of the sensitivity and specificity of diffuse PRAME expression in the determination of malignancy for special-site lesions.

Acknowledgment—The authors thank the pathologists at the University of Virginia Biorepository and Tissue Research Facility (Charlottesville, Virginia) for their skill and expertise in performing immunohistochemical staining for this study.

REFERENCES

1. VandenBoom T, Gerami P. Melanocytic nevi of special sites. In: *Pathology of Melanocytic Tumors*. Elsevier; 2019:90-100. doi:10.1016/B978-0-323-37457-6.00007-9
2. Hosler GA, Moresi JM, Barrett TL. Nevi with site-related atypia: a review of melanocytic nevi with atypical histologic features based on anatomic site. *J Cutan Pathol*. 2008;35:889-898. doi:10.1111/j.1600-0560.2008.01041.x.
3. Brenn T. Melanocytic lesions—staying out of trouble. *Ann Diagn Pathol*. 2018;37:91-102. doi:10.1016/j.anndiagpath.2018.09.010
4. Ikeda H, Lethé B, Lehmann F, et al. Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. *Immunity*. 1997;6:199-208. doi:10.1016/s1074-7613(00)80426-4
5. Epping MT, Wang L, Edel MJ, et al. The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. *Cell*. 2005;122:835-847. doi:10.1016/j.cell.2005.07.003
6. Alomari AK, Tharp AW, Umphress B, et al. The utility of PRAME immunohistochemistry in the evaluation of challenging melanocytic tumors. *J Cutan Pathol*. 2021;48:1115-1123. doi:10.1111/cup.14000
7. Lezcano C, Jungbluth AA, Nehal KS, et al. PRAME expression in melanocytic tumors. *Am J Surg Pathol*. 2018;42:1456-1465. doi:10.1097/PAS.0000000000001134
8. Gill P, Prieto VG, Austin MT, et al. Diagnostic utility of PRAME in distinguishing proliferative nodules from melanoma in giant congenital melanocytic nevi. *J Cutan Pathol*. 2021;48:1410-1415. doi:10.1111/cup.14091
9. Googe PB, Flanigan KL, Miedema JR. Preferentially expressed antigen in melanoma immunostaining in a series of melanocytic neoplasms. *Am J Dermatopathol*. 2021;43):794-800. doi:10.1097/DAD.0000000000001885
10. Raghavan SS, Wang JY, Kwok S, et al. PRAME expression in melanocytic proliferations with intermediate histopathologic or spitzoid features. *J Cutan Pathol*. 2020;47:1123-1131. doi:10.1111/cup.13818
11. McBride JD, McAfee JL, Piliang M, et al. Preferentially expressed antigen in melanoma and p16 expression in acral melanocytic neoplasms. *J Cutan Pathol*. 2022;49:220-230. doi:10.1111/cup.14130