Analysis of PRAME immunocytochemistry in 109 acral malignant melanoma in situ

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ABSTRACT

Aims Preferentially expressed antigen in melanoma (PRAME) recently is a reliable immunohistochemistry (IHC) marker for distinguishing melanoma from other lesions. However, there are few articles focused on PRAME use in acral malignant melanoma, the most common type in Asians. This study investigated PRAME IHC expression in a large series of acral malignant melanoma in situ to add to the body of clinical knowledge.

Methods PRAME IHC was performed in unequivocal cases of primary acral lentiginous melanoma in situ (ALMIS), subungual melanoma in situ (SMIS) and acral recurrent nevi as the control. PRAME tumour cell percentage positivity and intensity were expressed as categorised in a cumulative score by adding the quartile of positive tumour cells to intensity labelling. The final IHC expression was interpreted as negative (0–1), weak (2–3), moderate (4–5) or strong (6–7).

Results In 91 ALMIS patients, 32 cases (35.16%) were strong, 37 (40.66%) were moderate and 22 (24.18%) were weak. In 18 SMIS patients, strong positivity of PRAME was observed in 4 (22.22%) cases, moderate in 10 (55.56%) and weak in the remaining 4 (22.22%). No melanoma sample was negative for PRAME. By comparison, only 2 of the 40 acral recurrent nevi cases were positive.

Conclusions Our study supports the ancillary value of PRAME for diagnosing ALMIS and SMIS with high sensitivity and specificity.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Preferentially expressed antigen in melanoma (PRAME) immunohistochemistry demonstrates high sensitivity and specificity in identifying primary and metastatic melanoma.

WHAT THIS STUDY ADDS

⇒ The present study investigated PRAME expression in a large series of acral melanoma in situ.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study might add to the knowledge base, verifies the diagnostic value of PRAME for this subtype of melanoma and an applicable threshold.

INTRODUCTION

Despite advances in diagnosis and treatment, malignant melanoma (MM) is an aggressive skin cancer with high morbidity, disability and death rates. Molecular pathology assays such as DNA fluorescence in situ hybridisation and single-nucleotide polymorphism help resolve the diagnostic challenge. Regarding melanoma in situ, unfortunately, the physiological background and cell-poor characteristics limit the use of these tests, and there is a need for a validated diagnostic immunohistochemistry (IHC) marker. Preferentially expressed antigen in melanoma (PRAME) is a non-classical cancer-testis antigen with restricted expression in somatic tissues but diffuse immunoreactivity in malignant neoplasms such as most melanomas, myxoid liposarcomas and carcinomas of various origins.[1–3] PRAME gene expression has been a noninvasive diagnostic parameter of MM for years; recent studies showed that diffuse PRAME IHC staining could distinguish non-spindle cell melanoma from benign melanocytic tumours, and atypical or dysplastic hyperplasia.[4–6]

Acral malignant melanoma is the most common melanoma subtype in Asians. This lesion has low sensitivity to chemotherapy, molecularly targeted drugs and immune checkpoint inhibitors. Subtle atypical cytological features and fewer tumour cells complicate the diagnosis of acral malignant melanoma, especially acral malignant melanoma in situ (AMMIS). PRAME is expected to be an adjunct tool for differential diagnosis of AMMIS; however, few studies focus on the topic. The cut-off value or threshold for a melanoma diagnosis is interpreted as PRAME diffuse positivity (>75%) since one of the first studies conducted by Lezcano *et al*.[5] However, this finding is controversial; there was a low diffuse positivity proportion in several subsequent studies.[7 8]

Therefore, the present study investigated the PRAME expression in an extensive series of acral melanoma in situ to add to the knowledge base, verify the diagnostic value of PRAME for this subtype and identify an applicable threshold.

MATERIALS AND METHODS

All cases with available paraffin blocks and clinical data between 2019 and 2021 were retrieved from our Department of Pathology database. We included 91 unequivocal cases of primary acral lentiginous melanoma in situ (ALMIS), 18 subungual melanoma in situ (SMIS) cases and 40 acral recurrent nevi cases as control. All original H&E and immunohistochemically stained slides were reviewed by HC and Y-PC, respectively.

IHC was performed on representative 4 µm, formalin-fixed, paraffin-embedded tissue. The sections were subjected to air drying, baking, dewaxing, antigen retrieval and immunohistochemical staining. PRAME rabbit monoclonal antibody (Abcam, USA) was diluted at 1:400 for 30 min at room temperature. 3,3′-diaminobenzidine or FAST RED was used as chromogen, and haematoxylin was used as the counterstain. Both chromogens were employed when results were equivocal (eg, in heavily pigmented lesions).

PRAME expression was as usual, interpreted as nuclei positive percentage of tumour cells and scored as follows: no staining (0), 1%–25% (1), 26%–50% (2), 51%–75% (3) and >75% (4). The intensity of expression was evaluated as weak (1), moderate (2) or strong (3). PRAME final IHC score was derived from the sum of the two scores: 0–1: negative expression; 2–3: weak expression; 4–5: moderate expression and 6–7: strong expression. The scoring was performed in a double-blinded manner by two dermatopathologists (QM and HC) with expertise in melanocytic neoplasms, and a consensus was reached in cases of disagreement. When the staining intensity was heterogeneous, the greatest intensity was recorded. SPSS software (V.25.0, IBM) was used for the statistical analyses. A $p<0.05$ was considered statistically significant.

RESULTS

PRAME IHC was performed on a series of 131 cases. These included 91 ALMIS and 18 SMIS from 37 male and 72 female Chinese patients with a median age of 54 years (range 30–82 years). The remaining 40 cases of acral recurrent nevi represented the control group from 14 male and 26 female patients. The nuclei of the melanocytes of the melanoma in situ were immunoreactive for PRAME, while the melanocytes of the adjacent normal skin were not.

In 91 ALMIS samples, 32 cases (35.16%) showed strong (figure 1), 37 cases (40.66%) showed moderate and 22 cases (24.18%) showed weak PRAME immunoreaction. In 18 SMIS samples, strong positivity of PRAME was observed in 4 (22.22%) cases (figure 2), moderate in 10 (55.56%) cases and weak in the remaining 4 (22.22%) cases. No melanoma samples were negative for PRAME. In specimens with few neoplastic cells, the staining was restricted to the tumour cells, corresponding to the H&E impression (figure 3). Conversely, this study's 38 cases of acral recurrent nevi lacked any staining (figure 4A,B); the two positive cases were displayed in figure 4C,D. The difference in PRAME percentage and combined scores between melanomas and nevi was statistically significant ($p<0.001$). No significant difference in PRAME performance was observed between skin and nail lesions. An overview of clinical features and PRAME expression scores in each subset is displayed in online supplemental tables 1,2.

Using a cut-off value of the percentage score of >75%, PRAME correctly categorised only 6 of 91 ALMIS and 1 of 18 SMIS samples. When the threshold decreased to50%, PRAME categorised 49 ALMIS and 5 SMIS samples, increasing sensitivity increased to 53.85% and 27.78%, respectively. When the cut-off value was set to the combined score of 5, PRAME recognised 51 of 91 ALMIS and 7 of 18 SMIS cases with sensitivities of 56.04% and 38.89%, respectively. The sensitivities were 75.82% (69/91) and 77.78% (14/18), respectively, when the threshold was set to a combined score of 4; meanwhile, the specificity was 98% (39/40). This finding suggests that PRAME can be incorporated into a diagnostic clinicopathological matrix.

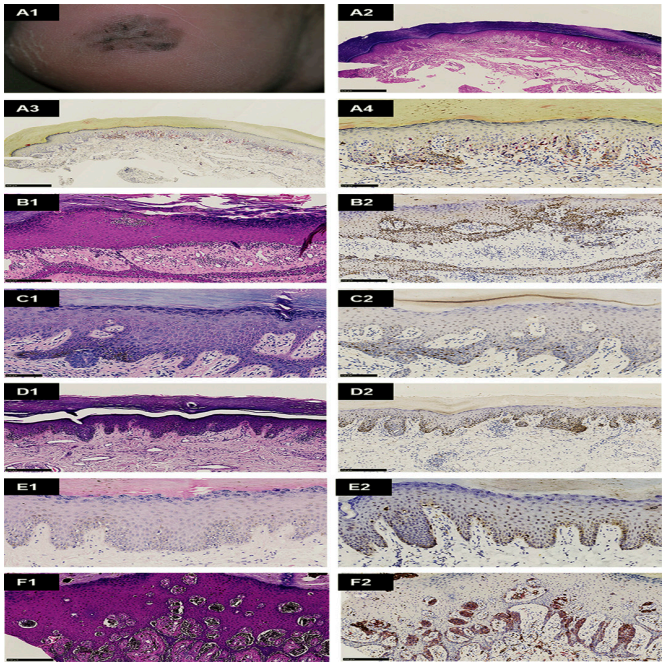

Figure 1 Representative examples of ALMIS. The clinical image (A1), H&E (A2, ×50), and PRAME staining of a case of ALMIS is displayed (A3, ×50; A4×200). The H&E sections (B1, D1 and F1, ×100; C1 and E1, ×200) from the other five different cases show atypical melanocytes in situ with corresponding PRAME intense nuclear staining displayed in the right column (B2, D2 and F2, ×100; C2 and E2, ×200). Fast red was used as chromogen in panels A3, A4 and F2. ALMIS, acral lentiginous melanoma in situ; PRAME, preferentially expressed antigen in melanoma.

DISCUSSION

The PRAME gene, first recognised in autologous T cells in a patient with metastatic cutaneous melanoma, belongs to a family of leucine-rich repeat proteins with diverse functions.[1] PRAME is an independent prognostic marker in MM and provides an accurate assessment of excision margin status. It also helped to determine candidacy for immunotherapy in various clinical trials.[9–12] PRAME IHC can be used in the differential histopathological diagnosis of MM, demonstrating high sensitivity and specificity to identify primary and metastatic melanoma.[5 13 14]

Acral melanoma accounts for more than 40% of cutaneous melanoma in Asian countries. It is still a diagnostic challenge for pathologists due to the thin and atypical characteristics such as pagetoid migration of melanocytes, scattered cellular atypia and mitotic figures. PRAME was found diffusely expressed (>75% labelling) in 94.4% of acral melanomas by Lezcano *et al* despite the small number of samples (n=18).[5] However, in a recent study, Hu *et al* conducted a large-sample study on PRAME IHC expression of the ALM (n=75) and acral nevi; the sensitivity was only 69.3% with a cut-off value decreased to 50% positivity.[15] Likewise, diffuse PRAME IHC expression was identified only in 55% of subungual melanomas in Rothrock *et al* study cohort (n=22).[16] Santandrea *et al* performed PRAME IHC in 107 acral and 20 nail melanocytic lesions and categorised PRAME tumour cell percentage positivity and intensity in a cumulative score as in our study; they reported a correct identification of 82.5% of benign and 87.1% of malignant lesions with the threshold set to a median combined score of 5.[8] Small numbers of AMMIS were included in these studies.

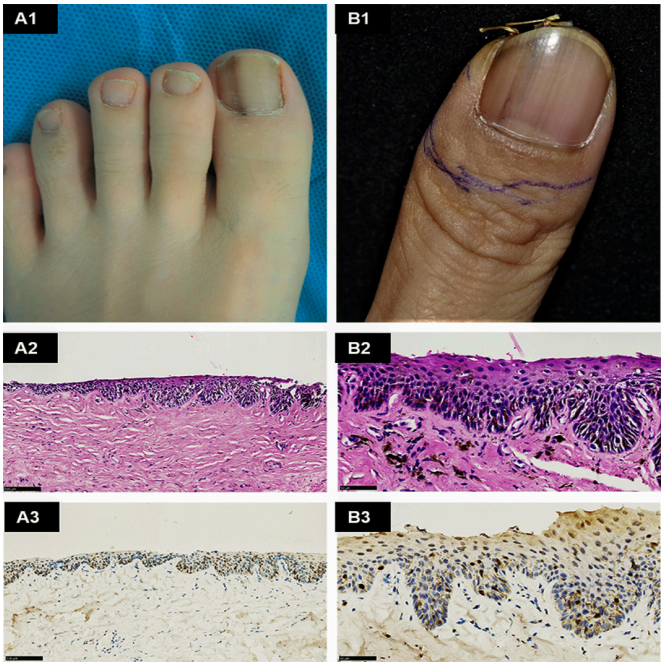

Figure 2 Representative two cases of SMIS (A1 and B1). H&E staining and PRAME immunostaining reveal diffuse atypical melanocytes of the nail matrix from two cases. (A2 and A3, ×200; B2 and B3, ×400). PRAME, preferentially expressed antigen in melanoma.

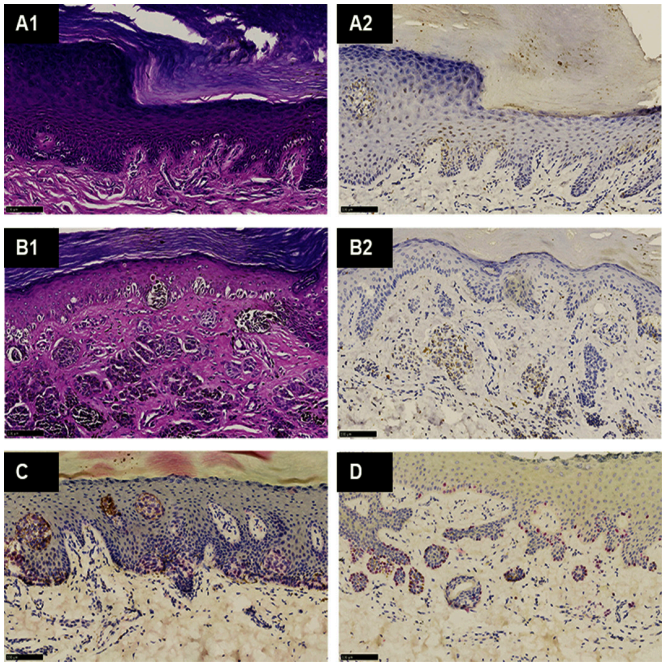

Figure 4 Representative PRAME expression in acral recurrent nevi. The proliferative nevus cells lack immunostain of PRAME (A1 and B1, H&E staining ×200; A2 and B2, PRAME ×200). The two PRAME weakly or strongly positive cases are shown in C and D, respectively (×200). PRAME, preferentially expressed antigen in melanoma.

In agreement with these studies, we found that PRAME IHC expression is a sensitive and specific marker for distinguishing acral melanomas (figure 5). However, the diffuse positivity proportion is not nearly as high as reported previously. This discrepancy could be because we focused on the specific subset of AMMIS, a challenging field represented by small, thin and atypical samples, often with clinicopathological discrepancy.

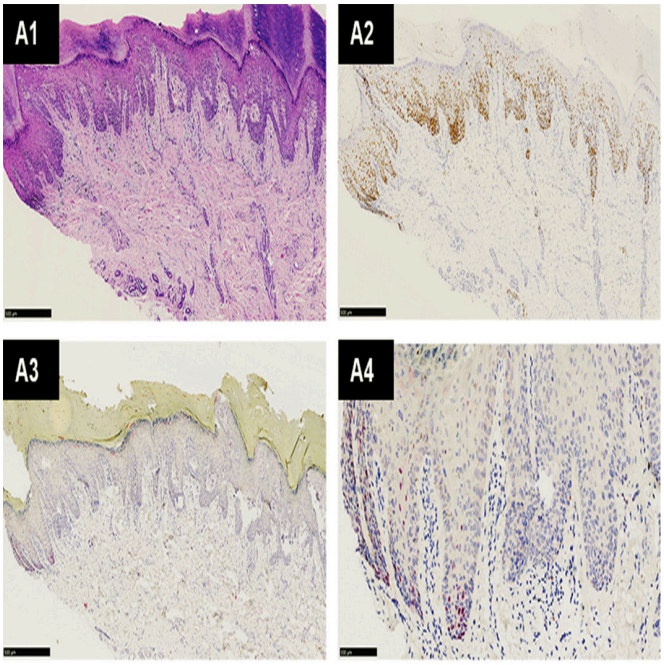

Figure 3 A case of ALMIS that the marginal tumour cells are barely visible on H&E staining (A1, ×50). SO×10 indistinguishably highlights the melanocytes (A2, ×50). PRAME crisply stained residual tumour cells with high specificity (A3, ×50; A4, ×300). ALMIS, acral lentiginous melanoma in situ; PRAME, preferentially expressed antigen in melanoma.

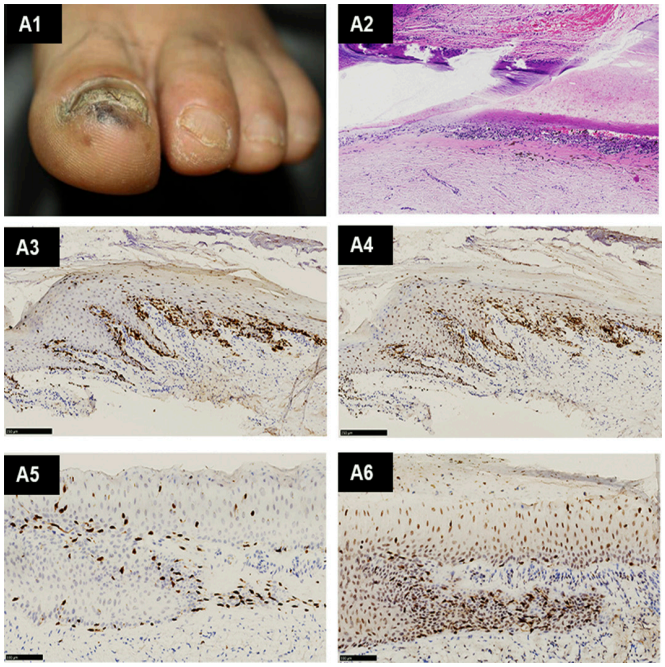

Figure 5 A completely detached nail unit from a case of SMIS was stained by H&E (A2, ×50), and PRAME IHC showed diffusely labeled tumour cells with high sensitivity and specificity (A4, A6×100) compared with SO×10 (A3, A5×100). IHC, immunohistochemistry; PRAME, preferentially expressed antigen in melanoma; SMIS, subungual melanoma in situ.

Gradecki *et al* first performed a large-scale assessment of PRAME IHC expression in lentigo maligna and found 58.9% diffuse positivity (n=77), similar to 58.6% in the Gassenmaier *et al* study on thin melanomas (n=70); very few acral samples were included in these studies.[9 17] There are no extensive studies on PRAME IHC of AMMIS. The Santandrea *et al* cohort covered the most significant number of AMMIS (n=37) and observed a heterogeneous picture with 18.9% of cases showing negative/focal expression.[8] We selected a threshold of moderate positivity (combined score ≥4) to prioritise the ability of PRAME to identify cases of AMMIS (83/109) without simultaneously impairing the specificity (98%). Differences in thresholds may be attributable to variances in staining methodology, interobserver reliability of assessment and (most likely) the specific subtype.

Given its advantage of moderate sensitivity and high specificity, lower cost, and faster turnaround than cytogenetic tests, PRAME IHC has practical value as an ancillary tool for the distinction of MM. Our findings demonstrate the value of moderate to strong PRAME positivity in cases of AMMIS. Nevertheless, it should be noted that non-diffuse PRAME expression by no means excludes melanoma, as this pattern was observed in a significant portion of our study cohort. PRAME IHC could be supportive evidence for melanoma with other markers in clinical practice and should be interpreted in context with other histopathological and clinical features. Additional work is necessary to validate the reliability of PRAME IHC in acral melanoma in situ and determine whether the combined score threshold set in our study is appropriate.

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Contributors The planning was guided by author Y-PC and HC. JZ and X-BS provided technical support. The interpretation and analysis of data was completed by QM and HC. The manuscript was written by QM, checked by JS and guaranted by HC.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The study was approved by the Ethics Committee of the Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College (approval no. 2013-LC/KY-033). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information.

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