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PRAME expression in spindle cell melanoma, malignant peripheral nerve sheath tumour, and other cutaneous sarcomatoid neoplasms: a comparative analysis

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Diagnosis of spindle cell/sarcomatoid melanoma may be challenging due to frequent loss of expression of melanocytic marker(s) and histomorphologic resemblance to various mesenchymal tumours, particularly malignant peripheral nerve sheath tumour (MPNST). Overexpression of PReferentially expressed Antigen in MElanoma (PRAME) supports a diagnosis of melanoma when evaluating challenging melanocytic tumours. PRAME expression in MPNST and other cutaneous sarcomatoid neoplasms, however, has not been well characterised. We aimed to determine the utility of PRAME immunostain in distinguishing spindle cell melanoma from MPNST and other sarcomatoid mimics. PRAME expression was scored by extent (0 to 4+) and intensity (0 to 3) of staining. A strong positive correlation was observed between the extent and intensity scores (r = 0.84). An extent score of 4+, defined by staining in 76–100% of tumour cells, was seen in 56% (23/41) of spindle cell melanomas, 18% (7/38) of MPNSTs, 15% (4/27) of cutaneous sarcomatoid squamous cell carcinomas (SCCs), 33% (5/15) of poorly differentiated cutaneous angiosarcomas, 12% (4/33) of atypical fibroxanthomas (AFXs), 4% (1/25) of pleomorphic dermal sarcomas (PDSs), and none (0/16) of the high-grade cutaneous leiomyosarcomas. A significant difference was found between spindle cell melanoma and all other examined sarcomatoid neoplasms except angiosarcoma. While diffuse (and often strong) PRAME expression is more frequently observed in spindle cell melanoma than MPNST, sarcomatoid SCC, AFX, PDS, and high-grade leiomyosarcoma, its limited sensitivity and specificity caution against its use as a standalone diagnostic marker. PRAME may complement other epigenetic or lineage-specific markers and should only be used as part of an immunohistochemical panel when evaluating these sarcomatoid neoplasms.

Keywords: malignant peripheral nerve sheath tumour, melanoma, PRAME, sarcomatoid

Introduction

PRAME (PReferentially expressed Antigen in MElanoma) is a tumour-associated antigen in the family of cancer testis antigens. Its expression is minimal in normal adult human tissues and is largely limited to the gonads.[1,2] PRAME expression has been reported in various neoplasms including melanoma, breast carcinoma, renal cell carcinoma, non-small cell lung carcinoma, neuroblastoma, acute leukaemia, and several types of sarcomas.[2–10] Overexpression of PRAME helps distinguish melanoma from melanocytic nevi,

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which has led to the increasingly popular use of PRAME immunohistochemistry (IHC) in routine dermatopathology practice.[1,6,11] Recent studies have reported only minimal PRAME expression in soft-tissue tumours with melanocytic differentiation, including perivascular epithelioid cell tumours and clear cell sarcomas,[12,13] thus supporting the additional utility of PRAME IHC in discriminating melanoma from these histologic mimics.

Malignant peripheral nerve sheath tumour (MPNST) is a rare, aggressive soft-tissue sarcoma that shows variable differentiation towards nerve sheath components.[14,15] Diagnosis of MPNST is challenging due to the lack of pathognomonic histopathologic features, specific immunophenotype, or diagnostic molecular signatures.[14] In particular, differentiation between MPNST and spindle cell/sarcomatoid melanoma is notoriously difficult, as these tumours may share strikingly similar histomorphology and immunophenotype.[16,17] S100 and SOX10 are often the only melanocytic/schwannian markers expressed in these tumours,[17,18] and about half of MPNSTs even lack expression for at least one of these markers.[17,19–21] A relatively new immunohistochemical tool is H3K27me3, an epigenetic marker frequently lost in MPNST and retained in its histologic mimics, including melanoma.[22–24] Such loss of expression has been reported in some conventional melanomas but only rarely in spindle cell melanomas.[14,25,26] A newer marker, H3K27me2, has shown similar sensitivity and superior specificity for MPNST compared to H3K27me3.[27] Because spindle cell melanoma and MPNST have different clinical behaviours and therapeutic implications, making an accurate diagnosis is crucial, and the search for additional helpful discriminators continues.

We have encountered rare sarcomatoid tumours with only focal SOX10 or S100 staining and retained H3K27me3 expression, in which metastatic melanoma and MPNST were the leading diagnostic considerations. PRAME IHC was entertained in these instances, although its utility in such a setting was unclear. To our knowledge, PRAME expression in MPNST has only been recently examined in two previous studies. Cadwell *et al*. reported PRAME staining in 66% of MPNST cases, compared to 0% of benign peripheral nerve sheath tumours (schwannomas and neurofibromas).[1] In their study, positivity was defined as staining in at least 5% of tumour nuclei, which is significantly lower than the widely accepted >75% threshold in the evaluation of melanocytic lesions.[11,13,28] That study also did not include any histologic mimics of MPNST other than benign nerve sheath tumours. Another study by Albertsmeier *et al*. reported "high expression" of PRAME in 38% of MPNST cases, without clearly defining the criteria for high expression.[29]

In addition to MPNST, various primary cutaneous sarcomas and sarcomatoid carcinomas may occasionally enter the differential diagnosis of spindle cell melanoma. Of these, atypical fibroxanthoma (AFX) and pleomorphic dermal sarcoma (PDS) are undifferentiated tumours that can be difficult to distinguish from rare cases of undifferentiated or dedifferentiated melanoma.[30–32] Cutaneous high-grade leiomyosarcoma and poorly differentiated angiosarcoma are other neoplasms that may mimic sarcomatoid melanoma on histopathology. Herein we examine the utility of PRAME IHC in distinguishing spindle cell melanoma from MPNST and various cutaneous sarcomatoid mimics.

Materials and methods

This study was conducted according to a protocol previously approved by the Institutional Review Board at our institution. Three tissue microarrays (TMAs) previously constructed from formalin-fixed paraffin-embedded spindle cell melanomas (excluding desmoplastic melanomas), conventional MPNSTs, cutaneous sarcomatoid (SCCs), AFXs, PDSs, cutaneous high-grade leiomyosarcomas, and poorly differentiated cutaneous angiosarcomas were used. Each case was represented by triplicate 1.0 mm cores (MPNST) or 0.6 mm cores (all other examined tumours). Our pathology archive was also searched for the above tumour entities to include additional whole-section cases. Spindle cell melanomas included in this cohort differed from desmoplastic melanomas in that the former showed high cellularity, prominent cytologic atypia, and brisk mitotic activity, whereas the latter is characterised by abundant fibrotic stroma, variable cytologic atypia, and scattered lymphoid aggregates.[33]

Sections of 4-μm thickness made from each TMA and selected whole-section blocks were deparaffinised, and heat-induced epitope retrieval was performed on the Ventana Benchmark Ultra immunostainer using cell conditioning 1 (CC1) buffer from Ventana Medical Systems (Tucson, AZ, USA). Following blocking of endogenous peroxidase activity, the slides were incubated with a PRAME monoclonal antibody (EP461, Cell Marque, prediluted) for 60 min at room temperature. Immunoreactivity was detected using the OptiView universal DAB detection kit (Ventana Medical Systems).

Each case was assessed by a board-certified dermatopathologist (M.P.C.) and assigned the following scores: (1) "Extent score" based on percentage of tumour nuclei staining positively for PRAME (0 = no staining; 1+ = 1–25% of nuclei staining; 2+ = 26–50%; 3+ = 51–75%; 4+ = 76–100%),[6] (2) "Intensity score" based on staining intensity (0 = negative; 1 = weak; 2 = moderate; 3 = strong), and (3) "Combined score" by adding the extent score and the intensity score (0 to 7). A Wilcoxon rank-sum test was used to compare scores between groups. The chi-square test was used to compare proportions of cases meeting preset cutoffs. Statistical significance was defined as a *P*-value of <0.05. The Pearson correlation coefficient (r) was calculated to determine correlation between extent and intensity scores.

Results

Extent, intensity, and combined PRAME scores for all tumour categories are summarised in Table 1 and Figure 1. Extent score and intensity score were found to be highly positively correlated (r = 0.84).

A total of 41 spindle cell melanomas (38 TMA cases and three whole-section cases) were evaluated for PRAME expression. Over half (56%) of these cases demonstrated an extent score of 4+ (Figure 2A,B). The remaining cases showed a range of 0 to 3+ (Figure 2C), resulting in a mean extent score of 2.8. The mean intensity of staining was 2.0, with 51% of cases receiving a score of 3. Finally, 56% of spindle cell melanomas had a combined score of ≥6 (Figure 2A).

PRAME expression was evaluated in 38 soft-tissue conventional MPNSTs (26 TMA cases and 12 whole-section cases). Histologic grade was known in 35 cases, of which 32 were high-grade and three were low-grade. This group of cases demonstrated fairly even distribution of extent scores from 0 to 4+ (Figure 2D–F). Diffuse (4+) expression was seen in 18% of cases, and the mean extent score was 1.7. Staining intensity tended to be weak, with a mean intensity score of 1.3. Only 18% of cases had a combined score of ≥6 (Figure 2D). As a group, MPNST displayed significantly lower mean extent score, mean intensity score, and mean combined score compared to spindle cell melanoma.

Twenty-seven cutaneous sarcomatoid SCC cases (20 TMA cases, seven whole-section cases) were included. The extent of staining was relatively limited, with the majority of cases showing 1+ or negative staining. Four (15%) cases showed 4+ staining. The mean extent score was 1.5. The intensity of staining was also consistently low, with a mean intensity score of 1.1 (Figure 2G). Only three (11%) cases had a combined score of ≥6. Overall, sarcomatoid SCC demonstrated significantly lower mean extent score, mean intensity score, and mean combined score compared to spindle cell melanoma.

Thirty-three AFX cases (22 TMA cases, 11 whole-section cases) were examined. Four (12%) of these cases displayed an extent score of 4+, and only one (3%) case showed an intensity score of 3 (Figure 2H). Of the 25 PDS cases (19 TMA, six whole-section) examined, only one (4%) case showed an extent score of 4+. None of the PDS cases had an intensity score of 3 (Figure 2I). Both AFX and PDS showed

Table 1. PRAME expression in spindle cell melanoma and sarcomatoid mimics

Tumour type	Total *n*	Extent score						Intensity score					Combined score		
		0	1+	2+	3+	4+	Mean	0	1	2	3	Mean	<6	≥6	Mean
Spindle cell melanoma	41	6	3	6	4	23	2.8	6	8	7	21	2.0	19	23	4.8
MPNST	38	10	8	9	4	7	1.7	10	13	8	7	1.3	31	7	3.0
Sarcomatoid SCC	27	7	9	5	2	4	1.5	7	14	3	3	1.1	24	3	2.6
AFX	33	14	7	7	1	4	1.2	14	16	2	1	0.7	32	1	2.0
PDS	25	13	7	2	2	1	0.8	13	9	3	0	0.6	24	1	1.0
Leiomyosarcoma	16	11	4	0	1	0	0.4	11	5	0	0	0.3	16	0	0.8
Angiosarcoma	15	5	2	1	3	5	2.1	5	4	3	4	1.4	9	6	3.5

AFX, atypical fibroxanthoma; MPNST, malignant peripheral nerve sheath tumour; PDS, pleomorphic dermal sarcoma; SCC, squamous cell carcinoma.

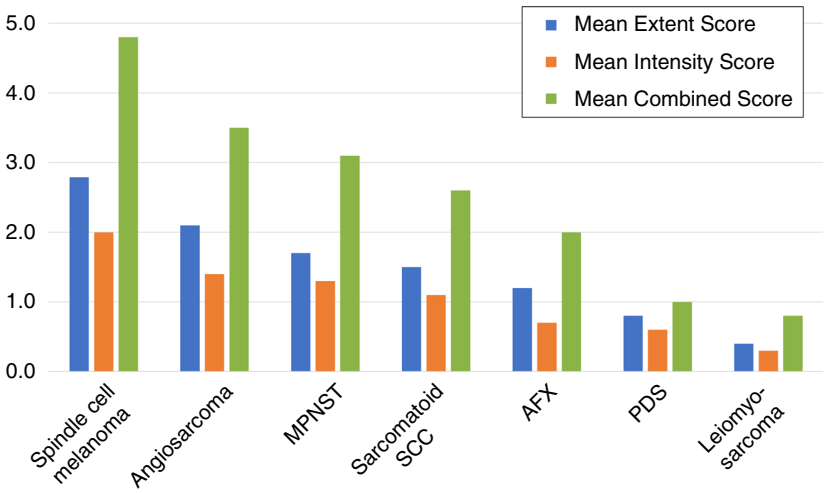

Figure 1. Mean extent, intensity, and combined PRAME scores across different tumour types. AFX, atypical fibroxanthoma; MPNST, malignant peripheral nerve sheath tumour; PDS, pleomorphic dermal sarcoma; SCC, squamous cell carcinoma.

significantly lower extent, intensity, and combined scores compared to spindle cell melanoma.

Sixteen leiomyosarcoma cases (14 TMA, two whole-section) showed the lowest extent and intensity scores of all tumour groups examined (Figure 2J). In contrast, 15 poorly differentiated cutaneous angiosarcomas (13 TMA, two whole-section) demonstrated the highest degree of PRAME staining after spindle cell melanomas (Figure 2K). There was no statistically significant difference in the extent, intensity, or combined scores between poorly differentiated cutaneous angiosarcoma and spindle cell melanoma. Furthermore, no significant difference was identified when comparing primary ($n = 9$) and secondary ($n = 6$) cutaneous angiosarcoma cases.

Sensitivity and specificity data are shown in Table 2. An extent score of 4+ showed a sensitivity of 56% for spindle cell melanoma. Using the same cut-off, specificity for spindle cell melanoma was 82% when compared with MPNST; 85% when compared with sarcomatoid SCC; 88% when compared with AFX; 96% when compared with PDS;100% when compared with high-grade leiomyosarcoma; and 67% when compared with poorly differentiated angiosarcoma. An intensity score of 3 and a combined score of ≥6 showed generally similar sensitivities and specificities.

Discussion

Spindle cell/sarcomatoid melanoma poses a significant diagnostic challenge due to its histologic resemblance to various mesenchymal tumours. Many of these melanomas are poorly differentiated, expressing only S100 or SOX10, while expression of other melanocytic markers is diminished or absent.[18,34] Such characteristics render the distinction between spindle cell melanoma and MPNST particularly difficult, especially when S100 or SOX10 expression is patchy or focal, or in the setting of metastatic disease.[14,16,17] PRAME IHC has recently become a widely adopted ancillary tool in the evaluation of challenging melanocytic tumours. Overexpression of PRAME typically supports a diagnosis of melanoma, whereas benign melanocytic neoplasms and soft-tissue tumours with melanocytic differentiation are usually negative for overexpression.[1,6,11,13] In light of these findings, we aimed to determine the utility of PRAME in the differential diagnosis of spindle cell melanoma and MPNST by characterising the frequency, extent, and intensity of PRAME staining in both malignancies. We also examined PRAME expression in other cutaneous sarcomatoid neoplasms that may enter the histologic differential diagnosis of spindle cell melanoma.

We scored each case using three different measurements of PRAME expression: extent, intensity, and combined scores. Interestingly, comparative analysis of spindle cell melanoma with other tested tumour types yielded fairly consistent *P*-values across all three scoring methods, and Pearson correlation revealed a strong association between extent and intensity scores, suggesting these scoring methods are similarly useful in the evaluation of these tumours. In other words, diffuse expression is typically strong, and focal expression is usually weak. As most previous reports on melanocytic lesions utilised a >75% threshold of

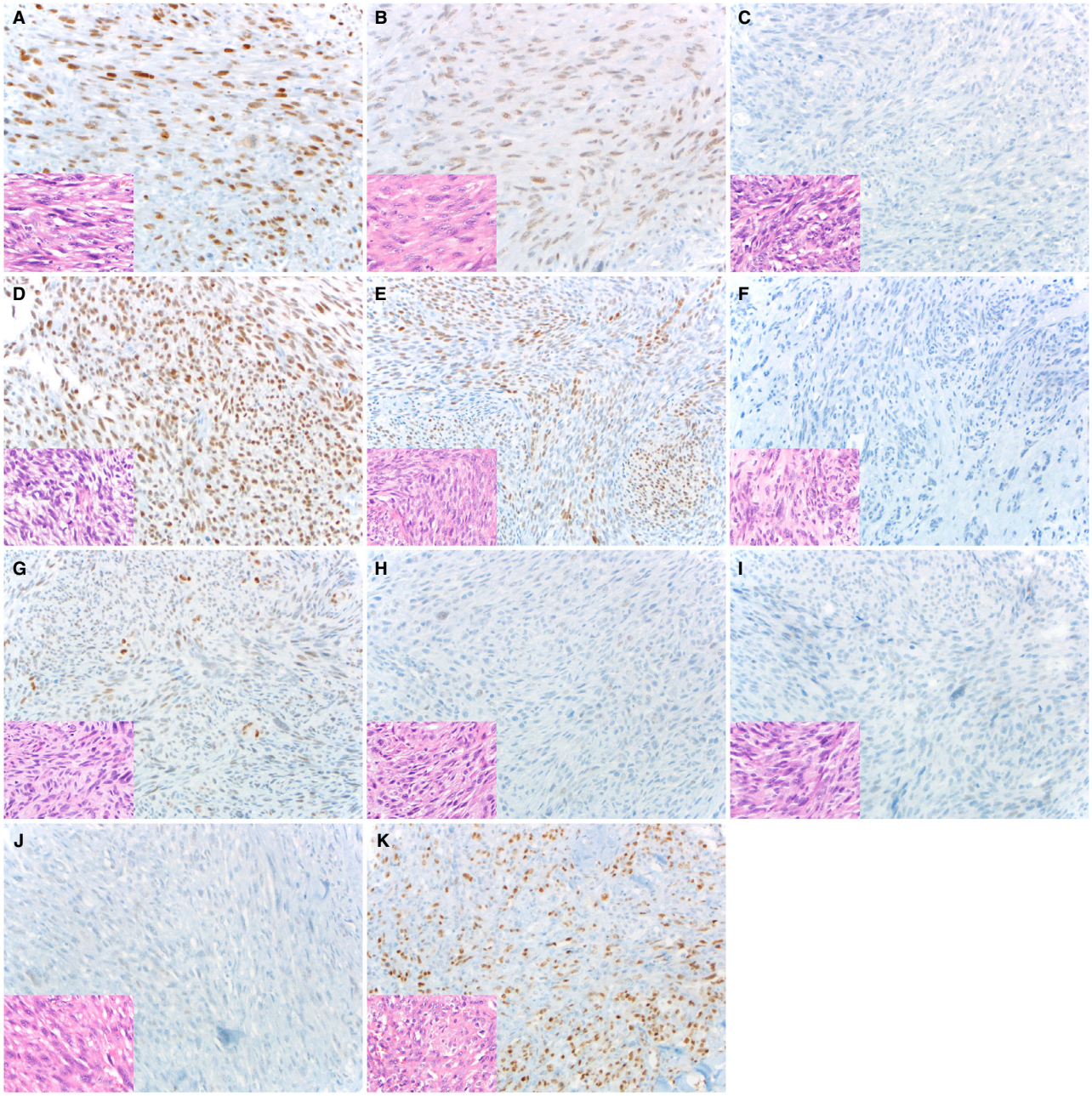

Figure 2. PRAME expression in selected spindle cell melanomas and sarcomatoid mimics. **A**: A spindle cell melanoma shows strong PRAME staining in >75% of tumour cells (extent score 4+, intensity score 3). **B:** Another spindle cell melanoma shows moderate intensity of staining in >75% of tumour cells (extent 4+, intensity 2). **C:** This spindle cell melanoma is negative for PRAME expression. **D:** A malignant peripheral nerve sheath tumour (MPNST) displays diffuse and strong staining (extent 4+, intensity 3). **E:** Another MPNST shows patchy moderate staining (extent 2+, intensity 2). **F:** This MPNST is negative for PRAME expression. **G:** A sarcomatoid squamous cell carcinoma demonstrates weak staining in most tumour cells (extent 4+, intensity 1). Minimal to absent staining is observed in an atypical fibroxanthoma (**H**), a pleomorphic dermal sarcoma (**I**), and a high-grade leiomyosarcoma (**J**). **K**: A poorly differentiated cutaneous angiosarcoma shows strong staining in 51–75% of tumour cells (extent 3+, intensity 3) (original magnification, ×200).

PRAME nuclear immunoreactivity (extent score of 4+) to support a diagnosis of melanoma over nevus,[6,11,13,35] we recommend adherence to this common scoring system and cutoff. If taking intensity into account, we would suggest using a combined score cutoff of ≥6, as our data demonstrated this to be >80% specific for spindle cell melanoma compared to all other tested tumour types, except poorly

Table 2. Comparative analysis of PRAME expression in spindle cell melanoma and sarcomatoid mimics

	Extent score of 4+			Intensity score of 3			Combined score of ≥6		
Tumour type	Sensitivity	Specificity	*P*-value*	Sensitivity	Specificity	*P*-value*	Sensitivity	Specificity	*P*-value*
Spindle cell melanoma	56%	—	—	51%	—	—	56%	—	—
vs. MPNST	—	82%	0.0017	—	82%	0.0075	—	82%	0.0019
vs. Sarcomatoid SCC	—	85%	0.0006	—	89%	0.0008	—	89%	0.0004
vs. AFX	—	88%	<0.0001	—	97%	<0.0001	—	97%	<0.0001
vs. PDS	—	96%	<0.0001	—	100%	<0.0001	—	96%	<0.0001
vs. Leiomyosarcoma	—	100%	0.0005	—	100%	<0.0001	—	100%	<0.0001
vs. Angiosarcoma	—	67%	0.1542	—	73%	0.0943	—	60%	0.112

AFX, atypical fibroxanthoma; MPNST, malignant peripheral nerve sheath tumour; PDS, pleomorphic dermal sarcoma; SCC, squamous cell carcinoma.
*Comparison of proportions of cases meeting cutoff.

differentiated cutaneous angiosarcoma, which is discussed further below.

Lezcano *et al.* have shown a lower rate of PRAME overexpression (extent score of 4+) in melanomas with spindle morphology, including desmoplastic melanomas (35%), compared to those with epithelioid morphology (90%).[6] Another group examined acral lentiginous melanomas and found decreased PRAME positivity when the tumour cells were spindle.[36] Our cohort of nondesmoplastic spindle cell melanomas corroborated these findings, with only 56% of cases displaying diffuse (4+) PRAME positivity. Despite this limited sensitivity for spindle cell melanoma, the mean extent, intensity, and combined scores were still significantly higher in spindle cell melanoma than in MPNST. Of note, the scarcity of low-grade MPNST in our cohort precludes correlation of PRAME expression with histologic grade. The two prior studies examining PRAME expression in MPNST used different scoring methods than ours and did not include melanoma for comparison. Based on our data, diffuse and strong PRAME expression would favour melanoma over MPNST, although such distinction is not without caveat, as almost one-fifth of MPNSTs exhibited the same degree of staining. Proper workup of this differential diagnosis should therefore include other immunohistochemical markers such as Melan-A and H3K27me3 or H3K27me2.[14,27]

We limited our cutaneous angiosarcoma cohort to poorly differentiated tumours, because well differentiated angiosarcomas are much less likely to be confused with melanoma. A surprising finding was the relatively high expression of PRAME in this group. More specifically, 33% of these cases showed an extent score of 4+, and 40% had a combined score of ≥6. These data are concordant with a previous study that showed PRAME expression in 33% (3/9) of angiosarcoma cases.[29] No difference in PRAME expression was observed between primary and secondary cutaneous angiosarcomas in our small cohort. Additional studies are needed to further characterise the degree of PRAME expression in different grades and subtypes of angiosarcoma. Based on our findings, PRAME is not useful in distinguishing spindle cell melanoma and poorly differentiated angiosarcoma. Vascular markers such as ERG and CD31 should be included when angiosarcoma is a diagnostic consideration.[37]

Our study showed fairly low PRAME expression in AFX, PDS, and cutaneous high-grade leiomyosarcoma, indicating that diffuse and strong PRAME expression is generally reliable in excluding these entities. In a previous report, all 10 AFX and seven leiomyosarcoma cases showed <25% nuclear PRAME positivity.[38] Another study reported PRAME expression in 6% of leiomyosarcomas and 7% of undifferentiated pleomorphic sarcomas, although the scoring criteria were not clearly defined.[29] Because AFX and PDS are diagnoses of exclusion, additional immunohistochemical markers and careful attention to histopathologic clues are needed to rule out melanoma as well as other sarcomatoid neoplasms.[34] When an undifferentiated or dedifferentiated melanoma is suspected, PRAME and BRAFV600E immunostains could be particularly helpful, as positivity would support a diagnosis of melanoma and argue against AFX and PDS.[39–41]

To our knowledge, only one prior study has assessed PRAME expression in poorly differentiated

cutaneous SCC, which showed <50% nuclear positivity in all 15 cases examined.[38] Although the majority of our cutaneous sarcomatoid SCC cases also showed minimal staining, a small subset (15%) demonstrated diffuse expression. This slight discrepancy with the prior study could be due to the fact that our cohort included only sarcomatoid SCCs, which are presumably of higher histologic grade. Increased PRAME expression has been associated with higher histologic grade in several other tumours, including head and neck SCC and breast cancer.[3,42–44] Our finding of 4+ PRAME staining in some sarcomatoid SCCs cautions against the use of PRAME alone to exclude this diagnosis.

Our study has several limitations. The use of TMAs potentially introduced sampling error, which was in part overcome by triplicate sampling of each tumour, and inclusion of additional whole-section cases. Some of the sarcomas included in this study had relatively small sample sizes due to the rarity of these tumours. Nevertheless, our findings in AFX, PDS, leiomyosarcoma, and angiosarcoma are largely consistent with the limited data available in the literature.

In conclusion, PRAME has shown some utility in differentiating spindle cell melanoma from MPNST and other sarcomatoid tumours of the skin, but should only be used in conjunction with other immunohistochemical markers due to its incomplete sensitivity and specificity. It is expected to complement H3K27me3 or H3K27me2 in the challenging differential diagnosis of spindle cell melanoma and MPNST. Although PRAME expression was significantly more common in spindle cell melanoma than in sarcomatoid SCC, AFX, PDS, and high-grade leiomyosarcoma, various lineage-specific markers are likely more reliable discriminators in these contexts. Finally, given the overexpression of PRAME in a significant subset of poorly differentiated cutaneous angiosarcomas, more studies are needed to explore its possible role in distinguishing benign and malignant vascular neoplasms.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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