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PRAME and LEF1 in combined deep penetrating nevus and combined blue nevus: utility and pitfalls

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Abstract

Deep penetrating nevi (DPN), particularly those showing combined features, or combined deep penetrating nevi (CDPN), may show histopathological resemblance to blue nevus (BN) and melanoma. PRAME (Preferentially Expressed Antigen in MElanoma) is a marker that helps distinguish melanoma from benign melanocytic lesions. LEF1 (Lymphoid enhancer-binding factor 1) has been proposed to be used in conjunction with β-catenin for diagnosis of DPN.

The immunohistochemical expression of PRAME and LEF1 was evaluated in 10 DPN (including 6 CDPN and 2 DPN-like proliferations with atypical features), 16 BN (including combined and cellular BN), and 2 melanomas with features of DPN or BN.

PRAME was negative in most DPN (n=10/10, n=9/10, one case with discrepancy between readers) and all BN (n=16/16), while the 2 melanomas included were positive (n=2/2). All DPN were positive for LEF1 (n=9/9) while only a subset of BN were positive (n=6/16, p=0.0028; n=5/16, p=0.001, per both readers).

LEF1 appeared to be easier to interpret than β-catenin because of its nuclear pattern of expression. The expression of LEF1 in the regular nevus component of combined BN presents a potential pitfall in practice since it may lead to misinterpretation of LEF1 as positive in the BN component of the lesion. However, a subset (approximately one third) of combined BN appeared to show true LEF1 expression. Taking into account pitfalls in interpretation, the combinatorial panel of

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PRAME and LEF1, in addition to conventional histopathological features, may be useful to distinguish CDPN from combined BN and other benign and malignant mimics.

Keywords

Deep penetrating nevus; blue nevus; melanoma; PRAME; LEF1

Introduction:

Deep penetrating nevus (DPN) was first described in 1989.[1] The differential diagnosis for DPN includes other heavily pigmented lesions such as blue nevus (BN), cellular blue nevus (CBN), plexiform spindle cell nevus, clonal/inverted type A nevus, pigmented epithelioid melanocytoma (PEM), and melanoma.[1–4] Combined nevi, which contain at least two populations of melanocytes in one lesion can also prove to be challenging to characterize. In particular, combined DPN (CDPN) are relatively frequent and may be difficult to differentiate from other biphenotypic lesions such as combined BN or cellular BN (CBN) as they are histologically similar. Importantly, melanoma arising within a nevus is within the differential diagnosis for combined nevi, particularly CDPN. Generally, DPNs are expected to behave in an indolent manner, although atypical DPN, or deep penetrating melanocytoma, has been associated with occasional spread to lymph nodes. Thus, accurately differentiating DPN from other melanocytic lesions that are heavily pigmented and extend deep into the dermis is important and may be challenging on histopathological grounds alone, particularly in small biopsies.[4–7]

Recent studies have characterized the molecular underpinnings of DPN. Specifically, it has been determined that DPNs harbor mutations in both the mitogen-activated protein kinase (MAPK) pathways as well as the WNT/β-catenin pathway, resulting in the expression of both cyclin D1 and β-catenin.[7,8] It has also been demonstrated that melanomas arising within DPN contain these as well as additional mutations, such as *TERT* promoter mutation or biallelic loss of *CDKN2A*.[7] With the activation of the WNT/β-catenin pathway, β-catenin enters the nucleus of melanocytes and interacts with different transcription factors including lymphoid enhancer-binding factor 1 (LEF1). LEF1 has been implicated in the facilitation of the epithelial-mesenchymal transition in tumorigenesis.[9,10] LEF1 has been found to be expressed in DPN and can be used in conjunction with β-catenin for the accurate diagnosis of DPN.[8,9,11,12]

Preferentially Expressed Antigen in MElanoma (PRAME) is a relatively new melanoma-associated antigen preferentially expressed in melanoma cells and less commonly in other melanocytic lesions. It was identified through autologous T-cell epitope cloning in a patient with metastatic cutaneous melanoma and has since been found to be expressed in ocular melanoma as well as other non-melanocytic malignant neoplasms.[13–18] PRAME is a repressor of retinoic acid (RA) receptor signaling and it inhibits RA-induced processes including apoptosis and differentiation.[17] Given that PRAME is still a relatively new marker, the literature surrounding the expression of PRAME in DPN, CBN, blue nevi and DPN-like

lesions, including atypical DPN, is relatively sparse. Furthermore, PRAME expression in combined lesions, such as CDPN and combined BN has not been extensively studied.

Early studies have determined that PRAME is generally not expressed in DPN, BN, CBN or Spitz nevi.[18–22] However, Kline et al. (2022) have recently determined that borderline CBN and DPN demonstrate low PRAME expression.[18] The goal of this study is to characterize the expression of PRAME and LEF1 in DPN and BN and to explore the utility of these markers for the differentiation of DPN, and in particular CDPN, from histologic mimics, including those showing combined features.

Materials and Methods:

After IRB approval, our institution's pathology files were reviewed to identify cases of DPN (including CDPN) and BN (including combined and cellular variants). Two melanomas showing plexiform (DPN-like), and BN-like architecture were also included as controls for expression of PRAME. Terms used to search our institutional database included: "blue nevus", "deep penetrating nevus", "combined deep penetrating nevus", "combined blue nevus" with a focus on these terms appearing in any combination within the "final diagnosis" or "comment" sections of the pathology report. The lesions included in our study, and in particular the DPNs included, had confirmatory β-catenin and cyclin D1 staining prior to beginning our analysis. Clinical variables collected include gender, age, and site of neoplasm.

Immunohistochemical studies were performed on freshly cut unstained slides prepared from the selected formalin-fixed paraffin-embedded (FFPE) tissue blocks, using previously validated immunostaining methods (Leica BOND-III, Leica Biosystems, Wetzlar, Germany) at our CLIA-certified clinical laboratory, with the following antibodies: PRAME antibody (EPR20330; 1:250 dilution, Abcam), LEF1 (EPR2029Y; 1:100 dilution, Abcam).

We analyzed 16 BN (combined BN, n=7; cellular BN, n=6; BN with atypical features such as cytologic atypia and increased proliferation rate, n=2; BN with features of DPN, n=1) and 10 DPN (CDPN, n=6; DPN, n=2; DPN with atypical features such as cytologic atypia and increased proliferation rate, n=2). In addition, 2 melanomas with DPN or BN features were deemed appropriate for comparison. Lesions were scored as either positive or negative for PRAME and LEF1. Scores were determined based on the independent evaluation of two dermatopathologists (CAT and KV). PRAME was interpreted as positive if greater than 75% of the lesional cells showed nuclear positivity. Positive staining for LEF1 was defined as homogenous or heterogenous nuclear staining of melanocytes deep to papillary dermis and away from adnexal structures, as previously defined by Raghavan et al (2020).[8] Of note, conventional nevus components were not scored if available in the lesions of interest. Nuclear staining for PRAME and LEF1 was defined as intensity equal to or greater than positive internal control as applicable. Internal controls for LEF1 staining included superficial conventional nevus component and lymphocytes. Internal control for PRAME included normal sebocytes/adnexal elements.

Statistical Analysis:

Fisher exact tests were used to assess the association between PRAME and LEF1 expression for the BN and DPN groups, for each pathologist (reader 1 and reader 2). Concordance between readers for PRAME and LEF1 expression data was also assessed using Cohen's kappa statistic. Analyses were not conducted on the melanoma cases due to the small number of samples. All statistical analyses were performed using R version 4.2.2.

Results:

Demographics and Clinicopathologic Characteristics (Table 1):

Patients with standard and CDPN (n=8) had equal sex distribution and a median age of 50.1 years (Table 1). The location of the lesions was either the trunk (n=5), head and neck (n=1) or upper extremity (n=2). The two patients with DPN with atypical features were both women (ages 28 and 30 years) and these lesions were located on the upper extremity and trunk. Among the patients with BN (n=16), most were women (n=12, 75%) and median age was 46.8 years. Anatomic distribution of these lesions was the head and neck (n=5), upper extremity (n=4), trunk (n=4), and lower extremity (n=3). Patients with the melanomas with features of DPN or BN were women (ages 44 and 80 years). These lesions were located on the head and neck and upper extremity.

Immunohistochemistry (IHC):

By immunohistochemistry (IHC), both DPN and BN were essentially negative for PRAME (Figure 1, Table 1, Table 2). The two DPN with atypical features were negative for PRAME, although one case showed focal staining (less than 10% of the cells) by both readers (Table 1). Both melanomas were positive for PRAME (Figure 1, Table 1).

All the standard DPN, CDPN and DPN with atypical features available for analysis were positive for LEF1 (Figure 2, Table 1, Table 2). One DPN was not interpretable after further processing due to depletion of candidate tissue. It was noted in all combined DPN cases that the associated conventional nevus showed either LEF1 positivity in the superficial aspect and loss of labeling with descent ("maturation pattern with depth") or negative staining throughout. Many BN tended to be negative for LEF1 (n=10/16; n=11/16) (Figure 3, Table 1, Table 2). A case interpreted as cellular BN with DPN features (based on histology and negative expression of β-catenin and cyclin D1) had discrepant interpretations of LEF1 by the two readers. The melanoma with DPN-like features was positive for LEF1 while the other melanoma was not interpretable after further processing and repetition of IHC due to folding and subsequent depletion of the candidate tissue. Of note, the melanoma with DPN-like features was positive for *BRAF* V600E, *ATRX* G2018E, and *MLH1* S362F mutations by next-generation sequencing and subsequently developed a regional lymph node metastasis. No molecular data is available for the melanoma with features of blue nevus. Clinical data and results are summarized in Table 1 and 2.

Statistical analyses for the PRAME and LEF1 expression in BN and DPN are summarized in Table 2. For PRAME, all BN were found to be negative by both readers. Reader 1 found all DPN to be negative for PRAME; while reader 2 determined that 1 of 10 DPN was positive

for PRAME. The DPN deemed positive for PRAME was a CDPN consisting of a compound conventional nevus with DPN. Overall, LEF1 expression was found to be more frequent in DPN than in BN, with all DPN found to be positive for LEF1 by both readers. This association was found to be statistically significant for both readers (p=0.0028; p=0.001).

Concordance between pathologists was assessed using Cohen's kappa statistic. For PRAME, kappa was 0.781 and for LEF1, kappa was 0.92 indicating substantial and almost/near perfect agreement between both pathologists for both immunohistochemical studies respectively.

Discussion:

The differential diagnosis for DPN includes benign, atypical or borderline lesions and malignant neoplasms. At times, diagnosis can be challenging even for experienced dermatopathologists. Although histopathologic examination remains the mainstay for diagnosis, ancillary studies including IHC, FISH and other molecular techniques have proven to be effective diagnostic adjuncts.[19,21,23–25]

DPNs typically demonstrate WNT pathway activation via gain-of-function mutations of *CTNNB1* (exon 3), which encodes the β-catenin protein.[4,8] WNT activation leads to an increase in melanocyte size and pigmentation, contributing to the characteristic appearance of DPN. The mutated β-catenin interacts with LEF1 in the nucleus, making LEF1 expression a potentially useful candidate for demonstration of WNT activation and diagnosis of DPN.[4,7,8]

DPN shares several histopathological features with BN and Spitz nevus and can demonstrate diffuse expression of HMB45 by IHC, as with BN. However, *GNAQ* and *GNA11* mutations are specific for BN and not DPN. *HRAS* mutations, present in a subset of Spitz nevi, have also been identified in DPN, suggesting a possible shared lineage or relationship between these two lesions; in contrast, *ALK* rearrangements are not readily identified in DPN.[23,24]

Atypical DPNs demonstrate similar mutations as DPN. Manca et al. (2021) determined via next generation sequencing (NGS) that atypical DPN demonstrated mutations within both the β-catenin and MAPK pathways as well as IDH mutations in 33% of cases.[25] Generally, atypical DPN do not demonstrate cytogenetic abnormalities via FISH and CGH.[6,26] However, abnormal profiles in these lesions have been described.[8,27] Atypical DPNs may show unremarkable cytogenetic profiles initially, but later demonstrate chromosomal aberrations once they have progressed to melanoma.[6,27]

Mutations in *BRAF* or *MEK* have been shown to give rise to conventional nevi. The development of a subsequent *CTNNB1* mutation results in the development of a DPN. With additional molecular alterations in genes such as *CDKN2A* and *TERT*, DPN-like melanomas may arise.[7] DPN-like melanoma typically have histomorphologic features of both DPN and melanoma. Like DPN, DPN-like melanomas usually exhibit activation of the WNT pathway. However, different mutations within different regions of these lesions have been described. Giubellino et al. (2022) described *BRAF* and *PTEN* mutations in both DPN and melanoma components of a biphenotypic DPN-like melanoma. However, a *CTNNB1* mutation was

only appreciated in the DPN-like regions of the tumor.[27] In their study, Yeh et al. (2017) detected mutations in either *BRAF* or *NRAS* in five of five DPN-like melanomas; while activating mutations in β-catenin were detected in only three of five cases.[7] Additional oncogenic alterations have been reported in DPN-like melanoma including in *CDKN2A, TERT, TP53, ARID1A, TET2, IDH1, ERBB4, BRCA2*, and *RET*, to name a few.[4,7,26]

Recently, PRAME has emerged as a potentially useful biomarker for distinguishing between melanomas and benign melanocytic nevi as most melanomas show diffuse PRAME expression (i.e. nuclear immunoreactivity in >75% of tumor cells) by immunohistochemistry whereas most benign nevi show little to no reactivity.[19] Gene expression studies have also demonstrated that PRAME is readily expressed in melanomas when compared to benign nevi and it is included in gene expression diagnostic tests for cutaneous melanomas.[28,29]

Although well studied in the context of benign melanocytic nevi and conventional melanomas, PRAME expression in other challenging and borderline melanocytic lesions has not been extensively studied.[28] It has been demonstrated in early studies that DPNs are generally negative for PRAME.[18,19] Our results provide additional evidence that PRAME may be of diagnostic utility in the evaluation of CDPN and combined BN, lesions in which a diagnosis of melanoma arising in association with a nevus may be entertained.

It has been demonstrated that DPNs harbor mutations in β-catenin as well as driver mutations in the MAPK-pathway. β-catenin activates LEF1, leading to the expression of different genes.[30–32] The expression of LEF1 is purely nuclear and as such it can be easier to interpret than β-catenin, which in contrast commonly demonstrates strong membranous/cytoplasmic staining in addition to diagnostic nuclear staining. At times, additional heavy intracytoplasmic melanin can make the evaluation of β-catenin nuclear expression, required for the accurate diagnosis of DPN, difficult in daily practice. Through the application of the criteria described, our results demonstrate that all DPN, including those with atypical features, express LEF1 while the majority of BN were negative. In contrast to what has been previously described by Raghavan et al. (2020), who determined that none of the BN in their study stained for LEF1[8] ; our two readers found several BN expressing LEF1 (Table 1). Specifically, Reader 1 identified 6 BN expressing LEF1 (1 cellular blue nevus with features of deep penetrating nevus, 3 combined BN, 1 combined BN with atypical features and 1 BN with atypical morphologic features and cellular morphology) and Reader 2 identified 5 BN expressing LEF1 (3 combined BN, 1 combined BN with atypical features and 1 BN with atypical morphologic features and cellular morphology). Specifically, predominantly heterogeneous LEF1 staining toward the middle to base of the lesion in these cases was observed. Although the lesions were carefully evaluated to make sure that LEF1 labeling did not correspond to the conventional nevus portion of the lesion, this finding may be explained by the biphenotypic and/or atypical appearance of these lesions, which made interpretation of LEF1 staining difficult. For example, mature conventional nevus positive for LEF1 toward the middle of a combined BN with spindled morphology similar to BN may have confounded interpretation in spite of best efforts. In fact, initial evaluation of the cases rendered more combined BN interpreted as positive due to the presence of positive cells, mainly superficial, in the conventional nevus portion of the lesions. This phenomenon has been reported in other benign lesions, such as acral nevi and congenital nevi[33], and

has also been previously described with β-catenin, further supporting the relationship between β-catenin and LEF1.[8,9] LEF1 may be best used in more conventional BN cases without atypia and in combined BN with clear delineation between components. In contrast, LEF1 in the DPN component of the combined nevi was expressed throughout. Interpreting positivity of LEF1 in the associated conventional nevus component of combined BN as supportive evidence for DPN is therefore a potential pitfall that should be avoided and can be solved by further evaluating the deep aspects of the lesion. In addition, care must be taken when interpreting LEF1 in inflamed nevi or in nests near adnexal structures as normal T-cells and melanocytes near adnexal structures tend to express LEF1.[8] One melanoma in our cohort was positive for LEF1. It has been documented that melanomas, particularly those with DPN-like features demonstrate activation of the WNT-pathway and so LEF1 expression would not be unexpected.[6,7] The inclusion of two melanomas, although primarily for comparison and control purposes, is a limitation to the current study. In future studies, consideration to the inclusion of additional melanoma cases (especially those with morphologies reminiscent of DPN or BN) as well as other melanocytic lesions should be given.

In summary, PRAME appears to be negative in BN and most DPN, including CDPN and DPN with atypical features, in contrast to what is reported for melanoma. The restricted expression of LEF1 in the nucleus may facilitate the diagnosis of CDPN over combined BN, especially in cases with equivocal nuclear expression of β-catenin since most combined BN are negative. In some combined BN, however, LEF1 may display expression in the conventional nevus component of the lesion, representing a potential pitfall when evaluating this stain. Looking for a “maturation pattern” of labeling may help in these cases.

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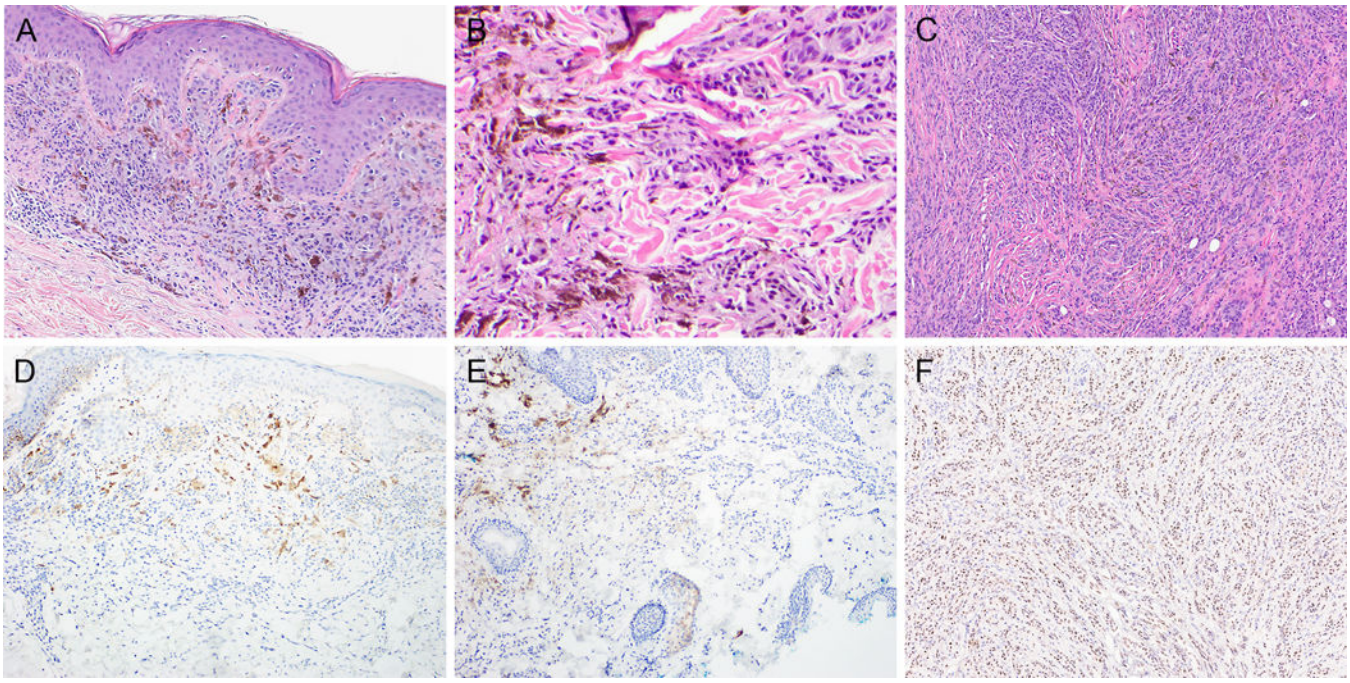

Figure 1:

Expression of PRAME in deep penetrating nevus (DPN) and DPN-like lesions. A: Deep penetrating nevus with combined intradermal nevus. A proliferation of spindle and epithelioid melanocytes arranged in a plexiform architecture is present, associated with heavily pigmented melanocytes (H&E, x 10), B: Combined blue nevus (BN). The blue nevus component of the lesion shows similar morphological features to those seen in the DPN (H&E, x 20), C: Melanoma with DPN-like features. This malignant proliferation of melanocytes also shows a plexiform pattern of growth and associated melanophages (H&E, x 10) D: Both components of this combined DPN are negative for PRAME (x10). E: Both components of this combined BN are negative for PRAME (x 20); F: In contrast to DPN and BN, the DPN-like melanoma shows diffuse positivity for PRAME (x 10).

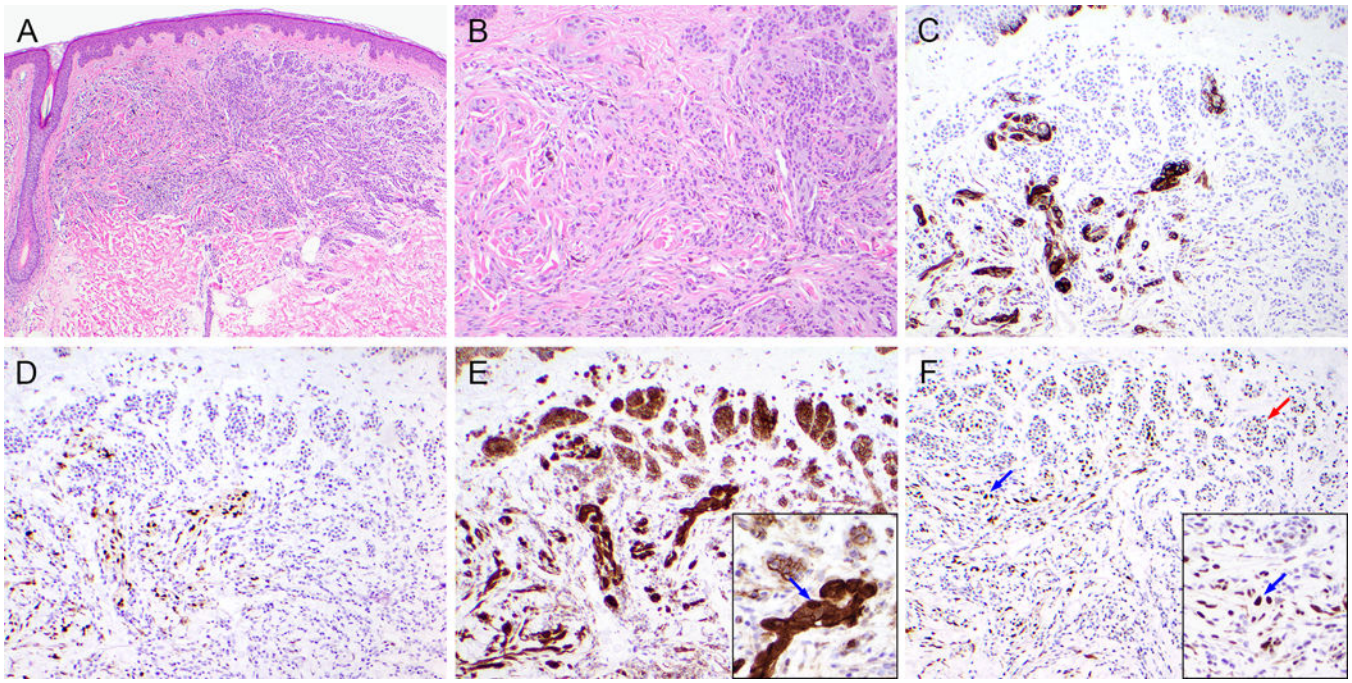

Figure 2:

Combined deep penetrating nevus (CDPN). A: The lesion shows a wedge-shaped architecture and biphenotypic cytology (H&E, x 4). B: The melanocytes display spindle cell (DPN) and nevoid (intradermal nevus) morphology (H&E, x 10). C: HMB45 reveals strong labeling in the DPN component while the conventional nevus cells are negative (x 10). D: The DPN cells are strongly positive for cyclin D1, in contrast to the conventional nevus cells (x 10). E: β-catenin shows strong cytoplasmic labeling in both components of the lesion, which may make interpretation difficult. The DPN cells appear to also exhibit nuclear staining (x 10) Inset: Nuclear expression of β-catenin in the DPN cells (blue arrow). Notice the membranous pattern of expression of surrounding regular nevus cells. Even at high magnification, evaluation of nuclear staining of β-catenin can be difficult (x 40). F: LEF1 shows clean and diffuse nuclear labeling in the DPN cells (blue arrow). The inset shows higher magnification of cells displaying nuclear expression of LEF1 (blue arrow) and adjacent melanophages (x 40). The conventional nevus cells show nuclear positivity predominantly in the superficial cells (red arrow), while the deeper cells appear to be negative (“maturation pattern”) (x 10).

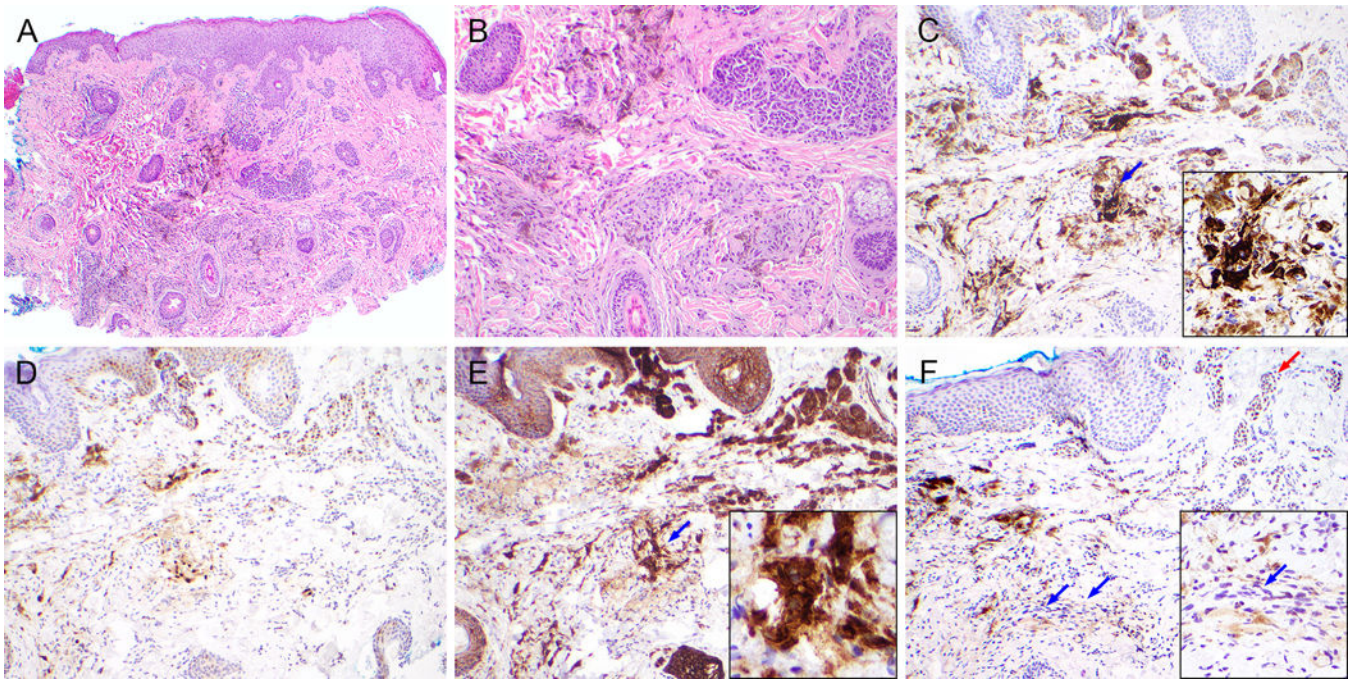

Figure 3:

Combined blue nevus (BN). A: The lesion shows a biphenotypic appearance, similar to the combined DPN seen in Figure 2 (H&E x4), B: The blue nevus is composed of spindle and epithelioid cells admixed with melanophages. A conventional intradermal nevus is present in the upper right quadrant of the figure (H&E x 10), C: HMB45 is strongly expressed by the blue nevus cells. Inset shows a higher magnification view of the blue nevus cells that are strongly positive for HMB45 and are associated with abundant melanophages (x 40). The adjacent conventional nevus cells reveal loss of HMB45 with descent (“maturation pattern”) (x10), D: cyclin D1 labels scattered cells, mostly in the upper portions of the conventional nevus component of the lesion (x 10), E: β-catenin shows diffuse cytoplasmic staining in both blue nevus and conventional nevus cells, which may make interpretation difficult (x 10). Higher magnification (inset) fails to show unequivocal nuclear expression of the marker, although evaluation is still challenging (x40), F: LEF1 reveals lack of nuclear staining in the blue nevus cells (blue arrows; Inset: a higher magnification view, (x40). The conventional nevus cells show labeling in the superficial portion (red arrow) (x 10).

Table 1:

Patient demographic information, histopathological description and expression patterns of PRAME and LEF1 in melanocytic lesions.

	PRAME expression (Reader 1)	LEF1 expression (Reader 1)	PRAME expression (Reader 2)	LEF1 expression (Reader 2)	Tumor Type	Gender (F= female; M= male)	Age (years)	Anatomic site
Blue nevus (BN) group								
1	Negative	Positive	Negative	Positive	Combined: compound and blue nevus with atypical features	F	33	right upper leg
2	Negative	Negative	Negative	Negative	Combined: compound with congenital features and blue nevus	F	47	right upper arm
3	Negative	Positive	Negative	Positive	Combined: compound and blue nevus	F	52	left upper back
4	Negative	Positive	Negative	Positive	Combined: blue and intradermal nevus	M	62	upper medial abdomen
5	Negative	Negative	Negative	Negative	Combined: blue and compound dysplastic nevus	F	32	right upper back
6	Negative	Negative	Negative	Negative	Combined: blue and compound nevus	F	62	right lower eyelid
7	Negative	Positive	Negative	Positive	Blue nevus with atypical features and cellular morphology	F	76	left anterior upper arm
8	Negative	Negative	Negative	Negative	Cellular blue nevus	M	32	left radial dorsal hand
9	Negative	Negative	Negative	Negative	Cellular blue nevus	F	58	right foot
10	Negative	Negative	Negative	Negative	Cellular blue nevus	F	58	right dorsal foot
11	Negative	Negative	Negative	Negative	Cellular blue nevus	M	70	left supra-auricular
12	Negative	Negative	Negative	Negative	Cellular blue nevus	F	42	left superior frontal scalp
13	Negative	Negative	Negative	Negative	Cellular blue nevus	F	12	right forearm
14	Negative	Positive	Negative	Negative	Cellular blue nevus with features of deep penetrating nevus	F	32	left frontal scalp
15	Negative	Positive	Negative	Positive	Combined: blue and intradermal nevus	F	26	right cheek
16	Negative	Negative	Negative	Negative	Combined: blue and compound nevus	M	55	right midline abdomen above umbilicus
Deep penetrating nevus-like with atypical features								
1	Negative	Positive	Negative	Positive	Atypical melanocytic proliferation with features of atypical blue nevus and deep penetrating nevus	F	28	left anterior distal upper arm

	PRAME expression (Reader 1)	LEF1 expression (Reader 1)	PRAME expression (Reader 2)	LEF1 expression (Reader 2)	Tumor Type	Gender (F= female; M= male)	Age (years)	Anatomic site
2	Negative (focal positive in <10% of cells)	Positive	Negative (focal positive in <10% of cells)	Positive	Deep penetrating nevus with atypical features	F	30	right mid back
Deep-penetrating nevus (DPN) group								
1	Negative	Positive	Negative	Positive	Combined: deep penetrating and compound dysplastic nevus	M	76	left mid back
2	Negative	Positive	Negative	Positive	Combined: deep penetrating and compound nevus	F	40	left cheek
3	Negative	n/a	Negative	n/a	Deep penetrating nevus	F	31	right forearm
4	Negative	Positive	Positive	Positive	Combined: compound with deep penetrating nevus	F	43	left arm
5	Negative	Positive	Negative	Positive	Combined: blue and deep penetrating nevus	M	63	right mid back
6	Negative	Positive	Negative	Positive	Combined: deep penetrating and focal intradermal nevus	M	55	left lower back
7	Negative	Positive	Negative	Positive	Deep penetrating nevus	M	11	Right lateral back
8	Negative	Positive	Negative	Positive	Combined: blue nevus and deep penetrating nevus	F	82	left lateral back
Melanoma								
1	Positive	Positive	Positive	Positive	melanoma with features of deep penetrating nevus	F	80	left temple
2	Positive	n/a	Positive	n/a	melanoma with features of blue nevus	F	44	right anterior proximal upper arm

Table 2:

Summary of statistical analyses expression of PRAME and LEF1 in blue nevus (BN) and deep-penetrating nevus (DPN) groups.

	Expression	BN (n)	% BN	DPN (n)	% DPN	Total BN and DPN (n)	Combined BN and DPN (%)
PRAME Expression Reader 1	Negative	16	100	10	100	26	100
	Positive	0	--	0	--	0	--
LEF1 Expression Read 1	Negative	10	62.5	0	--	10	40.0
	Positive	6	37.5	9	100	15	60.0
***p* = 0.0028**							
PRAME Expression Reader 2	Negative	16	100	9	90.0	25	96.2
	Positive	0	--	1	10.0	1	3.8
***p* = 0.38**							
LEF1 Expression Reader 2	Negative	11	68.8	0	--	11	44.0
	Positive	5	31.2	9	100	14	56.0
***p* = 0.001**							