J Cutan Pathol 2009: 36: 1074–1076
doi: 10.1111/j.1600-0560.2008.01223.x
John Wiley & Sons. Printed in Singapore

Journal of
Cutaneous Pathology

Caveats in BerEP4 staining to differentiate basal and squamous cell carcinoma

Background: Superficial skin biopsies of basal cell carcinoma (BCC) represent some of the most common dermatopathology specimens. Superficial shave biopsies containing partial samples of lesions with squamatization present difficulties in distinguishing BCC from squamous cell carcinoma (SCC). BerEP4 has been employed as a dependable marker in differentiating between BCCs and SCCs.
Methods: We collected 12 cases of superficial biopsies of BCC with centrally located cords and strands suggesting squamous differentiation at the Yale Dermatopathology Laboratory over a 3-month period and stained them with BerEP4.
Results: We found that all cases (12 out of 12) showed membranous and cytoplasmic staining with BerEP4 in the obvious areas of basaloid differentiation at the periphery of the tumors, while cords and strands of enlarged cells with squamoid features in the center and surface of the biopsy failed to label with BerEP4.
Conclusions: BerEP4 labeling is not reliable in superficial biopsies of BCC with squamoid features. It is important to be aware of this caveat in interpreting BerEP4 staining for BCC and SCC.

Yu L, Galan A, McNiff JM. Caveats in BerEP4 staining to differentiate basal and squamous cell carcinoma.
J Cutan Pathol 2009; 36: 1074–1076. © 2009 John Wiley & Sons A/S.

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Accepted for publication November 20, 2008

Superficial biopsies of basal cell carcinoma (BCC) represent some of the most common skin specimens received in pathology laboratories. With adequate biopsies, diagnosis is usually straightforward, based on peripheral palisading of basaloid nuclei, with cleft artifact between tumor islands and a specialized stroma. In some cases, however, superficial shave biopsies containing partial samples of lesions present difficulties in distinguishing BCC from squamous cell carcinoma (SCC), especially when only the surface of the lesion is visualized, and shows squamatization. This phenomenon seems most prevalent in small biopsies of ulcerated BCCs, in which the more obvious basaloid features expected at the periphery of the tumor are not contained in the biopsy. In such cases, small cords and strands of enlarged pale cells without basaloid features sometimes predominate. Moreover, this caveat seems under-recognized in major dermatopathology textbooks.[1–3]

In recent years, anti-human epithelial antigen (BerEP4) has been widely used as a reliable marker for BCC. Published data suggest that BerEP4 is a dependable marker in differentiating BCC from other neoplasms, most notably SCC, but also including actinic keratosis and microcystic adnexal carcinoma (MAC).[4–7] In this study, we evaluated BerEP4 labeling in cases of BCCs with superficial squamous differentiation to evaluate its accuracy and reliability in distinguishing such cases from superficial samples of SCC.

Materials and methods

Twelve superficial biopsies of BCC with centrally located cords and strands suggesting squamous differentiation were retrieved from the histopathology archives at the Yale Dermatopathology Laboratory

over a 3-month period. In addition, one case diagnosed as SCC during the study period, and identified as truly representing the surface of BCC on re-excision, was also included.

Serial recut sections of all cases were labeled BerEP4 monoclonal mouse anti-human antibody (DakoCytomation GmbH) as the primary antibody, at a concentration of 1:1000 with an incubation time of 30 min, followed by a biotinylated secondary antibody for 15 min and streptavidin peroxidase conjugate for another 15 min. 3-Amino-9-ethylcarbazol was used to visualize the end product.

Results

Twelve cases were included. See Table 1 for clinical information. Twelve of 12 cases showed membranous and cytoplasmic staining with BerEP4 in the obvious areas of basaloid differentiation at the periphery of the tumors, while uninvolved epidermis was not stained. In each case, cords and strands of enlarged cells without basaloid features in the center and surface of the biopsy failed to label with BerEP4 (Fig. 1). In a biopsy of BCC that was originally interpreted as SCC prior to re-excision, the squamoid cords and strands present in the biopsy were negative for BerEP4. In contrast, the more clear-cut BCC on re-excision was strongly positive for BerEP4.

Discussion

BerEP4 reacts with 34-kDa and 39-kDa glycoproteins present on the cell surface and in the cytoplasm of most human epithelial and carcinoma cells.[8] In the skin, BerEP4 stains normal adnexal epithelium, including the apocrine and eccrine epithelium, matrical and outer sheath epithelium of vellus anagen follicles and inferior segment epithelium of vellus telogen follicles.[4,9] BerEP4 reacts with cutaneous BCC, Merkel cell carcinoma, trichoepithelioma, tumors with sweat duct differentiation and follicular induction over dermatofibromas.[4,6,10] The usefulness of staining with BerEP4 to distinguish cutaneous BCC from SCC has been well shown. In a series with 22 cases of BCCs and 21 cases of SCCs, Tellechea et al.[4] showed diffuse and intense labeling of BerEP4 in all BCCs, but no expression in any of SCCs. In another study performed by Kist et al.,[6] 27 of 27 BCCs, including nodular, morpheaform/infiltrative and adenoid variants, were positive for BerEP4. BerEP4 defined areas of BCC in dense inflammation better than hematoxylin and eosin (H&E) stain in 13 of 27 BCCs and identified the final Mohs margins in two cases of infiltrative BCC that were otherwise appearing negative with routine H&E stain. Our experience of BerEP4 immunoreactivity in SCC is consistent with the literature. All (5 of 5) SCCs retrieved from the same period as BCCs in this study were negative for BerEP4. It has also been shown that BerEP4 reliably differentiates MAC from BCC to the same extent as it distinguishes the latter tumor from SCC.[5]

Table 1. Clinical information

Case	Age (years)	Sex	Site	Clinical diagnosis	Size (mm^3)
1	70	F	Left leg	AK, BCC	5 × 5 × 2
2	50	M	Left cheek	SCC	4 × 3 × 1
3	79	M	Forehead	SCC	5 × 2 × 2
4	50	F	Left chest	BCC	6 × 5 × 1
5	71	F	Nose	BCC	5 × 4 × 1
6	53	M	Nose	BCC	5 × 5 × 1
7	59	M	Face	SCC	5 × 4 × 1
8	62	F	Left leg	SCC	2 × 2 × 2
9	78	M	Left neck	BCC	5 × 5 × 1
10	88	F	Nose	BCC	7 × 5 × 3
11	71	M	Forehead	SCC	5 × 4 × 1
12	61	F	Nose	BCC	8 × 6 × 1

AK, actinic keratosis; BCC, basal cell carcinoma; SCC, squamous cell carcinoma.

BCC may show areas with morphologically distinct squamous cells, with or without keratinization. Those areas are usually located in superficial papillary dermis overlying more typical foci of palisaded epithelium and can make it challenging to diagnose BCC when only superficial biopsy is conducted. It is important to note that the clinical impression was 'SCC' in several of our cases, potentially misleading the pathologist in histologically ambiguous cases. To the best of our knowledge, no study has been done to evaluate BerEP4 expression in these cases. The data in this study suggest that staining for BerEP4 differentiate between BCC and SCC in such ambiguous cases is not reliable.

The terms 'basosquamous carcinoma' and 'metatypical carcinoma' are somewhat controversial and confusing but are most often applied to carcinomas with contiguous areas of both BCC and SCC, usually with less obvious peripheral palisading, and typically with an aggressive clinical behavior. Some authors feel that these tumors represent a transition between BCC and SCC.[2] Keratotic BCC also shows squamous differentiation, typically in the form of keratin pearls and differentiation toward hair follicles. However, these features are usually located in the center of tumors, in contradistinction to the pattern observed in this report affecting the surface only and under areas of ulceration. Some basosquamous carcinomas also exhibit epithelial pearls. In one study, positive BerEP4 staining was identified in all basosquamous carcinomas, while two of the basosquamous carcinomas were negative for BerEP4 in small foci that had well-developed epithelial pearl formation.[11]

In summary, we commonly receive superficial biopsies in which the precise classification of carcinoma is problematic. While an attempt can be made to favor either BCC or SCC on histological grounds, this

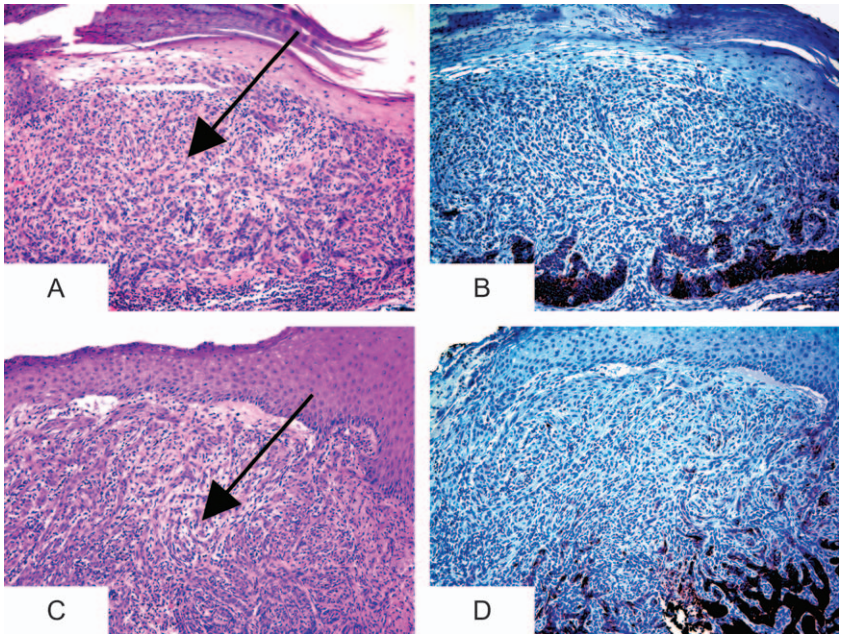

Fig. 1. BerEP4 labeled the obvious areas of basaloid differentiation at the periphery of superficial biopsies of basal cell carcinoma. Squamous differentiation in the center and surface of the biopsies (indicated by arrows) failed to label with BerEP4. A and B) H&E and BerEP4 stains of one biopsy (×400). C and D) H&E and BerEP4 stains of another biopsy (×400)

is not completely reliable. We hoped, based on results in the literature, that staining with BerEP4 would be a useful adjunct in these cases. Unfortunately, despite strong staining of the periphery of such BCCs with BerEP4, staining with this antibody is consistently negative in superficial areas of tumor showing a more squamoid appearance. Awareness of this caveat in BerEP4 staining for BCC and SCC is important.

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