Published in final edited form as:

Dermatol Surg. 2012 August ; 38(8): 1357–1366. doi:10.1111/j.1524-4725.2012.02417.x.

DIAGNOSTIC UTILITY OF CYTOKERATIN 17 IMMUNOSTAINING IN MORPHEAFORM BASAL CELL CARCINOMA AND FOR FACILITATING THE DETECTION OF TUMOR CELLS AT THE SURGICAL MARGINS

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Abstract

Background—The morpheaform subtype of basal cell carcinoma (BCC) often presents a diagnostic histological challenge, and its true margin may be difficult to determine with accuracy. This tumor may also be difficult to distinguish from other adnexal neoplasms having a benign clinical course. Previous work has shown that cytokeratin 17 (K17) expression is increased in BCC.

Objective—To confirm the expression of K17 across the subtypes of superficial, nodular and morpheaform BCC variants. Secondly, compare K17 expression in each of these subtypes of BCC to two other adnexal neoplasms.

Methods—Tissue specimens from each tumor category were randomly collected, immunolabeled, and scored for K17 expression by intensity and extent of immunostaining.

Results—Our results indicate that K17 is a very useful marker in the identification and outlining of BCC. Moreover, in morpheaform BCC, K17 immunostaining clearly detected individual tumor cells well away from the dermal tumor strands that otherwise seemed non-malignant by hematoxylin and eosin staining alone. In addition, we report that the expression of K17 in morpheaform BCC is capable (100% of specimens; $p < 0.0001$) of distinguishing this tumor from desmoplastic trichoepithelioma.

Conclusion—We propose that K17 immunostaining could improve the diagnostic and surgical management of these tumors.

INTRODUCTION

Basal cell carcinoma (BCC) normally does not present substantial diagnostic difficulties from a histological standpoint. However, certain BCC variants, such as the morpheaform

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The authors have indicated no significant interest with commercial supporters.

type, can be difficult to either diagnose or distinguish from other morphologically similar entities, such as desmoplastic trichoepithelioma. Dermal strands of tumor or isolated single tumor cells may also be difficult to detect. It would be useful to establish promising markers that would ensure complete removal of tumors present in sensitive areas such as the face, around the eyes, and the ears. There is background information suggesting that cytokeratin 17 (K17) may be such a useful marker.²¹⁻²⁶ However, no studies have addressed the usefulness of K17 in both differential diagnosis and tumor detection.

Keratin intermediate filaments are important structural proteins of epithelial cells. At least twenty different keratin peptides are known to occur diversely throughout human epithelia. There are two families of keratins, classified as either type I or type II based on biochemical properties. Members from the two different keratin families form obligate heteropolymers, meaning that a member from each family must be co-expressed to form a filament.^{1,2} Aside from providing structural support to epithelial cells, these filaments have been shown to be involved in cell signaling. Keratins can modulate the ability of cells to undergo apoptosis.^{3,4} In fact, Tong et al. have shown that, in mice, K17 modulates hair follicle cycling from anagen to catagen phases.

K17 is normally expressed in palmoplantar keratinocytes as well as in the nail bed, hair follicle, and sebaceous and sweat glands.⁶ Concerning its presence in the hair follicle, K17 is found admixed with several other keratins throughout the medulla of the hair shaft. Additionally, K17 expression is found in the outer root sheath, but its presence is limited to the isthmus and lower portion of this structure.⁶ K17 has also been found to be prominently expressed in human epidermal Merkel cell-rich sensory organs.⁷ K17 has been shown to be inducible by wounding or injury, and it is found to be upregulated in hyperproliferative conditions such as psoriasis.⁸⁻¹¹ Also, mutations in K17 can lead to a form of the rare disorder pachyonychia congenita, in which patients develop nail dystrophy, palmoplantar keratoderma, and other cutaneous findings associated with abnormal keratinization.^{12,13}

It has also been shown that K17 expression is increased in the epithelium of viral warts,¹⁴ in oral squamous cell carcinoma¹⁵, and in lesions of cervical intraepithelial neoplasia (CIN). Moreover, it has been demonstrated that enhanced positivity for K17 immunostaining is significantly correlated with increasing lesional grade for CIN.¹⁶ Meara et al. observed that K17 was over-expressed in odontogenic keratocysts. These cysts are locally destructive lesions of the jaw and are associated with the nevoid basal cell carcinoma syndrome (NBCC, Gorlin's syndrome). Also noted in that particular study was the finding that K17 immunostaining was stronger and more uniform in specimens from patients with the NBCC diagnosis than in those from patients without that condition.¹⁷

BCC is the most common malignancy of the skin.¹⁸ It is a peculiar neoplasm with distinctive traits in that it can undergo sustained local growth, but is much less likely to metastasize. However, the morpheaform or desmoplastic subtype of this tumor is notorious for being more aggressive, as it tends to invade the local tissues beyond the clinically apparent margins and tends to recur after excision. BCC develops almost exclusively on skin containing pilosebaceous units, with the face being a favored site.¹⁸ This finding suggests that these anatomic locations may be home to a greater number of precursor or progenitor tumor cells.¹⁸

Because keratin expression is generally regulated by cell activation and differentiation, it can be inferred that its expression may shed light on the differentiation trends of skin neoplasms. Several studies focused on keratin analysis have pointed to the hair follicle as the potential harbor of progenitor cells leading to BCC.¹⁹⁻²³ Moll et al. initially proposed that BCC is of follicular origin due to the presence of K17 in the pilosebaceous unit and in

lesions of BCC. Furthermore, the expression of K17 in BCC has been noted in other studies.²¹⁻²⁶ The presence of K17 has also been noted in other tumors of proposed follicular differentiation, such as trichilemmal carcinoma,²⁷ trichoblastoma, and trichoepithelioma.^{26,28,29}

Trichoblastoma and trichoepithelioma are adnexal neoplasms thought to display mostly follicular germinative differentiation. It is thought that trichoblastoma displays mostly follicular bulbar differentiation, and this tumor is considered by some to represent an immature form of trichoepithelioma. The term desmoplastic trichoepithelioma refers to a variant of tumor with extensive dermal sclerosis.³⁰ As previously mentioned, these lesions can present a diagnostic challenge and mimic BCC both clinically and histologically. However, these tumors generally do not carry the risk of local invasion or tumor recurrence associated with BCC.

In this report, our aim was to confirm the expression of K17 across the subtypes of superficial, nodular, and morpheaform BCC variants. Specifically, our intent was to determine whether the more aggressive morpheaform variant demonstrates a correspondingly higher expression of K17. Additionally, we sought to compare K17 immunostaining in BCC with that found in two other adnexal neoplasms of proposed follicular origin, namely trichoblastoma and desmoplastic trichoepithelioma.

MATERIALS AND METHODS

The study was conducted in full compliance with the Institutional Review Board (IRB). Fifty paraffin-embedded archived tissue samples from skin biopsy specimens (30 cases of BCC including 10 cases of each aforementioned subtype, and 10 cases each of trichoblastoma and desmoplastic trichoepithelioma) were identified, and their tissue blocks were obtained. Each case had previously been diagnosed microscopically by a dermatopathologist using standard hematoxylin and eosin (H&E) staining methods. The archived specimens were chosen in a sequential and unselective manner based on a pathology requisition number search. Histology and immunostaining were performed on all specimens simultaneously in the Department of Dermatology Laboratory and in the Roger Williams Center of Biomedical Research Excellence Imaging Core (PI, V. Falanga). Four-micron thick sections of formalin fixed tissue were used for immunoperoxidase analysis. After baking at 37°C overnight, deparaffinization and rehydration were performed (100% xylene x4 for 3 minutes each, 100% ethanol x4 for 3 minutes each and running water for 5 minutes). The sections were blocked for peroxidase activity with 1% hydrogen peroxide in methanol for 30 minutes and washed under running water for 5 minutes. The sections were pressure-cooked (Biocare Medical, Concord, CA) for antigen retrieval at 123°C in Citrate Buffer (DAKO Target Retrieval Solution, Carpinteria, CA). The sections were cooled for 15 minutes, and transferred to phosphate buffer saline (PBS). The sections with CK17 (NCL-CK17 from Novocastra, Newcastle upon Tyne, UK) were incubated overnight at 1:100 dilution at 4°C. The secondary antibody (DAKO Envision Mono K4007, Carpinteria, CA) was applied for 30 minute incubation. All the incubations were carried out in a humid chamber at room temperature. The slides were rinsed with TBS in-between each incubation. The sections were developed using 3,3'-diaminobenzidine (DAB from Sigma Chemical Company, St Louis, MO) as the substrate and counter-stained with Mayer's Hematoxylin. Human trachea was used as a positive control.

The K17 immunostained sections were examined using standard light microscopy and by comparing them to the H&E stained sections. Photomicrographs were taken of the sections, and the results were analyzed using a scoring system for the extent and intensity of immunostaining (see below). The assessment of the H&E and immunostaining was

performed by one of the authors (VF) with full training in histological methods and dermatopathology.

To perform CK-17 immunostaining using frozen sections, we used a standard protocol. Mohs frozen sections were cut at 4-microns and fixed in cold acetone for 10 minutes. After being washed in TBS, sections were incubated in 3% H₂O₂ for 5 minutes to block the endogenous peroxidase activity. Non-specific reactivity blocking was accomplished by incubating the sections in goat normal serum for 20 minutes. With no additional washing, the sections were incubated with the primary antibody (mouse anti human CK17 from Dako) for one hour. Sections were then raised to remove excess antibody and subsequently incubated for 30 minutes with the secondary antibody (goat anti mouse polymer-HRP from Dako EnVision system). Substrate DAB was used to develop the brown color, and hematoxylin was used for counterstaining.

RESULTS

K17 immunostaining has been previously reported in tissue sections of mostly nodular BCC.²¹⁻²⁶ In those previous reports, there was no attempt to analyze morpheaform BCC and its surgical margins, and no comparison was made with tumors having histological overlaps with morpheaform BCC. Therefore, our first goal was to confirm K17 expression in our own sequential and unselected tissue samples of BCC. In addition, we wanted to compare the immunostaining presence and intensity in different variants of BCC, as well as in trichoblastoma and desmoplastic trichoepithelioma. Using archived tissues, we retrieved 10 unselected and sequential archived specimens for 5 different tumor types or variants (categories): a) superficial BCC, b) nodular BCC, c) morpheaform BCC, d) desmoplastic trichoepithelioma, and e) trichoblastoma. In 3 of these categories of tumors, 9 of 10 specimens (instead of 10 out of 10) were available for analysis because of lack of tumor upon further sectioning of the specimen blocks.

The sections were scored for the intensity and the extent of K17 immunostaining. Briefly, the intensity of immunostaining was ranked on a scale of 0 to 3 as follows: 0 = absence of immunostaining; 1 = low; 2 = moderate; and 3 = high. The extent of immunostaining was ranked on a scale of 0 to 3 as follows: 0 = absence of immunostaining; 1 = focal or less than 10% of cells; 2 = patchy or 11-75% of cells; and 3 = diffuse or more than 75% of cells. The specimens were graded independently by one of the co-authors (Vincent Falanga; Table 1).

Table 1 summarizes the overall immunostaining presence and intensity in morpheaform BCC and the other tumors that were investigated. The scores represent the mean $\pm$ SEM. As shown in Table 1, the mean of intensity and extent of K17 immunostaining in superficial, nodular, and morpheaform BCC were more than two-fold and more than five-fold greater when compared to trichoblastoma and desmoplastic trichoepithelioma, respectively. In particular, K17 expression scoring was statistically greater in morpheaform BCC when compared to desmoplastic trichoepithelioma (student t-test; $p < 0.0001$). The differences in scoring among these two latter types of tumors and their variants with respect to K17 expression were quite dramatic. This is shown by representative photomicrographs (Figures 1-4) shown below and described in detail in the legends.

First of all, K17 immunostaining was strongly positive in a diffuse pattern throughout the lesions of superficial BCC in 100% (n=10) of the specimens analyzed. Figures 1a and 1b show representative images from this group of specimens. K17 immunostaining was similarly positive in a diffuse pattern throughout the lesions of nodular BCC in 100% (n=9) of the specimens analyzed. Figures 1c and 1d show representative images from this group of tumors.

An important novel goal of this work was to determine whether K17 immunostaining could identify and outline the strands of dermal tumor strands or cells typical of morpheaform BCC. The reasons for this goal lie in the not uncommon difficulties one encounters in the diagnosis and assessment of the extent of this tumor and the differential diagnosis from desmoplastic trichoepithelioma. We found that, indeed, K17 may be a very valuable marker for those purposes. K17 immunostaining was in fact strongly positive throughout the lesions of morpheaform BCC in 100% (n=9) of the specimens evaluated. These findings are shown in representative Figures 2a-d. We found that the K17 immunostaining could actually prove useful in identifying individual tumor cells located away from the morpheaform strands. This finding is illustrated by Figures 2e and 3d, where K17 immunostaining highlights individual cells in the dermis. We were able to detect these individual tumor cells away from the tumor strands in 78% (n=9) of the specimens. Likewise, K17 immunostaining on frozen sections of morpheaform BCC taken during Mohs micrographic surgery was strongly positive, and small tumor strands and cords were readily apparent (Fig. 5).

In contrast, K17 immunostaining was detectable at a mean level approximately 5 times less in the cells throughout the basaloid strands of all the desmoplastic trichoepithelioma specimens analyzed. Figures 4a-e show representative examples from these specimens. There was minimal to moderate nonspecific staining of horn cysts present in most of these lesions. K17 immunostaining was shown to be variable in the lesions of trichoblastoma and ranged from absent or low in some specimens to patchy and even diffuse in other specimens (Table 1; figures not shown).

DISCUSSION

In this study, we found that K17 is strongly expressed histologically throughout the lesions of BCC. This expression was present intensely throughout the lesional tissue, irrespective of tumor subtype. Interestingly, the results indicate that the intensity of K17 expression may not necessarily correlate with the more aggressive behavior generally associated with the morpheaform BCC variant. Thus, the morpheaform subtype of tumors we studied did not have a correspondingly higher expression of K17. Importantly, however, morpheaform BCC has a strikingly different expression pattern compared to that of desmoplastic trichoepithelioma, and K17 is able to highlight distinct and subtle tumor cells in the dermis of morpheaform BCC specimens. By assisting in defining the surgical margin on a Mohs section and highlighting subtle tumor strands, or by definitively differentiating morpheaform BCC from desmoplastic trichoepithelioma, our novel findings could prove useful in clinical and surgical management.

In previous efforts to identify immunohistochemical methods to distinguish between morpheaform BCC and desmoplastic trichoepithelioma, some differences in the immunostaining patterns have been noted. Expression of the anti-apoptotic protein bcl-2 has been reported throughout the lesions of BCC, while only being positive in the outer rim of epithelium in desmoplastic trichoepithelioma.³² In addition, it has been reported that a comparison of immunostaining for androgen receptors and cytokeratin 20 can help in making this distinction. Thus, BCC is usually positive for androgen receptors and negative for cytokeratin 20, while the reverse immunostaining pattern is found in desmoplastic trichoepithelioma.^{33,34} However, despite several investigations, there has been no single immunohistochemical stain to reliably and uniformly differentiate these tumors. Since K17 does not partially stain desmoplastic trichoepithelioma (unlike bcl-2) and does not require a comparison of two different antigens (i.e. androgen receptors and CK20), it may represent an advance in the ease of distinguishing these tumors. This advance may be especially important when the tumor biopsy sample is small.

As our results indicate, K17 immunostaining of trichoblastoma was quite variable, ranging from nearly absent in some specimens to diffuse in others. The reason for this finding is unclear, but the variable expression may be related to the maturational stage of each particular neoplasm. It should also be noted that, even in cases when the K17 immunostaining of this tumor was graded as diffuse, the intensity was much less than that found in the specimens of BCC (Table 1).

Overall, our work shows that the K17 antibody immunolabels basal cell carcinomas with a strong signal and minimal background. K17 expression may also differentiate basal cell carcinomas from such benign lookalikes as desmoplastic trichoepitheliomas and trichoblastomas. These differentiating features of K17 may have useful applications to dermatopathology and dermatological surgery.

We found that K17 immunostaining could be accomplished in both frozen and paraffin embedded sections. There are at least three applications of the K17 immunostaining when used in formalin-fixed, paraffin embedded tissue. First, it can be used for margin evaluation of tissue after standard surgical excision of morpheaform basal cell carcinomas. Second, K17 expression can be used for margin evaluation of en-face sections during the so-called “slow Mohs technique” when Mohs sections are evaluated as formalin-fixed, paraffin-embedded tissue. Third, K17 immunostaining can be used diagnostically, in ambiguous basaloid proliferations, where the differential diagnosis includes basal cell carcinoma, desmoplastic trichoepithelioma, and trichoblastoma. It should be noted from our published work³⁵ that rapid methods of K17 immunolabeling in Mohs surgery will further enhance the diagnostic value of K17 expression.

In summary, in this report we determined the diagnostic utility of K17 immunolabeling. Our data showed that K17 successfully immunolabeled basal cell carcinomas. It did so with high intensity, little background, and the ability to detect BCC cells at the margin. The K17 expression was very helpful in labeling basal cell carcinomas, while benign lookalikes such as trichoepitheliomas and trichoblastomas had low levels of intensity and/or distribution.

Acknowledgments

This work was supported from National Institutes of Health Grants (V. Falanga, Principle Investigator): NIAMS R01 grant #AR060342; NCCR/NIGMS Awards #P20RR018757 and #P20 GM103414, respectively; NIH COBRE Administrative and Imaging Cores.

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Figure 1. Cytokeratin 17 is intensively expressed in different sub-types of basal cell carcinoma (BCC)

a) Routine H&E stained superficial BCC showing basaloid nodules extending into the superficial dermis. 10X. b) K17 immunostaining of superficial BCC, showing diffuse expression throughout the tumor. 10X. c) Routine H&E stained nodular BCC, showing large nodules of basaloid cells in the dermis. 10X. d) K17 immunostaining of nodular BCC, showing diffuse expression throughout the tumor. 10X.

Figure 2. Cytokeratin 17 expression highlights morpheaform basal cell carcinoma (BCC)
a) Routine H&E staining of morpheaform BCC showing basaloid strand of cells in a sclerosing dermal stroma. 10X. b) K17 immunostaining of morpheaform BCC, showing diffuse expression throughout the tumor strands. 10X. c) Routine H&E staining of morpheaform BCC, showing scattered basaloid strands and small islands of tumor within the dermis. 4X. d) K17 immunostaining highlights the tumor strands and islands. 4X. e) Higher magnification of a portion of panel “d”, showing individual tumor cells (arrows) away from the tumor strands. 40X.

Figure 3. Cytokeratin 17 expression highlights a larger tumor of morpheaform basal cell carcinoma (BCC) and its margins

a) Routine H&E staining of morpheaform BCC, showing basaloid strands of tumor cells in a sclerosing dermal stroma. 10X. b) K17 immunostaining of morpheaform BCC, showing diffuse expression throughout the tumor strands. 10X. c) Higher magnification of a portion of panel “a”, showing scattered basaloid strands within the dermis. 40X. d) Higher magnification of a portion of panel “b”, at its margin, showing K17 immunostaining highlighting the tumor strands and individual neoplastic cells (arrows). 40X. (copyrighted, V. Falanga, 2011).

Figure 4. Cytokeratin 17 expression can distinguish morpheaform basal cell carcinoma (BCC) from desmoplastic trichoepithelioma (DT)

a) Routine H&E staining of desmoplastic trichoepithelioma, showing small basaloid strands and horn pseudocysts within a fibrotic stroma. 4X. b) K17 immunostained DT, showing absence of K17 expression in the basaloid strands comprising the tumor. 4X. c) Routine H&E staining of DT showing small basaloid strands and horn pseudocysts within a fibrotic stroma. 4X. d) K17 immunostained DT, showing absent K17 expression in basaloid strands. There is some nonspecific immunostaining of the horn pseudocysts within the lesion. 4X. e) Higher magnification of a portion of panel “d”, highlighting the absence of K17 expression in the basaloid strands of this DT section (arrows). 40X.

Figure 5. Cytokeratin 17 expression in frozen section of morpheaform basal cell carcinoma (BCC)

The photomicrograph shows distinct expression of cytokeratin 17 in a BCC tumor, including dermal cords and strands. 4X.

Table 1

Analysis and Scoring (mean $\pm$ SEM) of the Intensity and Extent of K17 Immunostaining in Basal Cell Carcinoma, Desmoplastic Trichoepithelioma, and Trichoblastoma

Tumor type	SBC [†]	NBCC [‡]	MBCC [*]	DT ^{**}	TB [§]
Number of specimens analyzed	10	9	9	9	10
Intensity of immunostaining ^a	2.8 $\pm$ 0.42	2.89 $\pm$ 0.33	2.9 $\pm$ 0.33	0.56 $\pm$ 0.72	1.3 $\pm$ 0.82
Extent of immunostaining ^b	3.0 $\pm$ 0.0	3.0 $\pm$ 0.0	3.0 $\pm$ 0.0	0.44 $\pm$ 0.53	1.3 $\pm$ 0.67

^a Intensity of immunostaining scored on a scale of 0 – 3 as follows: 0 = absence, 1 = low, 2 = moderate and 3 = high

^b Extent of immunostaining scored on a scale of 0 – 3 as follows: 0 = absence, 1 = focal or less than 10% of cells, 2 = patchy or 11% to 75% of cells, 3 = diffuse or more than 75% of cells

[†] superficial basal cell carcinoma

[‡] nodular basal cell carcinoma

^{*} morpheiform basal cell carcinoma

^{**} desmoplastic trichoepithelioma

[§] trichoblastoma