

# Practical immunohistochemistry of epithelial skin tumor

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## ABSTRACT

Skin tumors are tumors arising from keratinocyte and from adnexal structures. Immunohistochemistry is very helpful in diagnosis of difficult cases in epithelial skin neoplasms, especially basal cell carcinoma (BCC) which is positive for BerEP4, a keratin marker, and mostly negative for epithelial membrane antigen (EMA). Squamous cell carcinoma cells are positive for EMA and cytokeratin, which are of higher molecular weight than those found in BCC. In contrast to BCC, trichoblastoma and trichoepithelioma are negative for androgen receptors. Of the malignant dermal spindle cell lesions, spindle cell squamous carcinoma is positive to 34 betaE12, desmoplastic melanoma is positive to S100, and leiomyosarcoma is positive to desmin. Of the malignant pagetoid cells, Paget's disease is positive to CK7 and cam5.2, whereas the pagetoid variant of Bowen's disease is positive to CK 5/6. Melanoma *in-situ* is positive to both S100 and melan-A. Immunohistochemistry is an extremely valuable adjunct to standard morphologic diagnosis in diagnostic pathology. Diagnosis of epithelial tumor depends largely on morphological features but, in rare cases, immunohistochemical stains are needed for definitive diagnosis.

**Key words:** Adnexal, epithelial, immunohistochemistry, skin tumors

## INTRODUCTION

Skin tumors are tumors arising from keratinocyte and from adnexal structures. The latter either originate from hair follicles, eccrine, apocrine, sebaceous glands, or can be mixed. The histological diagnosis of these tumors is usually easy and straightforward. However, some cases are difficult, and show overlap features with other tumors, especially basal cell carcinoma (BCC). In this article, immunohistochemistry that is helpful in diagnosis of difficult cases in epithelial skin neoplasms were reviewed.

## KERATINOCYTIC TUMORS

### Basal cell carcinoma

BCC is positive for BerEP4, a keratin marker [Figure 1], and mostly negative for epithelial membrane antigen (EMA) [Table 1]. In one study, all cases of BCC were positive for BerEP4, which differentiate it from squamous cell carcinomas (SCC).<sup>[1]</sup> Fan *et al.* reviewed the immunoreactivity of 51 cases of nodular BCCs and they found moderate or strong BerEP4 expression in all cases with never less than 20% of the tumor staining. Expression of EMA is uncommon in BCC (moderate or strong in 8% of tumors) and was confined to keratotic or squamoid areas. Immunohistochemistry of 25 sebaceomas revealed unequivocal negative expression of Ber-EP4 in 24 of 25 cases. A single case exhibited focal weak Ber-EP4 staining, predominantly in mature sebocytes and in < 10% of the tumor cells. EMA was not expressed in the germinative cells of sebaceoma, but was expressed strongly in approximately 50% of mature sebocytes in all cases and highlighted the cytoplasmic vacuoles.<sup>[2]</sup> Another study showed that

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DOI:  
10.4103/0378-6323.102359

**How to cite this article:** Alhumaidi A. Practical immunohistochemistry of epithelial skin tumor. Indian J Dermatol Venereol Leprol 2012;78:698-708.

**Received:** September, 2011. **Accepted:** July, 2012. **Source of Support:** Nil. **Conflict of Interest:** None declared.

Ber-EP4 reliably differentiates microcystic adnexal carcinoma (negative) from BCC (positive).<sup>[3]</sup> However, there is another study with contradictory data in which 10 cases of locally aggressive adnexal carcinoma showed Ber-EP4 positive membrane staining confined to the glandular areas.<sup>[4]</sup>

Cytokeratin 20 (CK20), a marker for Merkel cells, has been used to differentiate some forms of trichoblastoma, trichoepithelioma (TE), or fibroepitheliomas as these have scattered CK20-positive Merkel cells [Figures 2 and 3] compared to BCC where they are rare or absent.<sup>[5,6]</sup> Izikson *et al.*'s study indicates that at least focal expression of androgen receptor (AR) was detected in 78% of BCCs and none of the trichoblastic tumors showed any AR immunoreactivity.<sup>[7]</sup> Immunohistochemical stains for AR and CK20 are useful to differentiate desmoplastic trichoepithelioma (DTE) from morpheaform/infiltrative BCC (mBCC). The AR-, CK20+ immunophenotype is sensitive (87%) and specific for DTE (100%). The AR+, CK20-immunophenotype is specific (100%) and moderately sensitive (61%) for mBCC<sup>[8]</sup> [Table 2]. A recent study indicates that immunohistochemistry for ARs and CK20 are helpful, but interpretation is difficult in some DTEs when few cells are immunopositive for these markers.<sup>[9]</sup>

Wagoner *et al.*<sup>[10]</sup> studied CD10 expression of 14 and 13 cases of BCC and SCC, respectively. They found that CD10 was strongly expressed in 14 of 14 superficial BCCs and failed to express in 2 of 2 deeply infiltrative BCCs. CD10 was negative in the tumor cells in 13 of 13 superficially invasive SCCs and SCC *in situ*. CD10 expressed weakly in the surrounding stromal cells of 2 of 13 SCCs.<sup>[10]</sup>

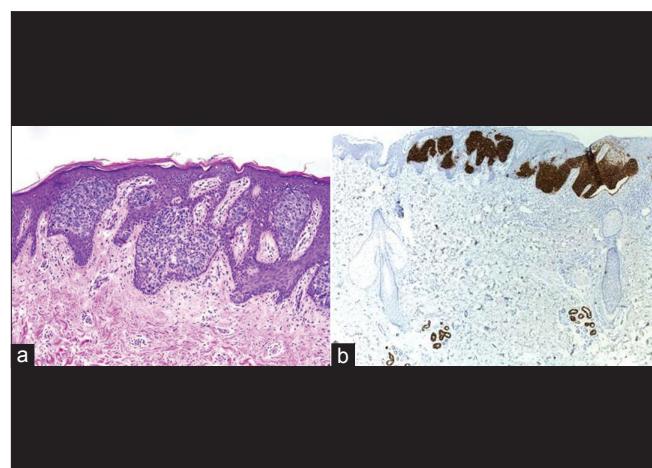
**Table 1: Basal cell carcinoma vs squamous cell carcinoma and sebaceous tumors**

	BerEP4	EMA	CD10
Basal cell carcinoma	+	±*	+
Squamous cell carcinoma	-	+	-
Sebaceous tumors	-	±*	±

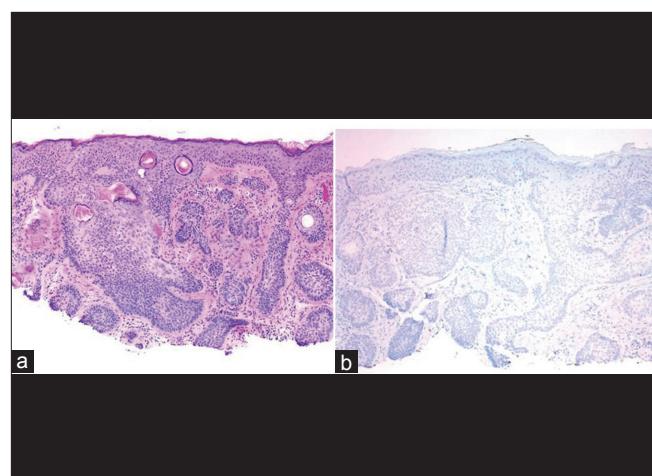
(\* ) EMA positivity is confined only to keratotic or squamoid areas in basal cell carcinoma and to mature sebocytes in sebaceous tumors

**Table 2: Basal cell carcinoma vs trichoblastoma, and trichoepithelioma**

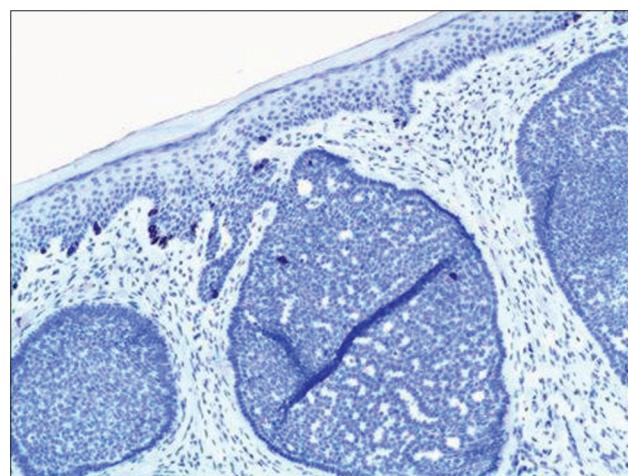
	CK20	Androgen receptor
Basal cell carcinoma	-	+
Trichoblastoma, and trichoepithelioma (in scattered Merkel's cells)	+	-



**Figure 1: (a) Superficial basal cell carcinoma mimicking Bowen's disease (H and E, ×200), (b) Same tumor showing strong Ber-EP4 positivity (immunohistochemistry, ×100)**



**Figure 2: (a) Basal cell carcinoma mimicking trichoepithelioma (H and E, ×100), (b) Cytokeratin 20 immunostain shows complete absence of Merkel cells (immunohistochemistry, ×200)**



**Figure 3: As comparison, trichoblastoma demonstrates scattered cytokeratin 20-positive Merkel cells (immunohistochemistry, ×200)**

A study of 32 patients with BCC showed that bcl-2 protein was positive in 26 patients (81.2%), bax protein was positive in 9 patients (28.1%), and p53 protein was positive in 13 patients (40.6%); there is a negative correlation between the expression of bcl-2 and bax protein ( $P < 0.05$ ). These findings suggest that apoptosis is suppressed which might cause the production of BCC.<sup>[11]</sup> An interesting aspect of BCC is that the forms that are more aggressive contain actin-positive cells, a finding that suggests that cell motility may play a role in tumor invasiveness.<sup>[12]</sup>

### Squamous cell carcinoma

Variants of SCC, e.g., adenoid, mucin-producing acantholytic [Figure 4], and spindle cell/desmoplastic [Figure 5] SCC are sometimes difficult to diagnose by routine hematoxylin and eosin (H&E) stain. The best evidence of SCC is demonstration of intra- and/or extracellular keratinization. The cells in SCC are positive for EMA and cytokeratin. The keratins are of higher molecular weight than those found in BCC.<sup>[13]</sup>

Cutaneous spindle cell squamous cell carcinoma (SCSCC) is an uncommon variant of SCC that must be distinguished from spindle cell/desmoplastic melanoma, cutaneous leiomyosarcoma, atypical fibroxanthoma (AFX), and scar. Morgan *et al.* studied 24 cases of SCC consisting of 12 spindle cell SCCs (SCSCCs), three AFXs, three leiomyosarcomas, three desmoplastic melanomas, and three scars were evaluated with a battery of immunohistochemical stains. The 12 SCSCCs show positive 34 beta E12 (12/12, 100%), p63 (10/12, 80%), AE1/AE3 (8/12, 67%), low-molecular-weight keratin (7/12, 58%), and P KER (4/12, 33%). The three AFXs were positive for CD68 and negative for all other stains, whereas the three leiomyosarcomas stained positively for desmin and smooth muscle actin and negatively for all other stains. The three melanomas stained positively for S-100 and negatively for all other immunohistochemistry. The scars were negative for all stains. So, this study shows that 34 beta E12 proved most promising in distinguishing SCSCC from the histological mimickers, AFX, spindle cell melanoma, scar, and leiomyosarcoma<sup>[14]</sup> [Table 3]. CD10 and procollagen-1 immunostaining can be used as a useful adjunct to supplement the differentiation of AFX (positive) and spindle SCC (negative) within the context of an immunoperoxidase panel.<sup>[15]</sup>

Adenosquamous carcinoma is a rare variant of SCC

arising from pluripotential cells related to acrosyringia, characterized by the formation of mucin-secreting glands. The tumors usually follow an aggressive course with the capacity for metastasis and local recurrence. The tumor cells are positive for cytokeratin and EMA, whereas those cells forming glands stain with carcinoembryonic antigen (CEA).<sup>[16]</sup>

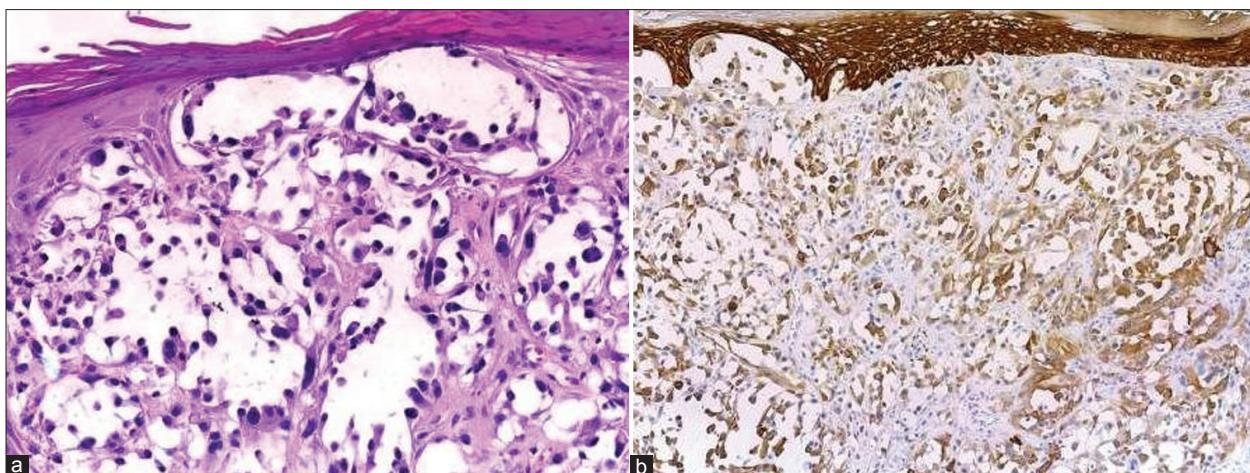
Dotto and Glusac's study showed that p63 was expressed diffusely in the nuclei of 100% (13/13) of SCSCC. Of controls, p63 showed focal labeling of two cutaneous leiomyosarcoma and two AFX. They concluded that p63 appears relatively specific to SCSCC and adds a useful nuclear marker to the available repertoire.<sup>[17]</sup>

CD44v6 and matrix metalloproteinase 1 (MMP-1), expressed in tumor cells, stromal cells respectively, are significant markers associated with the invasiveness of tumors in SCC and BCC of the skin, and that it will be helpful to evaluate the invasiveness by measuring the expression of these markers.<sup>[18]</sup>

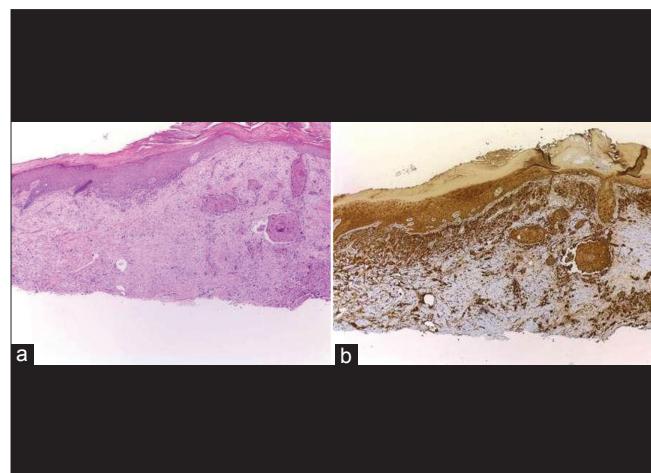
### Bowen disease

Bowen disease (BD) is a form of SCC *in situ*. It is a distinct clinicopathologic entity of the skin and mucocutaneous junction. The pagetoid variant of BD is sometimes difficult to distinguish from extramammary Paget disease. In the latter, mucicarmine, Cam 5.2, and CEA-positive tumor cells are present in the epidermis<sup>[16]</sup> [Table 4].

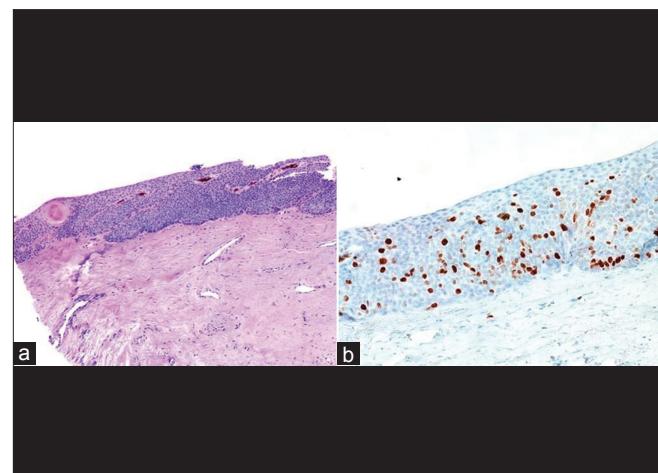
BD shows increased nuclear expression of p27 (a protein associated with cellular quiescence) and Ki-67 (a marker of proliferation) [Figure 6]. In one study, nuclear expression of p27 and Ki-67 was evaluated immunohistochemically in actinic keratosis (AK), BD, and SCC. They found that p27 labeling index was decreased in invasive aggregates of SCC ( $76.9 \pm 1.1\%$ ) when compared with those of normal epidermis ( $97.2 \pm 2.4\%$ ), AK ( $95.3 \pm 1.4\%$ ), and BD ( $98.0 \pm 0.5\%$ ). Ki-67 was expressed in a scattered to confluent linear pattern in the basal/parabasal cell layer of normal epidermis and AK. Keratinocytes in SCC exhibited Ki-67 in the peripheral layers of the neoplasm and frequently within the tumor aggregates. Ki-67 was observed in nuclei throughout the full thickness of the epidermis in BD. The staining pattern of Ki-67 in BD separated this entity from others under study. The combination pattern of p27 and Ki-67 staining can be used to support differentiation of AK, BD, and SCC.<sup>[19]</sup>



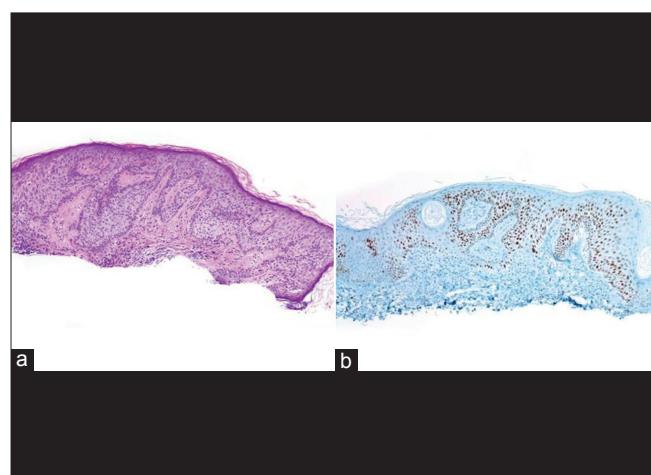
**Figure 4:** (a) Acantholytic SCC forms spaces lined by atypical cells mimicking angiosarcoma ((H and E,  $\times 400$ ), (b) Acantholytic SCC shows high-molecular-weight cytokeratin positivity (immunohistochemistry,  $\times 400$ )



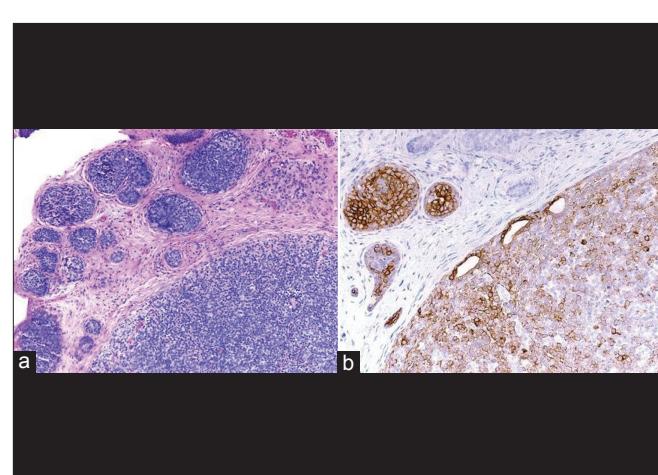
**Figure 5:** (a) Desmoplastic SCC is sometimes difficult to distinguish from desmoplastic melanoma and atypical fibroxanthoma by routine (H and E,  $\times 100$ ), (b) Desmoplastic SCC shows positivity of high-molecular-weight cytokeratin in epidermis and neoplastic cells (immunohistochemistry,  $\times 100$ )



**Figure 6:** (a) Bowen's disease of the nail (H and E,  $\times 100$ ), (b) Ki-67 immunostain shows high proliferation rate (Immunohistochemistry,  $\times 100$ )



**Figure 7:** (a) Bowen's disease mimicking seborrheic keratosis (H and E,  $\times 100$ ), (b) P53 immunostain shows a nearly full thickness epidermal positivity sparing the basal layer (immunohistochemistry,  $\times 100$ )



**Figure 8:** (a) Spiradenoma, well-circumscribed aggregates of blue tumor cells are seen in the dermis (H and E,  $\times 100$ ), (b) Cytokeratin 7 positivity indicates sweat gland ductal origin (immunohistochemistry,  $\times 100$ )

**Table 3: Immunohistochemistry of malignant dermal spindle cell lesions**

	34 beta E12	Procollagen-1	CD10	S100	Desmin
Spindle cell squamous carcinoma	+	-	-	-	-
Atypical fibroxanthoma	-	+	+	-	-
Spindle cell/desmoplastic melanoma	-	±	±	+	-
Leiomyosarcoma	-	-	-	-	+

**Table 4: Differential diagnosis of malignant pagetoid cells**

	CK7 and Cam 5.2	CK 5/6	S100 and Melan-A
Paget's disease	+	-	-
Pagetoid variant of Bowen's disease	-	+	-
Melanoma <i>in situ</i>	-	-	+

(+) positive, (-) negative, (+/-) positive but can be negative, (-/+) negative but can be positive

BD features atypical squamous cells in all portions of the epidermis but initially leaves basal cells in palisades along the basement membrane. Saglam *et al.* have evaluated Ki-67 and P53 immunohistochemistry in AK and palisading basal cells (PBC) in BD. AK stained for Ki-67 and p53 antibodies only in lower portion of epidermis and included the basal layer. BD with typical PBCs stained positive for both markers throughout the epidermis, except for the basal layer [Figure 7]. Four additional psoriatic biopsies stained positively for the two markers only in the basal and parabasal layers. Normal epidermis adjacent to the lesions in AK and BD biopsies stained sparsely in the basal layers.<sup>[20]</sup>

### Actinic keratosis

AK is a common intraepidermal neoplasm of sun-damaged skin characterized by variable atypia of keratinocytes. Ber-EP4 show negative result in AK as opposed to superficial BCC. Tope *et al.*<sup>[21]</sup> showed that abnormal keratinocytes in all specimens of superficial BCC (5 of 5) were Ber-EP4 positive; and all AK (10 of 10) and squamous intraepithelial neoplasia (8 of 8) were Ber-EP4 negative.<sup>[21]</sup>

### Keratoacanthoma

PCNA/MIB-1-labeled proliferating cells are found in the periphery of the squamous nests in keratoacanthoma (KA), in contrast to a more diffuse pattern in SCC.<sup>[15]</sup> In

a study, 80% of the KAs showed nuclear staining with anti-p53 antibody, distributed along the outermost layers of the aggregates of neoplastic cells, while 60% of the SCCs were p53 positive. Eight percent of the regressing KA also showed p53 positivity.<sup>[22]</sup> The latter finding is used to differentiate regressing KA from normal and reactive epidermis.

A study showed that a high index of staining of p53 favors the diagnosis of subungual SCC over subungual keratoacanthoma (SUKA). SUKAs do not express Ki67 strongly, whereas some subungual SCCs do. They conclude that immunohistochemistry for p53 and Ki67 may help distinguish between a subungual SCC and a SUKA.<sup>[23]</sup>

## TUMORS WITH DIFFERENTIATION TOWARD HAIR FOLLICLE STRUCTURES

### Trichoepithelioma and trichoblastoma

Trichoblastomas are benign neoplasms whose differentiation is toward the follicular germ—the embryonic structure that gives rise to the pilosebaceous-apocrine unit. TEs represent the most common form of trichoblastoma. Their most important differential diagnosis is with BCC, whose differentiation is similar. These tumors show scattered CK20-positive Merkel cells colonizing their peripheral layers [Figure 3]. Merkel cells are sparsely scattered in the basal layer of the epidermis and in hair follicles, so that their presence in trichoblastomas is a sign of follicular differentiation. Benign trichoblastic neoplasms are negative for AR proteins, further aiding in their distinction from BCC, which often expressed them. In a study done by Katona *et al.*,<sup>[24]</sup> immunohistochemistry for AR and CK20 was performed on 15 DTE and 31 mBCC. AR expression was seen in 13% (2/15) of DTE and 65% (20/31) of mBCC cases. CK20-positive Merkel cells were identified in 100% (15/15) of DTE and 3% (1/31) of mBCC. The expected pattern of AR-, CK20+ immunophenotype was present in 87% (13/15) of DTE cases. In mBCC, 61% (19/31) was AR+, CK20-. No DTE was AR+, CK20- and no mBCC was AR-, CK20+.<sup>[24]</sup> Another study concludes that immunohistochemistry for ARs and CK20 is helpful, but interpretation is difficult in some DTEs when few cells are immunopositive for these markers. This can be especially true in small biopsy specimens, which is a particular problem as these lesions are often present on the face.

CD10 staining pattern may be a useful adjunct marker in distinguishing between TE and BCC. In a study done by Pham *et al.*, 12/13 cases (92%) of TE showed positive stromal immunoreactivity. Of these, eight cases also demonstrated positivity of the papilla, and two showed positivity of the basaloid cells. No TE demonstrated epithelial expression alone. On the other hand, expression of CD10 by basaloid cells was identified in 20/23 (87%) cases of BCC. Stromal positivity was also identified in three cases of BCC.<sup>[25]</sup>

Lum and Binder studied the proliferative rate of basal-cell carcinoma and TE in small biopsy specimens. They found that BCCs qualitatively showed a greater proliferative fraction (using the antibody, Ki-67) compared to TE (50.0 vs 13.0%), as well as over-expression of p53. BCCs marked by p21 demonstrated scattered nuclear positivity compared to the virtual absence of staining in the TE.<sup>[26]</sup> Bcl-2 stains BCC in a diffuse pattern, whereas all of the TEs in one study showed staining of the outermost epithelial layer.<sup>[27]</sup>

#### Pilomatrixoma and pilomatrix carcinoma

These two neoplasms are proliferations of follicular matrical cells. The cells of the matrix rapidly proliferate and give rise to the inner root sheath and to the hair shaft. Pilomatrixoma and pilomatrix carcinoma are positive for the beta-catenin immunostain. Hassanein and Glanz reviewed 21 cases of pilomatrixoma and five cases of pilomatrix carcinoma. All 26 tumors displayed both nuclear and cytoplasmic staining of beta-catenin in the basaloid cells with focal membranous staining. Shadow cells were negative in all tumors. Normal control sections from the scalp displayed nuclear reactivity of the matrical cells, mostly concentrated in the supramatrical zone of hair follicles.<sup>[28]</sup>

#### Proliferating trichilemmal tumor

Proliferating trichilemmal tumor (PTT) shows p53 immunoreactivity that is not different from that seen in SCCs with trichilemmal differentiation (SCCT). On the other hand, p53 immunostaining is virtually absent in its much more common counterpart, trichilemmal cysts. The similar p53 immunoreactivity in both PTT and SCCT favors the interpretation of the former as carcinoma.<sup>[29]</sup> Most authors hold, however, that PTTs comprise a spectrum from neoplasms with little malignant potential to ones best considered as fully malignant carcinomas.<sup>[30]</sup>

#### Trichilemmoma and trichilemmal carcinoma

Trichilemmal carcinoma (TLC) is purportedly

carcinomas that differentiate toward the outer root sheath, which has clear cells. Its distinction from clear cell SCC can be problematic, and only a few cases in which this diagnosis is considered are likely authentic.<sup>[31]</sup> In reports of TLC, the lesions express CK 1, 10, 14, and 17, suggesting that TLC differentiates toward follicular infundibulum. In a comparison of CK expression between TLC and trichilemmoma, the absence of CK 15 and 16 in TLC may be related to transformation from trichilemmoma to TLC.<sup>[32]</sup>

Desmoplastic trichilemmoma (DT) is a variant of trichilemmoma, characterized by a central prominent desmoplastic component, which may simulate invasive carcinoma if looked at out of context. In DT, epithelial tumor cells showed CD34 immunostaining in contrast to BCC and SCC.<sup>[33]</sup>

#### TUMORS WITH SWEAT GLAND DIFFERENTIATION

The sweat glands consist of ductal and secretory portions. Only the secretory portion of both eccrine and apocrine glands expresses cytokeratins of low molecular weight such as CK7 and CAM 5.2. EMA and CEA stain the luminal aspect of the duct and show focal and somewhat weaker staining of the luminal aspect of the secretory glands. The myoepithelial cells are positive for S100 and smooth muscle actin (SMA). S100 also stain some secretory cells of eccrine glands.

Normal apocrine glands are stained with lysozyme, CD-15 (Leu M1), and gross cystic disease fluid protein-15 (GCDFP-15), while eccrine glands were positive only for GCDFP-15. In neoplastic tissue thought to be from apocrine tumors, antibodies rose against lysozyme and GCDFP-15 has a greater specificity (100%) for apocrine differentiation, while Leu M1 has a greater sensitivity (88%).<sup>[34]</sup>

There are histologic and immunophenotypic similarities between sweat gland carcinoma and breast cancer. Swanson *et al.*<sup>[35]</sup> assessed estrogen receptor protein (ERP) immunoreactivity in sweat gland tumors. They found that ERP was detected in 10 of 33 eccrine carcinomas, 3 of 10 eccrine hidradenomas, each of two examples of hidradenoma papilliferum, and 2 of 3 chondroid syringomas. Among immunoreactive eccrine neoplasms, eight of 10 carcinomas occurred in males. Another study done by Offidani *et al.* revealed that almost all the anogenital sweat glands showed immunoreactivity for estrogen receptor and, more weakly, for progesterone receptor, with immunolabeled

nuclear area ranging from 10% to 90%. Conversely, conventional sweat glands did not show any nuclear staining.<sup>[35]</sup> Coexpression of cytokeratin and vimentin may be frequently found in a variety of benign and malignant sweat gland tumors. In the majority of these neoplasms, vimentin-positive cells correspond to myoepithelial cells, as indicated by coexpression with alpha-SMA.<sup>[36]</sup>

In cylindroma, cytokeratin 7 predominantly labels the central basaloid cells, and SMA stain peripheral myoepithelial cells. Cylindromas do not express CK20, gross cystic disease fluid protein 15, or estrogen or progesterone receptor.<sup>[37-39]</sup> Recent study by Missall *et al.* concluded that both cylindromas and spiradenomas express CK7, a sensitive marker for the secretory coil [Figure 8]. They also found that syringomas express both CK6 (a marker for the inner ductal cells) and CK10 (a marker for the middle ductal cells). None of the studied tumors expressed SMA or CD10 (markers for myoepithelial cells).<sup>[40]</sup>

The tubular cells in both spiradenomas and cylindromas express human milk fat globulin and lysozyme, two markers associated with apocrine differentiation.<sup>[41]</sup> S100 and CD1a show scattered langerhans cells within these lesions, a finding that is confirmed by the electron microscopic observation of Birbeck granules.<sup>[38,42]</sup>

Poromas are benign adnexal neoplasms with terminal ductal differentiation. Although historically considered a neoplasm of eccrine differentiation, poromas can show either eccrine or apocrine lineage.<sup>[34]</sup> Most of the poroid cells are stained positively with antibodies against CKs 5 and 14, but none to very few cells (<5%) immunoreacted with antibodies against CKs 8/18 and CEA. Cuticular cells show a somewhat similar immunostaining pattern as poroid cells. The luminal edges of cuticle cells react with CEA.<sup>[43]</sup> Immunolabeling for EMA is present in the tumor cells of poroma. Thus, it is suggested that the constituent cells of these tumors originate from the outer cells of the intraepidermal and/or the upper portion of the intradermal eccrine ducts, as these cells are positive for EMA as well. There is little immunolabeling for EMA on the tumor cells of seborrheic keratosis and BCC.<sup>[44]</sup>

The tumor cells of mixed tumor or chondroid syringoma are positive for cytokeratins (AE1+AE3).

Glandular cells lining tubules are CK7 positive. The myoepithelial cells are S-100 protein, neuron-specific enolase, and glial fibrillary acidic protein.<sup>[45]</sup>

### Malignant sweat gland neoplasms

There are many recognized WHO types of sweat gland carcinomas. Superficial biopsy specimens of microcystic adnexal carcinoma can simulate infiltrative BCC and DTE. Hoang *et al.* found that the majority of microcystic adnexal carcinomas (MAC) (92%) and DTEs (100%) expressed CK15, whereas infiltrative BCCs and SCCs were all negative.<sup>[46]</sup> Another study showed that Ber-EP4 did not label any of the MAC (0/13), whereas 28/28 cases of BCCs were Ber-EP4 positive. 12/16 of DTEs were immunoreactive with Ber-EP4.<sup>[25]</sup> However, there is another study with contradictory data in which 10 cases of locally aggressive adnexal carcinoma showed Ber-EP4-positive membrane staining confined to the glandular areas.<sup>[26]</sup>

CEA and EMA highlight the ducts and the intracytoplasmic lumina of porocarcinoma and hidradenocarcinoma.<sup>[34,47]</sup>

Primary mucinous carcinoma of the skin can be mistaken histologically for a metastasis from extracutaneous sites, particularly the breast, or the gastrointestinal tract. Primary mucinous carcinoma of the skin is positive for cytokeratin 7, S100 protein, estrogen, and progesterone receptors, while negative for CK20.<sup>[48]</sup> Primary cutaneous mucinous carcinomas often have an area of carcinoma *in situ*, in which there are actin-positive myoepithelial cells at the edges of some of the aggregates.<sup>[49]</sup>

The most reliable histopathologic criteria for identifying apocrine skin carcinoma are decapsulation secretion, periodic acid-Schiff-positive diastase-resistant material in the cells or lumen, and immunoreactivity with gross cystic disease fluid protein 15. Apocrine carcinoma also expresses EMA and variably CEA.<sup>[50]</sup> Tubular carcinoma is the malignant counterpart of tubular adenoma, featuring apocrine differentiation with prominent tubular structures. Tubular carcinoma is positive for low-molecular-weight cytokeratins and the luminal cells express EMA and gross cystic disease fluid protein 15 (GCDFP-15). Expression of CEA is variable.<sup>[34]</sup>

### Mammary and extramammary Paget disease

Immunohistochemistry has been useful not only in the diagnosis of Paget's disease but also in attempting to clarify the cell of origin in Paget's disease. The most useful keratin markers for primary mammary and extramammary Paget disease are CAM5.2 and CK7 because they stain >90% of Paget cells but do not react with epidermal or mucosal keratinocytes<sup>[51]</sup> [Table 4]. CK7 also marks some Toker cells and Merkel cells, so morphological assessment of the labeled cells is necessary.<sup>[52]</sup> Secondary extramammary Paget disease arises from underlying adenocarcinomas, mostly from gastrointestinal tract. Such cases express CK20 and show negativity for CK7. GCDFP-15 is strongly expressed in cases of vulval and perianal extramammary Paget's disease without an underlying internal malignancy, and much less frequently in cases with an associated malignancy.<sup>[53]</sup> Rare cases of extramammary Paget's disease are associated with prostatic adenocarcinoma and are prostate-specific antigen (PSA) positive,<sup>[53,54]</sup> or from the urinary bladder.<sup>[55,56]</sup>

### TUMORS WITH SEBACEOUS DIFFERENTIATION

There is considerable overlap between the histological features of sebaceoma and BCC. Fan *et al.* showed that 24 of 25 sebaceomas are negative for Ber-EP4. EMA was not expressed in the germinative cells of sebaceoma, but was expressed strongly in approximately 50% of mature sebocytes in all cases and highlighted the cytoplasmic vacuoles. In contrast, all the<sup>[46]</sup> cases of BCC show moderate or strong BerEP4 expression with never less than 20% of the tumor staining. Expression

of EMA was uncommon in BCC (moderate or strong in 8% of cases) and was confined to keratotic or squamoid areas.<sup>[57]</sup> Immunohistochemical staining for AR is found to be a reliable marker of sebaceous differentiation. Bayer-Garner *et al.* found that all sebaceous neoplasms show diffuse positive nuclear AR staining, whereas approximately 60% of BCCs showed only focal positivity for nuclear AR immunoreactivity. Clear cell acanthomas and SCCs were uniformly negative.<sup>[58]</sup>

### PRIMARY VS METASTATIC CARCINOMA OF THE SKIN

Distinction of primary skin adnexal carcinomas from cutaneous metastasis of adenocarcinoma is sometimes challenging. In a study, podoplanin is positive in primary skin adnexal carcinomas and negative in metastatic adenocarcinomas.<sup>[59]</sup> Another study revealed that all primary cutaneous carcinomas, including adenocarcinomas, expressed p63. In contrast, none of the metastatic adenocarcinomas to the skin was positive for p63.<sup>[60]</sup> CK5/6 can also be used to distinguish primary skin adnexal carcinomas from metastatic adenocarcinoma. Plumb *et al.*'s study concluded that CK 5/6 was expressed by most (97%) of cutaneous adnexal neoplasms, while only 33% of metastatic adenocarcinomas showed positive expression.<sup>[61]</sup>

Estrogen and progesterone receptors and anti-gross cystic disease fluid protein 15 (BRST-2) are positive in metastatic breast carcinoma and can be positive in primary eccrine and apocrine neoplasms. Thus, these markers do not differentiate between primary and metastatic breast carcinoma.<sup>[62]</sup>

**Table 5: Some immunohistochemical markers and their usage**

Markers	Usage
Ki-67	<ul style="list-style-type: none"> <li>A marker of proliferation that stains throughout the epidermis in Bowen disease compared with actinic keratosis and benign epidermal proliferation.</li> </ul>
p53	<ul style="list-style-type: none"> <li>Tumor suppressor gene that stains throughout the epidermis in Bowen disease compared with actinic keratosis and benign epidermal proliferation.</li> <li>Positive in regressing keratoacanthoma compared with normal and reactive epidermis.</li> </ul>
p27	<ul style="list-style-type: none"> <li>A protein associated with cellular quiescence that shows decreased positivity in invasive aggregates of squamous cell carcinoma when compared with those of normal epidermis, actinic keratosis, and Bowen disease</li> </ul>
CEA	<ul style="list-style-type: none"> <li>Positive in luminal edges of cuticle cells of poroma</li> </ul>
Beta-catenin	<ul style="list-style-type: none"> <li>Positive in pilomatrixoma and pilomatric carcinoma</li> </ul>
CD34	<ul style="list-style-type: none"> <li>Positive in epithelial tumor cells of desmoplastic trichilemmoma in contrast to basal cell carcinoma and squamous cell carcinoma</li> </ul>
Podoplanin	<ul style="list-style-type: none"> <li>Positive in primary skin adnexal carcinomas in contrast with metastatic adenocarcinomas</li> </ul>
p63	<ul style="list-style-type: none"> <li>Positive in primary skin adnexal carcinomas including adenocarcinoma in contrast with metastatic adenocarcinomas</li> </ul>
TTF-1	<ul style="list-style-type: none"> <li>Positive in metastatic thyroid and lung adenocarcinoma</li> </ul>
RCC antigen	<ul style="list-style-type: none"> <li>Positive in metastatic renal cell carcinoma</li> </ul>
Glypican-3	<ul style="list-style-type: none"> <li>Positive in metastatic hepatocellular carcinoma</li> </ul>

There are some more specific markers that help pinpoint the origin of metastases to the skin. Examples of these include thyroglobulin (thyroid carcinoma), TTF-1 (thyroid or lung carcinoma), renal cell carcinoma antigen (renal cell carcinoma), and glypican-3 for hepatocellular carcinoma [Table 5].

## CONCLUSION

Immunohistochemistry is an extremely valuable adjunct to standard morphologic diagnosis in diagnostic pathology. Diagnosis of epithelial tumor depends largely on morphological features. In rare cases, immunohistochemical stains are needed for definitive diagnosis. Sometimes, a panel of markers is recommended instead of a single immunostain. A careful interpretation of the result and correlation with histological features will guide to correct and specific diagnosis.

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### Multiple Choice Questions

1. Which one of the following immunohistochemical markers is positive for basal cell carcinoma and negative for squamous cell carcinoma of the skin?
  - a. EMA
  - b. BerEP4
  - c. CK20
  - d. CD10
2. Which one of following immunohistochemical panels is useful to differentiate morpheaform/infiltrative basal cell carcinoma from desmoplastic trichoepithelioma?
  - a. CK20 and CD10
  - b. Ber-EP4 and EMA
  - c. Androgen receptor (AR) and CK20
  - d. Androgen receptor (AR) and CD10

**Contd...**

3. Which one of the following tumors is colonized by CK20-positive Merkel's cells?
  - a. Basal cell carcinoma
  - b. Trichoepithelioma
  - c. Sebaceous carcinoma
  - d. Squamous cell carcinoma
  
4. Which one of the following immunohistochemical epithelial markers is most sensitive for spindle cell squamous cell carcinoma?
  - a. 34 beta E12
  - b. AE1/AE3
  - c. Low-molecular weight keratin
  - d. P KER
  
5. Malignant dermal spindle cell tumor that is positive for pro-collagen and negative for S100, 34 beta E12, and desmin. The most likely diagnosis is:
  - a. Spindle cell squamous cell carcinoma
  - b. Cutaneous leiomyosarcoma
  - c. Atypical fibroxanthoma
  - d. Desmoplastic melanoma
  
6. Which one of the following markers is useful to differentiate regressing keratoacanthoma from normal and reactive epidermis
  - a. Ki67
  - b. P63
  - c. P27
  - d. P53
  
7. Malignant dermal poorly differentiated round cell tumor that is positive for beta-catenin. The most likely diagnosis is:
  - a. Proliferating trichilemmal tumor
  - b. Trichilemmal carcinoma
  - c. Spiradenocarcinoma
  - d. Pilomatrix carcinoma
  
8. Which one of the following markers is useful to differentiate desmoplastic trichilemmoma from basal cell carcinoma and squamous cell carcinoma
  - a. CD34
  - b. BerEP4
  - c. CD10
  - d. CK20
  
9. All of the following are reliable histopathologic criteria for identifying apocrine skin carcinoma except:
  - a. Decapitation secretion
  - b. BerEP4 positivity
  - c. Periodic acid-Schiff-positive diastase-resistant material in the cells or lumen
  - d. Gross cystic disease fluid protein positivity
  
10. Which one of the following markers is helpful to identify metastatic hepatocellular carcinoma to the skin
  - a. Thyroglobulin
  - b. TTF-1
  - c. Glypican-3
  - d. CK20

1. b, 2. c, 3. b, 4. a, 5. c, 6. d, 7. d, 8. a, 9. b, 10. c

**Answers**

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