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MINI-SYMPOSIUM: CUTANEOUS EPITHELIAL TUMOURS

The use of immunohistochemistry in the differential diagnosis of common epithelial tumours of the skin

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BCC;
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CD10;
Merkel cells

Summary Immunohistochemistry may be useful in the differential diagnosis of epithelial skin tumours in day-to-day practice. This article lists commonly available antibodies, summarizes immunostaining patterns reported in the literature for this group of tumours, highlights immunostaining patterns encountered in normal skin, and emphasizes the immunomarkers that the authors have found to be of particular value. The use of small immunopanels in different diagnostic settings is illustrated.

Introduction

Epithelial tumours of the skin are some of the most commonly encountered tumours by many histopathologists in their day-to-day surgical reporting practice. Familiarity and well-characterized diagnostic features on haematoxylin and eosin (H&E) sections mean that there is little diagnostic difficulty in the majority of cases. However, cutaneous tumours as a whole comprise a broad group of entities with histopathological diversity and this may lead to diagnostic uncertainty. The biological behaviour of groups of skin tumours is often similar and predictable, leading to a pragmatic approach of broadly grouping tumour types. However, there is considerable merit in an approach that identifies uncommon or rare tumour subtypes, particularly in those cases in which the special type relates to biological behaviour (risk of local recurrence or metastasis), or there is an association with other tumours or syndromes with genetic and/or familial implications.

The aim of this article is to highlight the utility of immunohistochemical (IHC) staining in the differential diagnosis of epithelial tumours of the skin.

Immunomarkers for epithelial skin tumours

Commonly available immunomarkers are listed below, along with a brief overview of the relevant

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literature, a description of the staining patterns in normal skin (which may give insight into the pathogenesis of epithelial skin tumours) and some personal observations. Small immunopanels of the more commonly used markers in the authors' practice are suggested for specific diagnostic problems.

BerEP4

BerEP4 is a monoclonal antibody to a 34/49-kDa glycoprotein on the surface of most epithelial cells, with the exception of superficial layers of squamous epithelia.[1] BerEP4 stains the vast majority of non-cutaneous epithelia and is highly conserved in the tumours derived from them, including non-cutaneous squamous cell carcinomas (SCC).[1] In normal skin, there is reported positivity of the lower part of telogen hairs (secondary hair germ), matrix and outer root sheath (ORS) of vellus hairs, early anagen of terminal follicles (but not any part of mature anagen follicles), and the lower part of the epithelial strand of late catagen follicles.[2,3] Similar strong positive staining is seen in lining cells of eccrine and apocrine coils, with more variable staining of sweat duct lining cells and the acrosyringium.[3] The epidermis, follicular mantle, sebaceous glands and all non-epithelial tissues are negative[1,3] (Fig. 1A–C).

BerEP4 has been shown repeatedly to stain all subtypes of basal cell carcinoma (BCC) but is negative in non-basaloid SCC.[3–10] The basosquamous variant of BCC retains strong positive staining in nearly all cases, although staining may be more variable and patchy, and therefore interpretation of weak staining in small biopsies may be problematic.[8,9] The authors have found the differential immunostaining patterns of BerEP4 used in combination with epithelial membrane antigen (EMA) (see below) to be invaluable in differentiating BCC and variants from SCC variants and other basaloid skin tumours (Fig. 1D–H). BerEP4 also stains most benign and malignant tumours of eccrine and apocrine origin (poroma, hidradenoma, hidradenoma papilliferum, mixed tumours and their associated carcinomas), mammary Paget's disease and metastatic adenocarcinomas.[3] In the authors' experience, BerEP4 highlights glandular/ductal differentiation in a pattern different to the luminal staining seen with other markers, in that the entire circumference of the cell membrane is stained, but staining may also be more widespread in gland-derived lesions. The utility of BerEP4 immunostain-

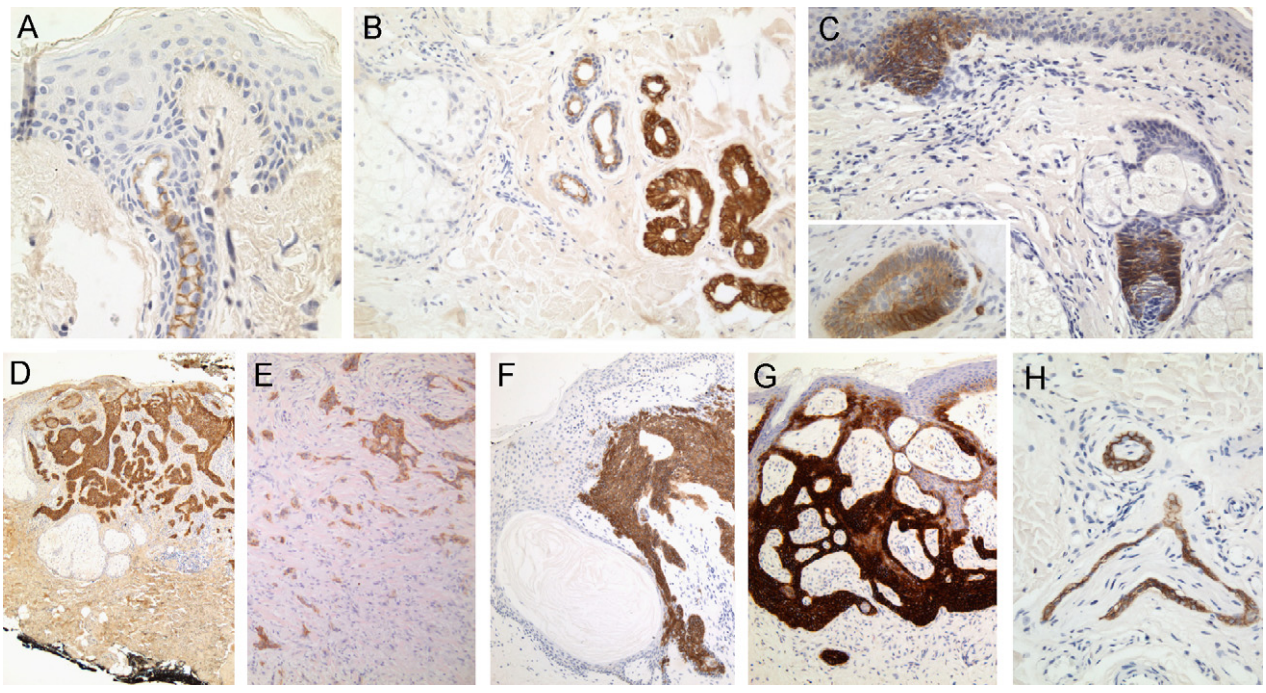

Figure 1 BerEP4 staining in normal skin and basal cell carcinoma (BCC): (A) weak staining in upper eccrine duct and acrosyryngium; (B) weak staining in lower sweat duct and strong staining in sweat gland coils; (C) outer root sheath telogen and anagen vellus buds (inset showing perifollicular Merkel cells); (D) nodular BCC; (E) metatypical and infiltrative BCC with weaker staining; (F) sebeorrhoeic keratosis (left side negative) in collision with superficial BCC; (G) fibroepithelioma of Pinkus; and (H) perineural invasion from a micronodular BCC.

ing in the differential diagnosis of sebaceomas (negative) from nodular BCC (positive) has been reported recently.[10] BerEP4 staining in Merkel cells located in the peripheral follicular infundibulum and in the adjacent perifollicular mesenchyme has also been noted [Fig. 1C (inset)]. BerEP4 immunostaining is widely recognized to be positive in small-cell neuroendocrine carcinomas from all sites, including 96% of cutaneous Merkel cell carcinomas.[11]

Epithelial membrane antigen

EMA is derived from glycoproteins isolated from human milk fat globulin membranes, and is a good marker of epithelial differentiation.[12] In normal skin, EMA strongly stains the cytoplasm of mature sebaceous glands (but not the germinative layer), the luminal membrane and canaliculi of sweat gland coils, the outer layer of sweat duct cells, the luminal lining of sweat ducts, Merkel cells and the epineurium of nerves (Fig. 2).[13] Plasma cells may also stain positively but other non-epithelial tissues are negative. The main use for EMA is in the distinction of SCC (infiltrative and basaloid types) and basaloid bowenoid epidermal dysplasia (EMA positive) from BCC (EMA negative except in foci of squamoid differentiation).[4,7–9] In the authors' experience, EMA staining can be quite focal and variable in both bowenoid dysplasia and SCC, so interpretation of negative staining in small biopsies may be problematic. Cytoplasmic staining of mature sebocytes by EMA is highly characteristic and can confirm focal sebaceous differentiation in basaloid tumours including BCC with sebaceous differentiation and sebaceomas. EMA is often positive in sebaceous carcinoma.[10] EMA is the single most useful marker for highlighting lumina in tumours with ductal or glandular differentiation, including BCC and sweat gland tumours.

Common acute lymphoblastic leukaemia antigen

Common acute lymphoblastic leukaemia antigen (CD10) is a 100-kDa cell-surface metalloendopeptidase involved in inactivation of a number of

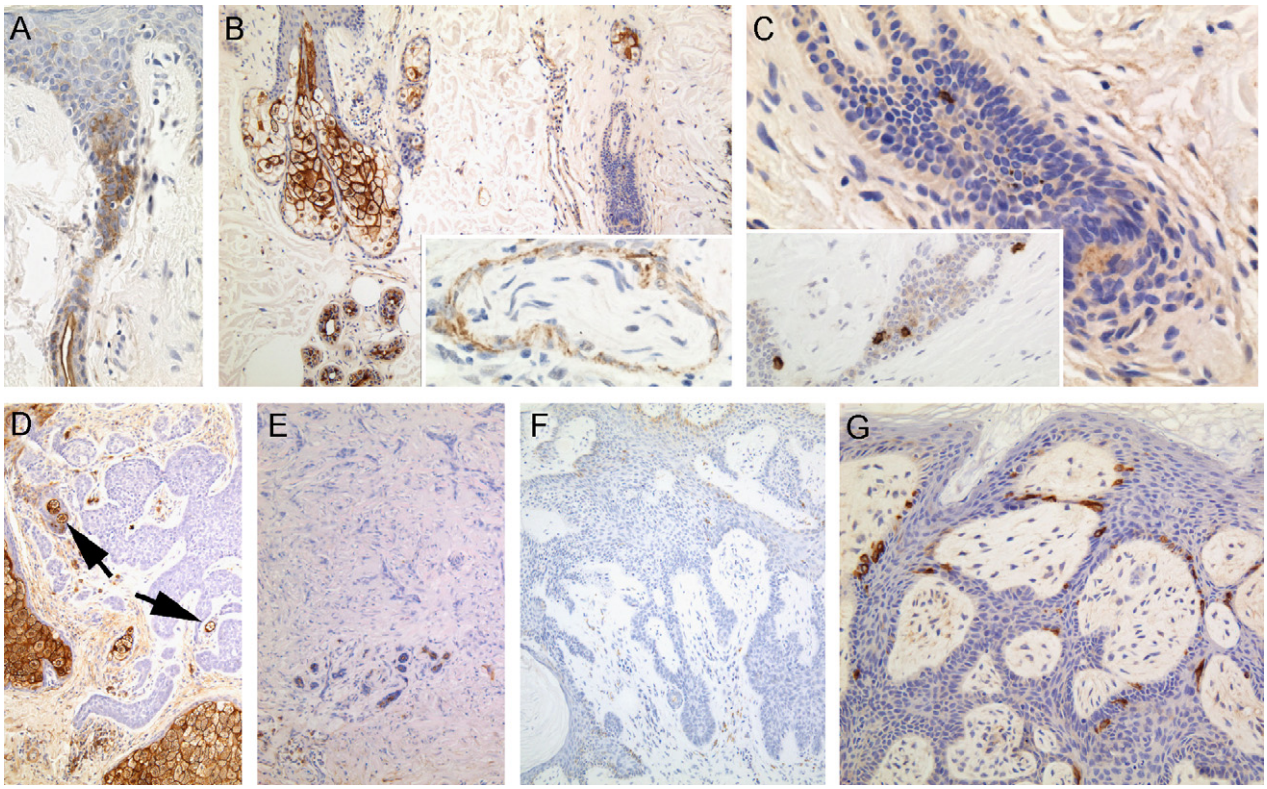

Figure 2 Epithelial membrane antigen staining in normal skin and basal cell carcinoma (BCC): (A) acrosyringium and luminal lining of eccrine duct are positive; the epidermis shows minimal staining; (B) strong staining in sweat glands, sweat gland coils and weak perineural staining (inset); (C) negative vellus follicle with Merkel cell and Merkel cells in outer isthmic region (inset); (D) nodular BCC with entrapped sebaceous cells (arrows); (E) metatypical and infiltrative BCC negative with internal control in sweat coils below; (F) weak staining in seborrhoeic keratosis overlying negative BCC (lower left); and (G) prominent Merkel cells in a Pinkus tumour.

biologically active peptides. It is expressed on the surface of a wide variety of normal (e.g. endometrial stromal cells) and neoplastic cells, including clear cell renal carcinoma, endometrial stromal sarcoma, mesonephric and trophoblastic tumours.[14–17] In normal skin, CD10 stains sebaceous glands (both nuclear and cytoplasmic membrane), myoepithelial cells of eccrine and apocrine glands, peri-adnexal mesenchymal spindle cells surrounding dermal appendages, occasional endothelial cells and vulval squamous epithelium.[15,17] Staining of nerve axons and strong staining of the hair papilla of vellus follicles has been noted (Fig. 3A–C).

CD10 is expressed in mesenchymal skin tumours, mainly dermatofibromas and, to a lesser degree, dermatofibrosarcoma protuberans and neurofibromas, in addition to metastatic and some primary melanomas.[18] CD10 immunostaining has recently been reported to be useful in distinguishing trichoepithelioma (TE) from BCC by differential staining of the stroma, including papillary mesenchymal bodies (a feature of TE), and predominantly the basaloid epithelium in BCC (Fig. 3D–F).[19] CD10 positivity has been reported in cutaneous metastases from renal cell carcinoma and some clear cell hidradenomas,[16,17] and may be of some diagnostic value in this setting in combination with other markers such as cytokeratin (CK) 5/6.[17] CD10 positivity has also been reported in a range of other cutaneous tumours including sebaceous adenomas, the myoepithelial layer of syringocystadenomas, tubular apocrine adenoma,[17] hidradenoma papilliferum and vulval 'ectopic' breast tissue,[15] with staining also reported in atypical fibroxanthomas[20] and balloon cell melanocytic naevi.[16] CD10 immunostaining therefore needs to be interpreted with caution, but can prove useful in certain situations, ideally within an immunopanel.

CD34

The CD34 antigen is a 110-kDa single-chain transmembrane glycoprotein selectively expressed on human lymphoid, myeloid haematopoietic progenitor cells and vascular endothelial cells in normal tissues.[21] In the skin, CD34 stains blood vascular endothelium (not lymphatic), perivascular and peri-adnexal spindle-shaped cells, and interstitial (dendritic) cells in the reticular dermis in greater density compared with the papillary dermis.[21,22]

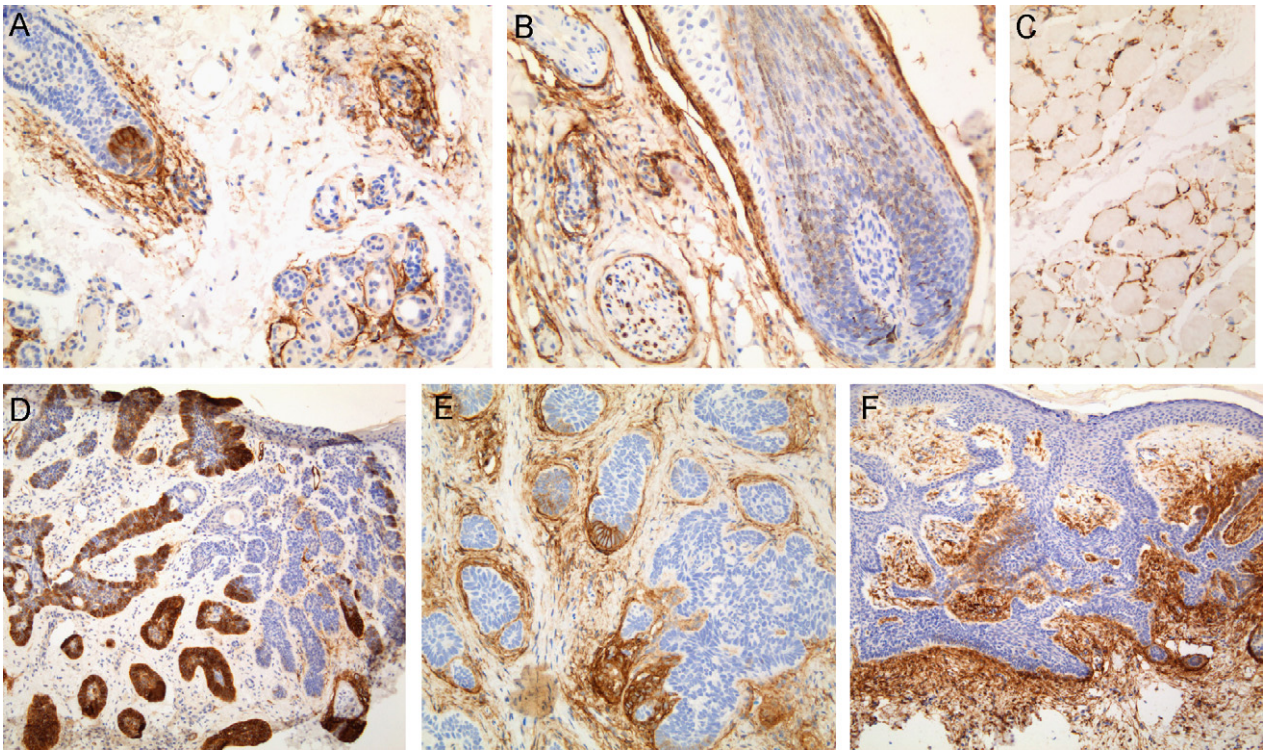

Figure 3 CD10 in normal skin, basal cell carcinoma (BCC) and trichoepithelioma (TE): (A) strong staining in the vellus hair papilla and peri-adnexal spindle cells; (B) negative in the papilla of a terminal follicle from the eyelid, strong tight perifollicular stromal staining, and staining of axons within a nerve (bottom left); (C) slender dendritic and spindle cells in the reticular dermis; (D) TE-like BCC with patchy but mainly epithelial staining with peripheral accentuation; (E) TE with mainly peritumoural stromal staining; and (F) Pinkus tumour with a similar pattern to TE.

CD34 is accentuated around the basement membrane zone of sweat gland coils, thought to be related to the dendritic processes of the spindle stromal cells.[21] There is also a distinctive population of perifollicular spindle cells in the mid portion of follicles (Fig. 4A).[21–23] In addition, CD34 stains the outer epithelial cells of the ORS in normal anagen-phase terminal hair follicles below the attachment zone of the erector pili muscle (Fig. 4A).[23]

A range of cutaneous soft tissue tumours usually stain with CD34, including Kaposi's sarcoma, dermatofibrosarcoma protruberans and, to a lesser degree, peripheral nerve sheath tumours, epithelioid sarcoma, clear cell sarcoma and malignant fibrous histiocytoma.[21,22]

CD34 has two main uses for epithelial tumours. The first is the proclivity to stain trichilemmomas, including the desmoplastic component of trichilemmomas,[22] and proliferating trichilemmal (pilar) tumours.[22] The authors have found CD34 particularly useful to highlight a diagnosis of trichilemmoma in rather ambiguous wart-like squamoid tumours that might otherwise be overlooked on H&E sections (Fig. 4B–D). Weak focal CD34 staining has been noted in some clear cell BCC, supportive of ORS differentiation in a proportion of these tumours.

The second putative use for CD34 is in the differential diagnosis of TE, including desmoplastic TE (DTE), from BCC and microcystic adnexal carcinomas, with most TE reported as showing characteristic tight peritumoural stromal positivity.[24,25] However, other investigators have found this to be an inconsistent and unreliable feature (summarized in Fig. 5B–G).[26–31]

Carcinoembryonic antigen

Carcinoembryonic antigen (CEA) is a glycoprotein initially described as a colonic oncofetal antigen,[32] but subsequently shown to be expressed in a variety of normal human tissues[33] including squames.[34] Anti-CEA antibodies are now designated 'CD66' with a subclassification a–e depending on which epitope of the antigen is recognized. In normal skin, membranous CEA positivity can occasionally be seen in suprabasal squamous cells of the epidermis, and on the inner lining of sweat gland coils (including canaliculi), dermal ducts and acrosyringium.[35] Monoclonal CEA can be used as a marker of ductal/glandular differentiation in skin tumours, and may also highlight mature squamous differentiation. However, in the authors' experience, immunostaining is sometimes inconsistent and less sensitive for lumina than EMA.

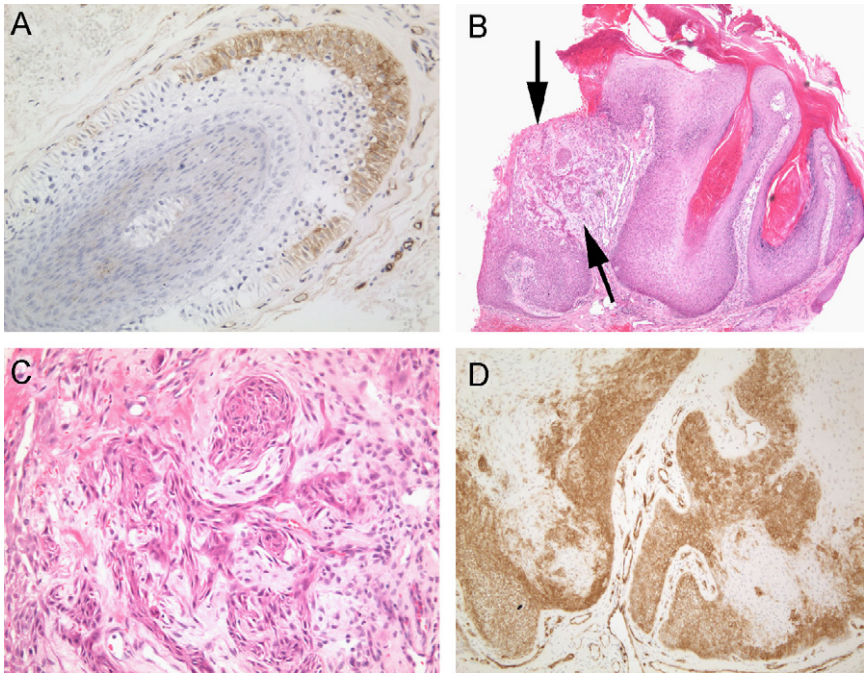

Figure 4 CD34 in normal skin and trichilemmoma: (A) CD34 in outer root sheath of terminal hair follicle (lower stem); (B) wart-like tumour lobular downgrowths and infiltrative focus (arrow); (C) infiltrative focus suggestive of squamous cell carcinoma; and (D) CD34 confirms a trichilemmoma with focus of desmoplastic trichilemmoma (cut out on this level).

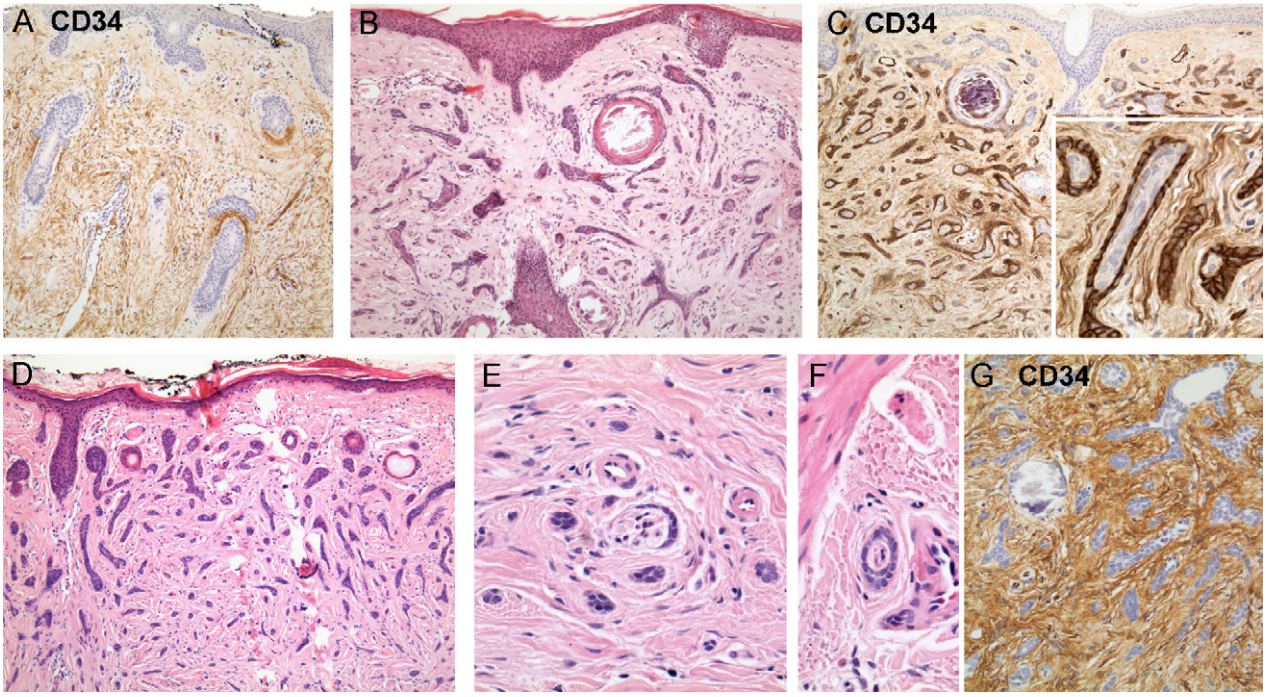

Figure 5 CD34 staining in normal skin, desmoplastic trichoepithelioma (DTE) and infiltrative basal cell carcinoma (BCC): (A) strong staining of perifollicular spindle cells around isthmic region of vellus follicles; (B) DTE with (C) relatively little peritumoural staining but strong peripheral epithelial staining (inset); (D) infiltrative BCC (DTE-like) with foci of perineural invasion (E, F), and (G) strong diffuse stromal staining for CD34.

Differential cytokeratins

Keratin filaments constitute type I (CK1–8, small, acidic) and type II (CK9–20, larger, neutral–basic) intermediate filaments and form part of the intracytoplasmic cytoskeleton in mammalian cells.[28,36] There are different keratins expressed in simple columnar (CK7, CK8, CK18 and CK19) and complex stratified squamous epithelia (epidermis CK1, CK2, CK5, CK10, CK14 and CK13/19 in internal squamous epithelium). Keratin expression in normal skin is complex, and is summarized in Table 1.[35,37–39]

The outer layers of the ORS are characterized by CK5/6, Cam5.2 (CK8, CK18 and CK19), CK14, CK15, CK16, CK17 and CK19, and the innermost layer of the ORS by CK6 and CK17 immunostaining.[37–39] Fetal follicles are positive for CK7.[38]

The staining patterns of cytokeratins in some tumours closely reflect the pattern in parts of the normal adnexae, for example, syringoma and the dermal eccrine duct (positive CK5/6, CK10, CK19 and EMA).[35] Nodular hidradenomas have a more variable pattern suggesting differentiation towards inner lining cells of ducts and secretory coils.[35] Clear cells in hidradenomas (expressing CK7 and Cam5.2) appear to show differentiation towards secretory coil cells.[35] Eccrine poromas (CK5/6 and EMA strong diffuse) comprise cells differentiating mainly towards outer cells of the eccrine duct/acrosyringium, but also show occasional lumina stained by CK6, CK7, CK19, Cam5.2 and EMA which are eccrine duct and coil lining markers.[35] The basaloid cells of TE show differentiation towards the outer layer of the ORS (CK5/6, CK14, CK15 and Cam5.2), and the squamous cells around keratocysts show differentiation towards the innermost layer of the ORS (CK6 and CK17) with heterogeneous staining for the ORS marker CK19.[35] The staining of BCC with Cam5.2 (CK8, CK18 and CK19) may reflect CK19 staining because BCC have been found to be negative for CK8 and CK18.[40,41] BCC, Pinkus tumour and TE have been found to have near-identical cytokeratin profiles (CK5/6, CK14, CK15, CK17 and CK19 positive; CK8, CK13 and CK18 negative; CK7 variable) consistent with ORS origin.[37,39,42]

Cam5.2 (CK8, CK18 and CK19) has been reported to be useful in the distinction of BCC from sebaceoma (latter negative), Paget's cells (positive) and Merkel cell carcinoma (characteristic dot pattern of positivity). Cam5.2 is usually negative in SCC and positive in a proportion of BCC and TE,[35] shows variable staining in glandular adnexal tumours, and may highlight luminal lining cells.[35]

Table 1 Cytokeratin (CK), epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA) expression in normal skin.

Epidermis including follicular infundibulum		Eccrine glands	
Basal layer	CK5/6, CK6*, CK14	Acrosyringium	CK6†, CK19†, EMA‡,§, CEA§
Suprabasal	CK10	Duct, inner cells	CK5/6, CK6, CK14, CK17¶, CK19, Cam5.2, S100¶, EMA¶, CEA
Hair follicles			
Inner root sheath	CK13	Duct, middle cells	CK5/6, CK10
Outer root sheath	CK5/6**, CK6††, CK10††, CK14‡‡, CK15** CK17††, CK19†, Cam5.2‡‡	Duct, outer cells	CK5/6, CK14, EMA
		Coil lining cells	CK19, Cam5.2, CK7, S100§§, GCDFP-15§, EMA§, CEA§
Sebaceous glands			
Peripheral cells	CK5/6, CK14	Coil: myoepithelia	CK5/6, CK14, CK17, SMA
Intermediate cells	CK5/6, CK6, CK7†, CK10, CK17	Merkel cells	Cam5.2, CK20, chromogrannin
Inner cells	CK5/6¶, CK7†, CK10, EMA		

Note: Usual partner for CK5 is CK14 and CK10 is CK1. Cam5.2, CK8, CK18 and CK19; SMA, smooth muscle actin; GCDFP-15, gross cystic disease fluid protein-15.

*Hyperproliferation.
†Variable.
‡Outer layer of acrosyringium.
§Luminal lining.
¶±.
**Innermost layer faint, outermost layer strong.
††Innermost layer of outer root sheath.
‡‡Outermost layer of outer root sheath.
§§Some cells. CK15 is a marker in the follicular bulge region of follicles.

CK7 expression is restricted to a subgroup of adenocarcinomas and can be used in conjunction with CK20 in the differentiation of adenocarcinomas of different origin.[36,43,44] CK7 can be helpful to confirm/highlight glandular differentiation in skin adnexal tumours, the staining often being focal and confined to the areas of gland formation.[12,44] In contrast, CK7 staining in metastatic adenocarcinomas expressing the marker is usually diffuse.[44] CK7 has also been reported to be positive in some BCC[31,40] and all TE,[31] but others have found CK7 to be negative in trichoblastomas including TE and DTE.[35,45] One study found CK7 in trichoblastic fibromas and BCC but not classical TE.[38] CK7 is diffusely positive in most mammary and extramammary Paget's disease, highlights glandular differentiation in malignant intra-epidermal eccrine poroma and is usually negative in bowenoid epidermal dysplasia with occasional exceptions.[46]

Variable CK10 positivity has been reported in the literature in bowenoid epidermal dysplasia but is consistently negative in Paget's disease and may be focally expressed in malignant intra-epidermal eccrine poroma.[46] CK15 may be a relatively specific marker for certain follicular tumours differentiating towards the follicular bulge regions in particular TE, a subset of BCC, inverted follicular keratoses, proliferating pilar tumours and pilar cysts, and negative in lesions differentiating from regions away from the follicular bulge including pilomatrixomas, trichilemmomas and pilar sheath acanthoma, and also negative in SCC and seborrhoeic keratoses.[39] CK19 is strongly diffusely positive in Paget's disease and most intra-epidermal malignant eccrine poromas, but usually focal or negative in bowenoid epidermal dysplasia.[46]

CK8,[47] Cam5.2 (CK8, CK18 and CK19) and CK20 stain Merkel cells in addition to chromogranin A. Cam5.2 shows dot-like cytoplasmic (or more widespread) positivity in small-cell neuroendocarcinomas of different origin, including cutaneous (Merkel cell) carcinomas. Merkel cells have rarely been reported in nodular hidradenoma[35] and more consistently in induction of follicles over

dermatofibroma,[47] basaloid proliferations in naevus sebaceous,[48] fibroepithelioma of Pinkus[49] and trichoblastomas (including classical TE, DTE and lymphadenoma),[35,42,48,49] but have been repeatedly shown to be absent in BCC.

Broad-spectrum and high-molecular-weight cytokeratins such as AE1/AE3 (CK10, CK14–16, CK19/1–8), CK 5/6, 34βE12 (CK1, CK5, CK10 and CK14) and MNF116 (CK5, CK6, CK18, CK17 and CK19) have been shown to be useful markers in demonstrating epithelial differentiation in cutaneous spindle cell squamous carcinoma, although up to one-third of these tumours show no evidence of epithelial differentiation using an expanded IHC panel.[50] There has been some evidence that CK5/6 may be useful to support a diagnosis of primary malignant cutaneous adnexal neoplasm (usually positive) in comparison with metastatic adenocarcinomas (usually negative or focal/weak).[51] CK5/6 is expressed relatively infrequently in non-cutaneous adenocarcinoma (9–15%) but is present in most cutaneous sweat gland tumours, and may be of help, in combination with p63 (see below), to distinguish primary from metastatic adenocarcinoma in the skin.[44]

β Catenin

β Catenin is a 92-kDa protein linking cell adhesion with gene expression associated with cellular proliferation and differentiation.[52] Nuclear translocation of β Catenin drives cellular proliferation and is a feature of a number of common cancers. In normal skin, membranous expression is seen in most epithelial cells but nuclear β Catenin expression is seen in the central matrix cells of the hair follicle bulb[53] and is thought to play a role in follicular morphogenesis. Nuclear positivity is reported in the more aggressive subtypes of BCC and in the proliferating matrix (basaloid) cells of pilomatrixoma and pilomatrix carcinomas (Fig. 6), associated with β Catenin mutation.[53–55] β Catenin immunostaining is of limited value in highlighting matrical differentiation in BCC.[56]

Bcl-2, p53 and Ki-67

Bcl-2 is an anti-apoptotic protein residing on the outer mitochondrial membrane. It is implicated in the pathogenesis of several common cancers by inhibiting programmed cell death. In normal skin, Bcl-2 stains the majority of keratinocytes in the basal epidermis, cells of the ORS, mesenchymal cells of the follicular papillae, and clear cells of eccrine glands.[57] Diffuse cytoplasmic Bcl-2 expression is reported in BCC[57,58] and is reported to be useful in the distinction of BCC (diffuse staining) from TE (staining of basal layer only)[59] and BCC and solar keratosis (latter negative).[60]

p53 is a tumour-suppressor gene and many p53 mutations result in a protein product that is unusually stable and becomes detectable by immunohistochemistry. Ultra-violet light is known to induce both overexpression of wild-type p53 and cause specific mutations in the p53 gene, and a discrepancy between cutaneous tumours with positive immunostaining and those with mutation has been reported.[61] Nuclear accumulation of p53 protein is a feature of the majority of malignant adnexal and epidermal tumours including BCC,[62] SCC and Bowen's disease,[63] and is confined to the areas of basal atypia in solar keratosis.[63] Hence, p53 immunostaining may have some utility in the differential diagnosis of intra-epidermal tumours (Fig. 7) and aids the distinction between basaloid proliferations (follicular induction) over dermatofibromas (rarely p53 positive) and the superficial subtype of BCC that they resemble.[64] p53 immunostaining is of lesser value in differentiating benign from malignant skin tumours, as evidenced by similar staining patterns reported between BCC and TE, and eccrine poroma and porocarcinoma.[65,66]

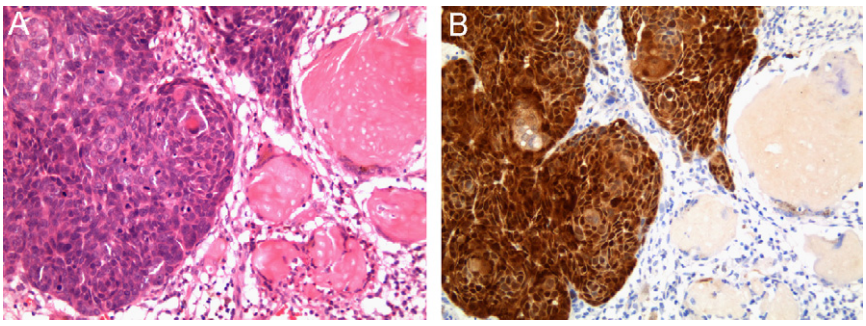

Figure 6 (A) Matricoma including basaloid and ghost cells; (B) nuclear positivity for β Catenin in basaloid cells.

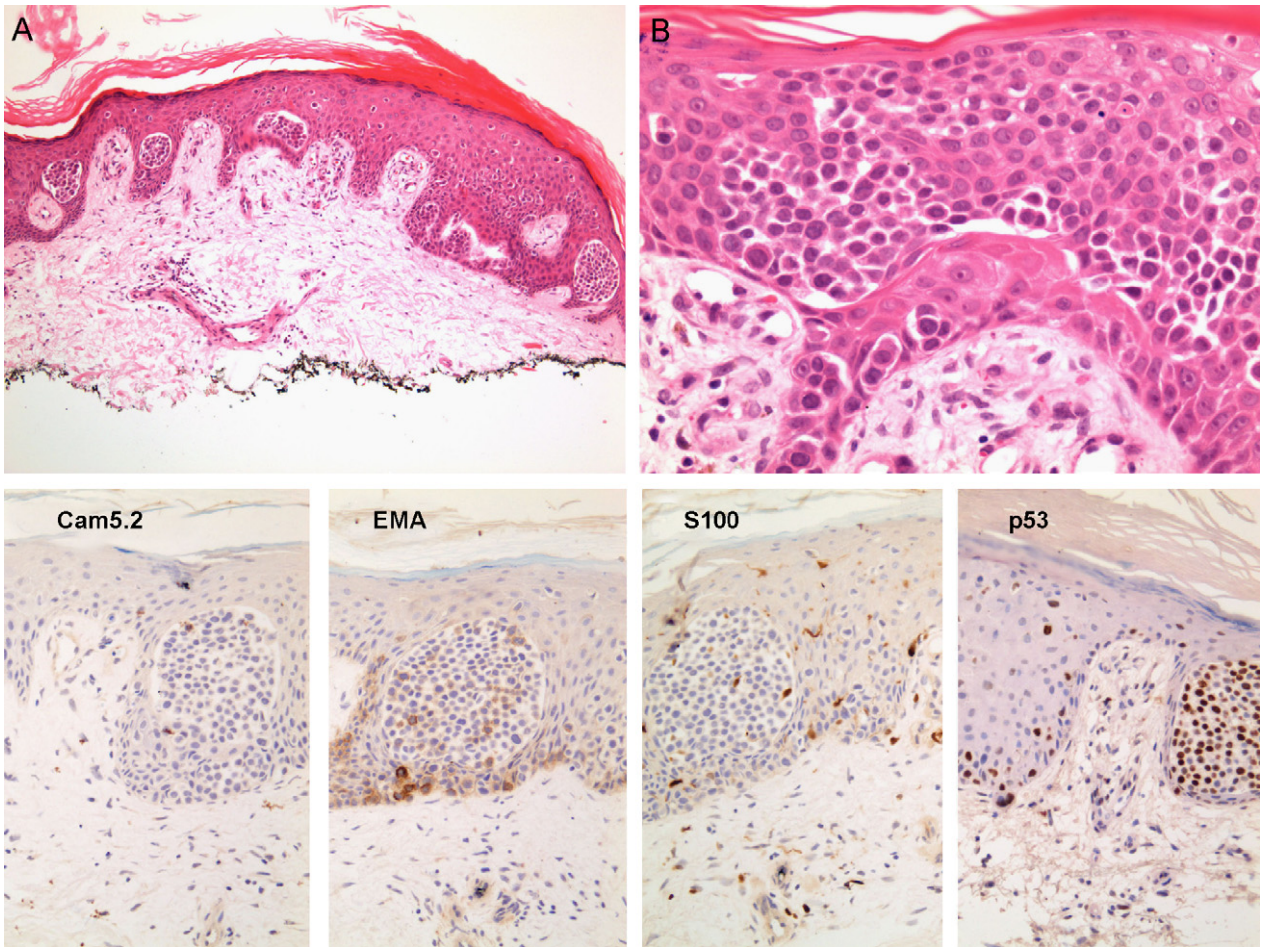

Figure 7 Clonal Bowen's: (A) low power of a clonal tumour and (B) high power showing uniform but atypical, acantholytic cells. Negative Cam5.2 is against extramammary Paget's and negative S100 against melanoma. p53 diffusely strongly positive.

Ki-67 (MIB 1) is a proliferation marker with a pattern of nuclear positivity. Demonstration of the proliferation index in skin tumours, in conjunction with the mitotic index, is used by many pathologists to help differentiate between benign and potentially malignant tumours. p53 positivity in conjunction with Ki-67 positivity is reported to be a feature of malignancy in the differential diagnosis of hidradenoma from hidradenocarcinoma, although histological parameters remain paramount.[67]

Miscellaneous immunomarkers

CD56 (neural cell adhesion molecule 1) is part of a family of cell-surface glycoproteins that play a role in embryogenesis and contact-mediated interactions between neural cells. Neural cell adhesion molecules are expressed specifically by neural, peripheral neuroectodermal and neuroendocrine tumours. They are also found in natural killer cells and seromucous glands. CD56 immunostaining has been found to be useful in lung neuroendocrine tumours,[68] and is useful in the diagnosis of primary neuroendocrine skin tumours and for highlighting focal neuroendocrine differentiation in skin tumours such as BCC.

Chromogranin A expression is related to the density of cytoplasmic neuroendocrine granules, and is positive in more differentiated neuroendocrine tumours.

S100 protein is expressed in a proportion of cells in the eccrine secretory coils, dendritic antigen presenting (Langerhan's) cells of the epidermis and dermis, melanocytes, nerves and adipose tissue. S100 is present in a proportion of adnexal tumours including weak, predominantly nuclear, staining in cylindroma, spiradenoma and, less commonly, nodular hidradenoma.[13] Tumours may also be populated by melanocytes (BCC, trichoblastoma) or Langerhan's cells.

Recent studies have shown that cutaneous adnexal neoplasms express p63 in contrast to metastatic adenocarcinomas that are generally negative.[44,69] Basaloid squamous carcinoma is diffusely positive in contrast to a compartmentalized pattern in adenoid cystic carcinoma,[70] and the combination of p63 positivity in combination with CK5/6 is highly predictive of a squamous origin in undifferentiated metastatic carcinomas.[71]

Smooth muscle actin (SMA) is present in the outer myoepithelial cells of secretory cells and may demonstrate myoepithelial differentiation in cylindroma and spriadenoma.[13]

Stromolysin-3 (a carcinoma-induced fibroblastic product) was present in stromal fibroblasts of 68% of morphoeic BCC but absent in DTE.[72] Involucrin stains the upper epidermis and follicular infundibular, the innermost layer of ORS and the inner root sheath, and the inner squamoid cells of keratinous cysts of trichogenic tumours.[45]

The autosomal-dominant disease Torre–Muir syndrome is characterized by a strong association between certain skin tumours, particularly sebaceoma, sebaceous carcinoma and keratoacanthoma, with colorectal cancer, and linked in turn with hereditary non-polyposis colorectal cancer.[73] In these patients, there is a germline mutation in one of the six common mismatch repair (MMR) genes; most commonly mutation in the hMSH2 gene followed by the hMLH1 gene. Immunostaining with the antibodies MSH2 and MLH1 can be used as an initial screen for Torre–Muir syndrome by highlighting loss of MMR gene protein expression in the nucleus of tumour cells (Fig. 8), implying germline mutation or hypermethylation.

Discussion and antibody panels

Understanding patterns of immunostaining in normal skin structures is key to the interpretation of immunostaining of epithelial skin tumours, and may give insight into the origin and pathogenesis of these tumours (Table 2).

The authors have found immunohistochemistry to be invaluable in the differential diagnosis of epithelial skin tumours, and use a more limited, practical number of immunomarkers in routine practice. Patterns of immunostaining using these markers in common intra-epidermal tumours are summarized in Table 3 and in epidermal and adnexal tumours in Table 4.

Immunostains are assessed optimally in conjunction with H&E appearances and antibodies most efficiently used in small immunopanels, according to the specific differential diagnosis. Commonly encountered diagnostic conundrums are highlighted below, together with a recommended immunopanel and commonly observed immunostaining patterns to help with the differential diagnosis.

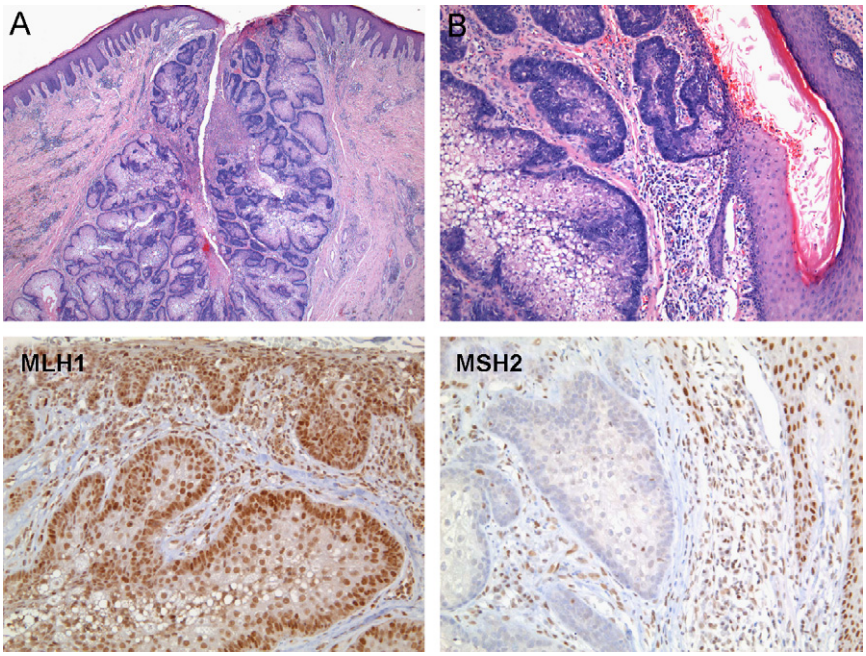

Figure 8 Sebaceous adenoma (A, B). Loss of nuclear staining for MSH 2.

Basal cell carcinoma vs. squamous cell carcinoma

Immunopanel—BerEP4, EMA
BCC invariably show strong diffuse membranous positivity with BerEP4 in 50% or more of tumour cells, whereas EMA is negative except for foci of squamoid differentiation. SCC shows essentially no BerEP4 positivity but show variable EMA positivity.

Table 2 Patterns of immunostaining in normal skin.

	EMA	BerEP4	CD10[a]	CD34	CEA	CK7
Epidermis						
Basal squames	—	—	—	—	—	—
Superficial squames	+/—	—	—	—	+	—
Hair follicles						
Inner root sheath	—	—	++	—	—	—
Outer root sheath	—	—	—	+	—	—
Germinative cells	—	++	—	—	—	—
Sebaceous glands	++[b]	—	—	—	—	+
Eccrine gland						
Acrosyringium	++	+/—	—	—	++	—
Dermal duct inner	++	+	—	—	++	—
Dermal duct outer	++	—	—	—	—	—
Secretory coil	++	++	—	—	+	++
Peri-adnexal dermis	—	—	++	+	—	—

EMA, epithelial membrane antigen; CEA, carcinoembryonic antigen; CK, cytokeratin.
[a]Papillary mesenchymal cells.
[b]Mature sebocytes.

Basal cell carcinoma vs. sebaceoma

Immunopanel—BerEP4, EMA, Cam5.2, MMR gene antibodies
BCC is BerEP4 and variably Cam5.2 positive, and EMA highlights sebaceous differentiation alone. In sebaceomas, the 'basaloid' cells are negative for BerEP4 and Cam5.2 with strong EMA positivity in sebaceous cells. Immunostaining for MMR gene proteins MLH-1 and MSH-2 may demonstrate loss of nuclear staining (more commonly of MSH-2) in sebaceoma tumour cells in cases of Torre–Muir syndrome.

Basal cell carcinoma with glandular differentiation vs. basaloid hidradenoma

Immunopanel—BerEP4, EMA, CEA, CK7
BCC strongly stains with BerEP4 as above, with EMA, CEA and CK7 variably highlighting foci of glandular/ductal differentiation alone within basaloid cells. In contrast, hidradenoma typically shows only patchy and weak BerEP4 positivity in basaloid cells, with the whole panel potentially highlighting ductal differentiation.

Basal cell carcinoma vs. trichoblastoma (trichoepithelioma)

Immunopanel—BerEP4, CD10, Bcl-2, CD34, CK20, Cam5.2
In trichoblastomas, CD10 typically highlights the peritumoral stroma, including papillary mesenchymal bodies, with minimal patchy staining of basaloid cells. In contrast, in BCC, the stroma is negative and basaloid cells variably strongly positive with CD10. Diffuse Bcl-2 positivity is reported in BCC, whereas the basal layer alone is highlighted in TE. The authors have found this to be variable and unreliable in practice. CD34 may highlight the

Table 3 Immunostaining of intra-epidermal epithelial malignancies.

	EMA	BerEP4	Cam5.2	CEA	CK7	CK20
Bowenoid actinic keratosis	++/+	—	—	—	—	—
Basaloid variant	++/+	++/— (50%)	—	—	—	—
Pagetoid variant	++/+	—	—	—	—	—
Paget's disease						
Nipple	++	++	++	+	++	+
Extramammary	++	++	++	+	++/—[a]	+/—[a]
Intra-epidermal porocarcinoma	++ and luminal	Var.+glands	+/—	+ luminal	+ glands	—

EMA, epithelial membrane antigen; CEA, carcinoembryonic antigen; CK, cytokeratin.
[a]Peri-anal/perineal with underlying rectal cancer CK7+, CK20+.

Table 4 Immunostaining of common epithelial skin tumours.

	EMA	BerEP4	CEA	CK7	CD34	CD10
Epidermal tumours						
BCC	+/− squamous/ sebaceous	++ diffuse	+ lumina	+ lumina, tumour variable	+ outer root sheath	+ tumour/ stroma−
SCC	+	−	+/− (squames)	?		?
Bowen's	+	−	Squames	?		?
Basaloid Bowen's	+	+(50%)	Squames	?		?
Sweat gland tumours						
Eccrine poroma	+	+ focal, glands	+ (lumina)	lumina		?
Hidradenoma	+ patchy/ glands	+, patchy, glands	+ (lumina)	+ (lumina)		?
Hair follicle tumours						
Tricholemmoma	+/−	−	Squames	?	++ clear cells	?
Trichoepithelioma (trichoblastoma)	n/a	−	Squames	Var		+ PMB/stroma, Epithelium−

EMA, epithelial membrane antigen; CEA, carcinoembryonic antigen; CK, cytokeratin; PMB, papillary mesenchymal bodies; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; ?, data not available.

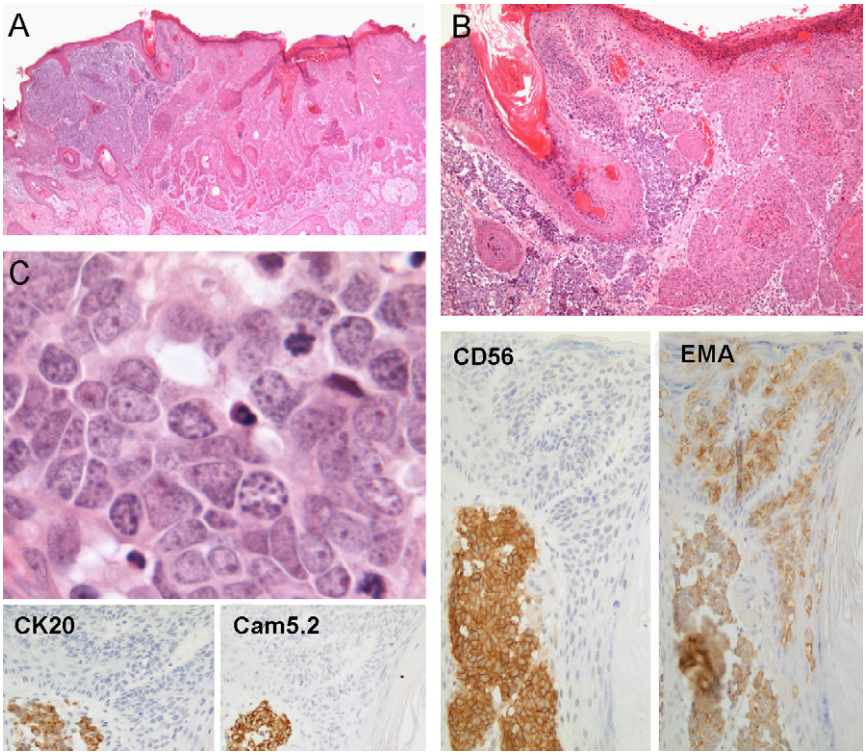

Figure 9 Merkel cell carcinoma (A, B left field) and squamous cell carcinoma (A, B right field). Merkel cell tumour only positive with CK20, Cam5.2 and CD56. Epithelial membrane antigen positive in both tumours.

peritumoural stroma in the desmoplastic variant of TE and not in infiltrative BCC, but this is also an unreliable finding in the authors' experience. Merkel cells can be highlighted with Cam5.2 or CK20 and are absent from BCC but increased in number in trichoblastoma.

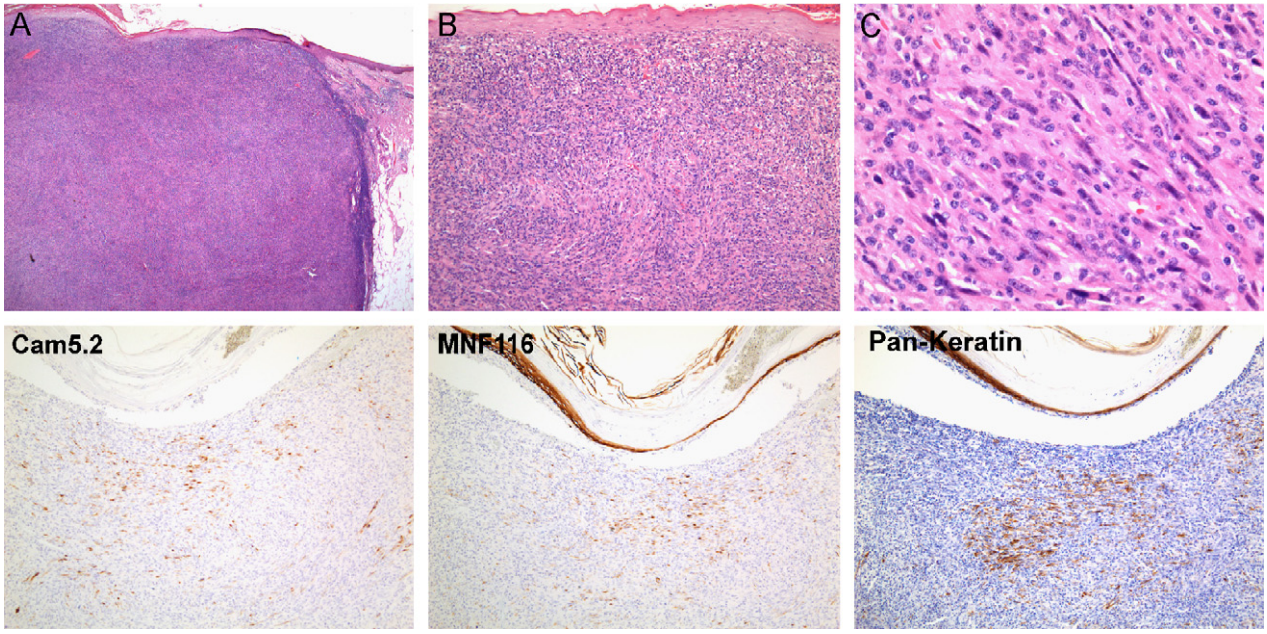

Figure 10 Spindle cell carcinoma: (A) low power, (B) medium power and (C) high power. Patchy positivity with Cam5.2, MNF 116, and pan-keratin.

Basal cell carcinoma vs. Merkel cell tumour vs. metastatic neuroendocrine carcinoma

Immunopanel—BerEP4, CD56, chromogrannin, CK20, Cam5.2, thyroid transcription factor 1 (TTF-1)

The combination of strong BerEP4 positivity with focal CD56 positivity and negative CK20 is suggestive of BCC with neuroendocrine differentiation. However, most neuroendocrine carcinomas are also BerEP4 positive. Merkel cell tumours of skin are usually CK20, CD56 and Cam5.2 positive (Fig. 9), the latter with a dot-like pattern of positivity. Metastatic neuroendocrine carcinoma (small-cell anaplastic carcinoma) from lung may also be TTF-1 positive.

Spindle cell squamous cell carcinoma vs. spindle cell melanoma vs. leiomyosarcoma vs. atypical fibroxanthoma

Immunopanel—EMA, pan-keratin or CK5/6 or AE3/AE1, S100, SMA, CD10, desmin

Spindle cell SCC may show positivity for EMA, pan-keratin or CK5/6 or AE1/AE3 (including Cam5.2) (Fig. 10), melanoma should be cytokeratin and EMA negative and S100 positive, and leoimyosarcoma may show variable SMA and desmin positivity. Atypical fibroxanthoma is commonly negative for all antibodies except 50% are SMA positive, many are CD68 positive (variable) and the majority are CD10 positive.

Clear cell hidradenoma vs. metastatic renal carcinoma

CD10 stains most renal cell carcinomas but only 20% of clear cell hidradenomas and 44% of sebaceous adenomas. It has been suggested that the panel of CD10 and CK5/6 (positive in skin adnexal tumours) may be of some value in distinguishing clear cell hidradenoma from metastatic renal cell carcinoma but this requires further study.

Practice points

- BerEP4 is a sensitive but non-specific marker for BCC and variants
- Basaloid cells in sebaceomas are BerEP4 negative, allowing differentiation from BCC with sebaceous differentiation
- EMA is the single most useful marker for highlighting lumina in a tumour with ductal/glandular differentiation
- CD10 immunostaining may help to differentiate TE (trichoblastoma) from BCC
- Merkel cells are reported to be absent from BCC but present in mimics such as trichoblastoma

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