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Neurofilament is superior to cytokeratin 20 in supporting cutaneous origin for neuroendocrine carcinoma

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Aim: Primary cutaneous neuroendocrine carcinoma, or Merkel cell carcinoma (MCC), cannot be distinguished morphologically from small-cell neuroendocrine carcinomas (SmCC) from other sites. Immunohistochemistry is required to confirm cutaneous origin, and is also used for detection of sentinel lymph node (SLN) metastases of MCC. Cytokeratin 20 (CK20) expression is commonly used for these purposes, but is negative in some MCC cases, and has unclear specificity. We evaluated immunohistochemistry for neurofilament and CK20 in MCC compared with SmCC from other sites.

Methods and results: We evaluated neurofilament expression in 55 MCC specimens from 39 unique patients, including nine CK20-negative MCC tumours. Neurofilament expression was observed in 42 of 55 (76.4%) MCC cases, including seven of nine (77.8%) CK20-negative MCC cases. Neurofilament was expressed in nine of 12 (75%) Merkel cell polyomavirus-positive tumours and five of 10 (50%) virus-negative tumours. Compared to a standard immunohistochemical panel (cytokeratin cocktail and CK20), neurofilament was 87.5% sensitive for detecting SLN metastases. Neurofilament and CK20 expression was also assessed in 61 extracutaneous SmCC from 60 unique patients, with primary sites including lung (27), bladder (18), cervix (3), gastrointestinal tract (3), sinonasal tract (2) and other sites (7). The specificity of neurofilament and CK20 for MCC versus non-cutaneous SmCC was 96.7% and 59.0%, respectively.

Conclusions: Neurofilament has superior specificity to CK20 in distinguishing MCC from non-cutaneous SmCC. Neurofilament is frequently expressed in CK20- and virus-negative MCC tumours. Limitations of neurofilament immunohistochemistry include lower sensitivity than CK20 and subtle staining in some tumours. However, our findings indicate that neurofilament is useful for excluding non-cutaneous SmCC.

Keywords: cytokeratin 20, Merkel cell carcinoma, Merkel cell polyomavirus, neuroendocrine carcinoma, neurofilament, sentinel lymph node, small-cell carcinoma

Introduction

Merkel cell carcinoma (MCC) is a rare but aggressive cutaneous malignancy that arises primarily on sun-damaged skin of elderly patients.[1] Metastatic disease to locoregional skin, regional lymph nodes and

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distant sites is a frequent occurrence. Estimated disease-specific mortality is 33–46%. Current evidence supports the existence of two subtypes of MCC: virus-positive MCC (VP-MCC), associated with the oncogenic Merkel cell polyomavirus (MCPyV); and virus-negative MCC (VN-MCC), with a high burden of ultraviolet (UV)-associated mutations.[2–5] Standard management for MCC includes surgery, radiotherapy and sentinel lymph node mapping.[6] As a small blue cell tumour with close morphological resemblance to certain other tumour types, immunohistochemistry has a critical role in diagnosis and accurate staging of MCC.

Considering the aggressive potential of MCC, accurate diagnosis is essential to timely management. MCC may be mistaken for other cutaneous tumours, and can be morphologically identical to metastasis from extracutaneous small-cell neuroendocrine carcinoma (SmCC), such as small-cell lung carcinoma (SCLC). In addition, MCC may present as a metastasis of unknown primary in lymph nodes or other extracutaneous sites, raising the differential diagnosis of SmCC from a range of sites. Standard diagnostic immunohistochemistry for MCC includes cytokeratin 20 (CK20), neuroendocrine markers and often thyroid transcription factor 1 (TTF-1). Neuroendocrine markers (including chromogranin A, synaptophysin and/or CD56) are expressed in MCC, but may also be expressed in other cutaneous carcinomas such as basal cell carcinoma,[1,7,8] and do not exclude extracutaneous SmCC or other poorly differentiated metastases. TTF-1 expression is relatively specific for SCLC in comparison to MCC, but is not expressed in approximately 15% of SCLC, and may be expressed in unusual cases of MCC.[9–13] MCPyV has been shown to be relatively specific for MCC compared to other cutaneous carcinomas and SCLC, but is less sensitive than other markers such as CK20,[1,14–17] and is often negative in CK20-negative MCC.[18]

Due to the high rates of lymph node metastasis for even small MCC tumours, sentinel lymph node biopsy is recommended by the National Comprehensive Cancer Network for all cases.[6] As any size of metastatic deposit is considered positive for staging purposes, immunohistochemistry (especially for CK20 and cytokeratin cocktail) is routinely used to assist in detection of small metastases, including isolated tumour cells.[1,19] Cytokeratin cocktail is sensitive, although staining of background lymph node fibroblastic reticular cells may present a challenge to evaluation for small metastases.[20,21] CK20 staining in lymph nodes is relatively specific for MCC; however, sensitivity may be limited due to low or absent expression in some tumours.[1] No single neuroendocrine marker is consistently expressed in MCC, limiting routine use of any given neuroendocrine marker in SLN evaluation.[1]

Given the limitations of other markers, CK20 is considered a key marker for MCC, with roles in diagnosis and sentinel lymph node evaluation. In MCC, CK20 is classically expressed in a paranuclear dot-like pattern, with or without cytoplasmic staining. Although CK20 is expressed in most MCC,[9–11,13,19,22–30] staining may be focal in some tumours, requiring careful interpretation. Approximately 5–10% of MCC cases completely lack expression of CK20, requiring more extensive immunophenotyping and clinical correlation for diagnostic confirmation. CK20 is considered to be specific for MCC relative to other cutaneous carcinomas.[1] Although CK20 expression has been reported to favour MCC over most extracutaneous SmCCs, a minority of SCLC may express CK20,[9,10,27,30] and CK20 is frequently expressed in parotid and cervical SmCC.[13,30,31]

Neurofilament is an intermediate filament expressed in the majority of MCC, classically in a paranuclear dot-like pattern similar to cytokeratins.[9,23,29,32,33] Limited reports suggest that neurofilament expression is specific to MCC relative to SCLC.[23,32] However, neurofilament expression has not been characterised in a spectrum of extracutaneous SmCCs. Although one study of sentinel lymph node biopsies for MCC that included five lymph node metastases found neurofilament to be less sensitive than CK20,[19] this finding has not been replicated in a larger cohort. In addition, studies have been limited regarding the relationship of neurofilament expression to CK20 expression and MCPyV status.[34]

To define the diagnostic utility of neurofilament for the diagnosis and staging of MCC more clearly, we examined neurofilament and CK20 immunohistochemical expression in a cohort of 116 neuroendocrine tumours, including MCC and SmCC from diverse anatomical sites.

Materials and methods

CASE COHORT

The study was conducted following a protocol approved by the Institutional Review Board at the University of Michigan (HUM00045834, approval date 8/11/2016). Cases of MCC and SmCC were identified using a retrospective search of the pathology database and previously assembled study sets at the University of Michigan. Cases were selected on the

basis of tumour adequacy for staining. Cases for the CK20-negative MCC cohort were required to have complete absence of CK20 expression (confirmed by repeat CK20 immunohistochemistry as described below). Haematoxylin and eosin (H&E)-stained sections were reviewed by P.W.H. to confirm the diagnosis. The MCC cohort consisted of 55 specimens from 39 unique patients, including nine previously characterised CK20-negative MCC tumours,[18,35] and a set of 16 matched primary tumour-sentinel lymph node metastasis CK20-positive MCC pairs. All metastases were at least 1 mm in maximal dimension. For 22 MCC cases from 14 unique patients, the results of MCPyV immunohistochemistry previously performed for other studies were available.[2,35,36] The SmCC cohort consisted of 61 tumours from 60 unique patients, with primary sites including lung (27), bladder (18), cervix (3), gastrointestinal tract (3), sinonasal tract (2), ovary (3), breast (1), prostate (1), thymus (1) and larynx (1). Five negative lymph nodes from non-MCC cases served as negative controls.

IMMUNOHISTOCHEMISTRY

Neurofilament (Cell Marque, Rocklin, CA, USA; mouse monoclonal clone 2F11, prediluted) and CK20 (Cell Marque; Confirm™rabbit monoclonal antibody, 1:200 dilution) immunohistochemistry was performed using the Ventana automated immunostainer (Ventana, Tucson, AZ, USA).[5–7] Neurofilament staining was performed on all tumours. CK20 staining was performed on all extracutaneous SmCC, all CK20-negative MCC and a subset of CK20-positive MCC. All parameters were scored independently by two dermatopathologists (P.W.H. and D.R.F.) in a blinded manner, with any major discordance resolved by a third independent scorer (M.P.C.). CK20 and neurofilament immunohistochemistry was scored as either negative or positive, with expression in a minimum of five tumour cells necessary to consider a tumour positive. CK20 and neurofilament staining pattern was assessed as paranuclear dot-like and/or cytoplasmic/membranous. Intensity of staining was scored (0–3). Final intensity values were averaged from individual scores and placed into the following categories: negative (0), weak (1–1.4), moderate (1.5–2.4) and strong (2.5 and above). Extent of staining (focal <10%, intermediate = 10–75%, diffuse >75%) was determined by consensus amongst independent scorers. Upon initial case review, there was a substantial rate of interobserver variability in distinction of intermediate from diffuse extent, and of moderate from strong intensity; therefore, these categories were grouped as intermediate/diffuse and moderate/strong for purposes of statistical analysis. Specific scores for each case are shown in Table S1.

STATISTICAL ANALYSIS

To determine significant difference between variables, Fisher's exact test or analysis of variance was used for categorical variables, and the Mann–Whitney *U*-test or Student's *t*-test was used for continuous variables. Statistical significance was defined as a *P*-value less than 0.05. Analyses were performed using Graphpad Prism version 7 software (Graphpad Software Inc., La Jolla, CA, USA).

Results

NEUROFILAMENT EXPRESSION IN MCC

To evaluate the sensitivity of neurofilament expression for the diagnosis and staging of MCC, we assembled a cohort of 55 MCC tumours, including primary-metastasis pairs and previously characterised CK20-negative MCC tumours (Table 1, Table S1).[18] Neurofilament expression was observed in 42 of 55 (76.4%) MCC cases, including seven of nine (77.8%) CK20-negative MCC cases (Figure 1A,B). Most cases displayed diffuse or intermediate extent of expression across the tumour, with 7.3% of cases staining focally (Figure 1C). Intensity of neurofilament staining was weak (34.5%) or moderate (41.8%). Staining pattern was consistently paranuclear dot-like when present. The extent and intensity of neurofilament staining in CK20-negative cases was similar to other MCC (Table 1, Figures 2 and 3). In all three tumours (from two patients) in our cohort that displayed a component of squamous differentiation (Table S1, cases 17A, 17B and 32), neurofilament was weakly expressed at an intermediate percentage in the neuroendocrine component and absent in the squamous component. Among cases previously characterised for the presence of MCPyV, neurofilament was expressed in nine of 12 (75%) VP-MCC tumours and five of 10 (50%) VN-MCC tumours (Table 1, Table S1). The intensity of neurofilament expression was weak in most cases of VN-MCC that displayed expression (Table 1).

NEUROFILAMENT EXPRESSION IN LYMPH NODE METASTASES

Compared to the gold standard (cytokeratin cocktail and CK20), neurofilament displayed a sensitivity of

Table 1. Neurofilament expression in MCC and SmCC

			Extent				Intensity		
Tumour	*n*	Positive cases	Negative	Focal	Intermediate/ diffuse	Positive, cannot assess extent*	Negative	Weak	Moderate/ strong
MCC									
All	55	42/55 (76.4%)	13/55 (23.6%)	4/55 (7.3%)	37/55 (67.3%)	1/55 (1.2%)	13/55 (23.6%)	19/55 (34.5%)	23/55 (41.8%)
MCC, $CK20^+$	46	35/46 (76.1%)	11/46 (23.9%)	3/46 (6.5%)	31/46 (67.4%)	1/46 (2.2%)	11/46 (23.9%)	15/46 (32.6%)	20/46 (43.5%)
MCC, $CK20^-$	9	7/9 (77.8%)	2/9 (22.2%)	1/9 (11.1%)	6/9 (66.7%)	0/9 (0%)	2/9 (22.2%)	4/9 (44.4%)	3/9 (33.3%)
MCC, $MCPyV^+$	12	9/12 (75%)	3/12 (25%)	0/12 (0%)	9/12 (75%)	0/12 (0%)	3/12 (25%)	1/12 (8.3%)	8/12 (66.7%)
MCC, $MCPyV^-$	10	5/10 (50%)	5/10 (50%)	1/10 (10%)	4/10 (40%)	0/10 (0%)	5/10 (50%)	4/10 (40%)	1/10 (10%)
SmCC									
All	61	2/61 (3.3%)	59/61 (96.7%)	1/61 (1.6%)	1/61 (1.6%)	0/61 (0%)	59/61 (96.7%)	1/61 (1.6%)	1/61 (1.6%)
Lung	28	1/28 (3.6%)	27/28 (96.4%)	1/28 (3.6%)	0/28 (0%)	0/28 (0%)	27/28 (96.4%)	0/28 (0%)	1/28 (3.6%)
Bladder	18	0/18 (0%)	18/18 (100%)	0/18 (0%)	0/18 (0%)	0/18 (0%)	18/18 (100%)	0/18 (0%)	0/18 (0%)
Cervix	3	0/3 (0%)	3/3 (100%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	3/3 (100%)	0/3 (0%)	0/3 (0%)
GI	3	0/3 (0%)	3/3 (100%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	3/3 (100%)	0/3 (0%)	0/3 (0%)
Sinonasal	2	1/2 (50%)	1/2 (50%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	1/2 (50%)	1/2 (50%)	0/2 (0%)
Ovary	3	0/3 (0%)	3/3 (100%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	3/3 (100%)	0/3 (0%)	0/3 (0%)
Larynx	1	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
Breast	1	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
Thymus	1	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
Prostate	1	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)

GI, Gastrointestinal; MCC, Merkel cell carcinoma; SmCC, Small-cell carcinoma; MCPyV, Merkel cell polyomavirus; CK20, Cytokeratin 20.
*Limited sampling of one tumour precluded definitive evaluation for extent of expression.

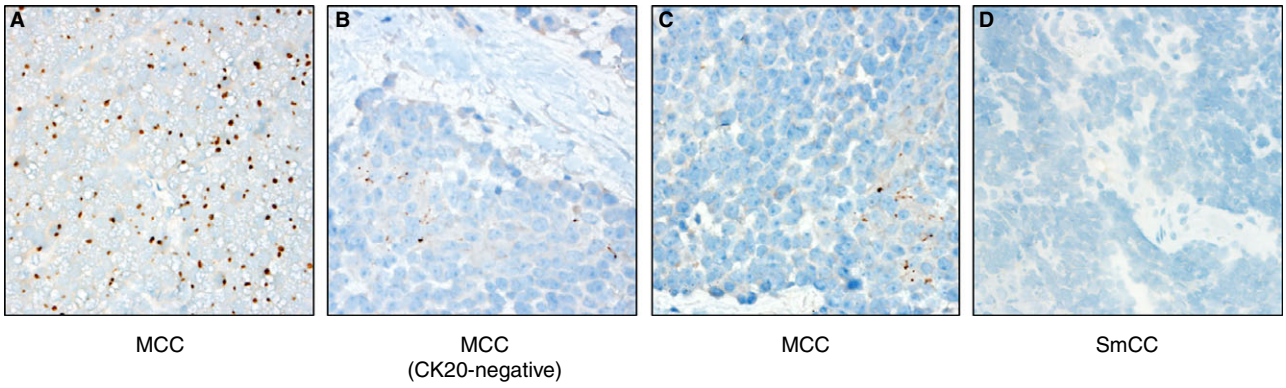

Figure 1. Representative cases of neurofilament expression in neuroendocrine carcinomas. **A**, Diffuse dot-like neurofilament expression in a CK20-positive Merkel cell carcinoma (MCC). **B**, Intermediate dot-like neurofilament expression in a CK20-negative MCC. **C**, Focal neurofilament expression in MCC. **D**, Absence of neurofilament expression in extracutaneous small-cell neuroendocrine carcinomas (SmCC).

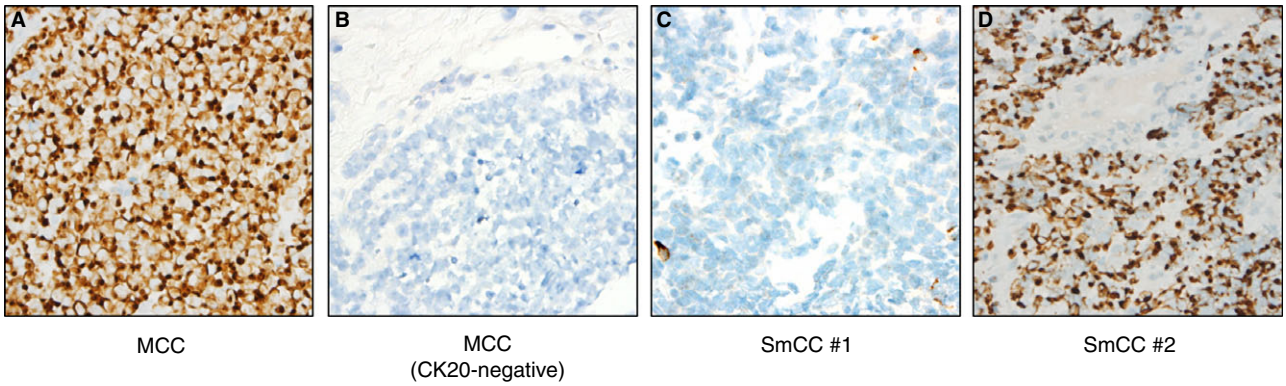

Figure 2. Representative cases of CK20 expression in neuroendocrine carcinomas. **A**, Diffuse strong CK20 expression in Merkel cell carcinoma (MCC). **B**, Absence of CK20 expression in CK20-negative MCC. **C**, Representative example of focal CK20 expression in an extracutaneous small-cell neuroendocrine carcinoma (SmCC). **D**, Representative example of diffuse CK20 expression in an SmCC.

87.5% and specificity of 100% for detecting lymph node metastases (Table S1). The presence or absence of neurofilament expression was consistent between the primary tumours and matched metastases in 10 of 16 pairs. Of the remaining pairs, five were negative for neurofilament expression in the primary tumour and positive in the matched metastasis (with intermediate to diffuse expression in the metastasis), whereas one pair was weakly positive for neurofilament in the primary tumour and negative in the matched metastasis (Table S1).

With the exception of neural structures, no background staining of lymph node elements by neurofilament was observed. Although neurofilament expression has been reported in cutaneous melanocytic nevi,[37] nodal nevi lacked neurofilament expression in all cases examined (none of five). Therefore, neurofilament expression was highly specific for MCC metastases.

NEUROFILAMENT AND CK20 STAINING IN DISTINGUISHING MCC FROM NON-CUTANEOUS SMCC

CK20 and neurofilament expression was evaluated in non-cutaneous SmCC to determine which immunohistochemical marker is best able to distinguish MCC from non-cutaneous neuroendocrine carcinomas. CK20 expression was observed in 25 of 61 (41.0%) non-cutaneous SmCC cases, resulting in a specificity of 59% for distinction from MCC (Table 2, Figures 1D, 2 and 3). CK20 staining was examined in one matched pair of SCLC tumours in which the primary tumour was negative for CK20, and a liver metastasis displayed focal CK20 expression.

Our criteria for considering tumours CK20 positive were relatively permissive (expression in at least five cells, with at least weak intensity staining), therefore we considered whether performance might be

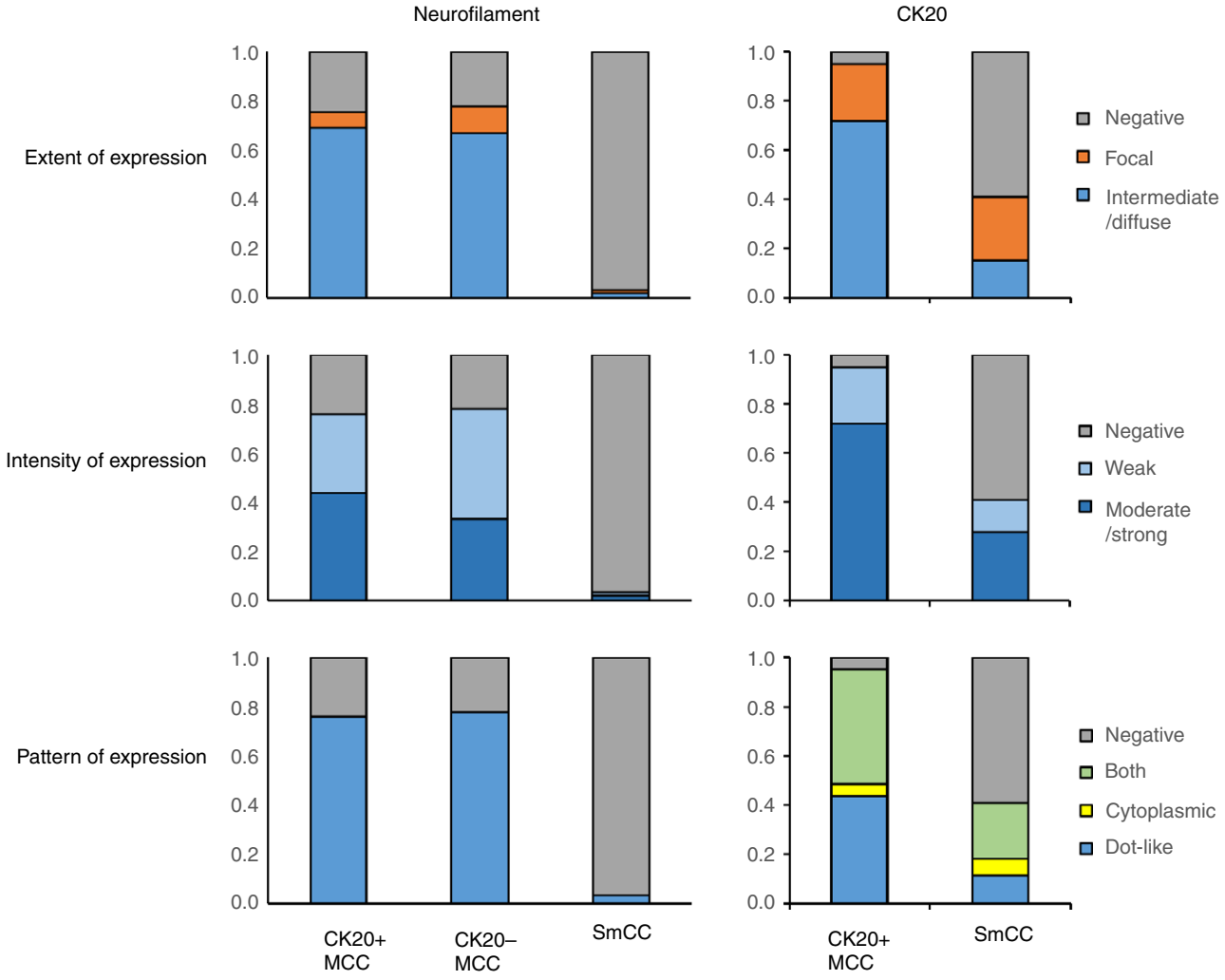

Figure 3. Extent, intensity, and pattern of immunohistochemical expression of neurofilament and CK20 in Merkel cell carcinoma (MCC) and non-cutaneous small-cell carcinoma (SmCC) tumours. Figures include cases for which the given parameter could be scored. [Colour figure can be viewed at wileyonlinelibrary.com]

improved by more stringent scoring criteria. Specificity for MCC was improved by requiring moderate/strong intensity of staining (specificity 71.8%) or expression in >10% of tumour cells (specificity 85.2%); however, these more stringent criteria also excluded 28.2% of MCC cases previously scored as positive by the original criteria. Requiring tumours to display a component of paranuclear dot-like CK20 staining improved specificity slightly (to 65.5%) and excluded relatively few MCC previously scored as positive (5.1%). Considering only tumours with positive CK20 staining, expression in >10% of tumour cells was significantly associated with MCC relative to SmCC ($P < 0.01$), whereas staining intensity and the presence of paranuclear dots were not significantly associated with cutaneous origin ($P = 0.56$ and 0.21, respectively).

Neurofilament was expressed much more frequently in MCC compared to non-cutaneous SmCC ($P < 0.0001$), and was 96.7% specific for this distinction (Table 1, Figures 1A–D and 3). Two non-cutaneous SmCC cases were positive for neurofilament: a CK20-negative sinonasal SmCC with focal weak expression and a SCLC with focal expression of both neurofilament and CK20 (Table S1). Both MCC and non-cutaneous SmCC displayed paranuclear dot-like staining for neurofilament when positive.

Discussion

Merkel cell carcinoma is an aggressive cutaneous carcinoma with high frequency of recurrence and

Table 2. Cytokeratin 20 expression in MCC and SmCC

			Extent			Intensity			Pattern		
Tumour	*n*	Positive cases	Negative	Focal	Intermediate/ diffuse	Negative	Weak	Moderate/ strong	Dot-like	Cytoplasmic	Both
MCC											
All											
MCC, CK20^{+}	39	37/39 (94.9%)	2/39 (5.1%)	9/39 (23.1%)	28/39 (71.8%)	2/39 (5.1%)	9/39 (23.1%)	28/39 (71.8%)	17/39 (43.6%)	2/39 (5.1%)	18/39 (46.2%)
MCC, CK20^{-}	9	0/9 (0%)	9/9 (100%)	0/9 (0%)	0/9 (0%)	9/9 (100%)	0/9 (0%)	0/9 (0%)	0/9 (0%)	0/9 (0%)	0/9 (0%)
SmCC											
All	61	25/61 (41.0%)	36/61 (59.0%)	16/61 (26.2%)	9/61 (14.8%)	36/61 (59.0%)	8/61 (13.1%)	17/61 (27.9%)	7/61 (11.5%)	4/61(6.6%)	14/61 (23.0%)
Lung	28	4/28 (14.3%)	24/28 (85.7%)	3/28 (10.7%)	1/28 (3.6%)	24/28 (85.7%)	1/28 (3.6%)	3/28 (10.7%)	2/28 (7.1%)	1/28 (3.6%)	1/28 (3.6%)
Bladder	18	12/18 (66.7%)	6/18 (33.3%)	8/18 (44.4%)	4/18 (22.2%)	6/18 (33.3%)	4/18 (22.2%)	8/18 (44.4%)	2/18 (11.1%)	2/18 (11.1%)	8/18 (44.4%)
Cervix	3	2/3 (66.7%)	1/3 (33.3%)	2/3 (66.7%)	0/3 (0%)	1/3 (33.3%)	1/3 (33.3%)	1/3 (33.3%)	1/3 (33.3%)	1/3 (33.3%)	0/3 (0%)
GI	3	2/3 (66.7%)	1/3 (33.3%)	1/3 (33.3%)	1/3 (33.3%)	1/3 (33.3%)	0/3 (0%)	2/3 (66.7%)	0/3 (0%)	0/3 (0%)	2/3 (66.7%)
Sinonasal	2	1/2 (50%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	1/2 (50%)
Ovary	3	2/3 (66.7%)	1/3 (33.3%)	1/3 (33.3%)	1/3 (33.3%)	1/3 (33.3%)	2/3 (66.7%)	0/3 (0%)	2/3 (66.7%)	0/3 (0%)	0/3 (0%)
Larynx	1	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
Breast	1	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)
Thymus	1	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
Prostate	1	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)

GI, Gastrointestinal; MCC, Merkel cell carcinoma; SmCC, Small-cell carcinoma; MCPyV, Merkel cell polyomavirus; CK20, Cytokeratin 20.

metastasis. Immunohistochemistry is critical to confirm the diagnosis of MCC and assist in excluding the possibility of extracutaneous SmCC. Metastatic MCC of unknown primary presenting in a lymph node or other extracutaneous site may be especially challenging to distinguish from non-cutaneous SmCC. Several diagnostic markers have been investigated for distinction of MCC from extracutaneous SmCC, including stains proposed to be expressed with relative specificity in MCC (CK20, neurofilament, TdT, MCPyV large T antigen) or SCLC (TTF1, MASH1 ASCL1).[1,9,11–13,16,17,23,25–27,32,33,38,39] With few exceptions, studies have not evaluated expression patterns in SmCC from anatomical sites other than lung. In addition, although substantial gene expression differences exist between VP-MCC and VN-MCC,[40] few studies have accounted for MCC viral status when comparing staining patterns with extracutaneous SmCC. As CK20-negative MCC are often also negative for MCPyV,[18] studies that address useful diagnostic markers in this subset of MCC are particularly necessary.

CK20 is a major diagnostic marker for MCC. CK20 expression has been proposed to be specific for MCC relative to extracutaneous SmCC. However, multiple reports have described CK20 expression in a minority of SCLC[9,10,27] and a significant percentage of parotid and cervical SmCC.[13,30,31] Our findings confirm and expand upon this observation, demonstrating that CK20 expression can occur in a minority of SmCC from multiple anatomical sites. CK20 was frequently expressed in cervical SmCC, bladder SmCC and a minority of SCLC. The specificity of CK20 staining for MCC was only slightly improved by requiring tumours to display a component of paranuclear dot-like staining. More stringent requirements for the extent and intensity of CK20 staining improved specificity, but also resulted in exclusion of a significant fraction of MCC cases. A limitation of our study is that CK20 expression was a controlled variable in our MCC cohort, precluding determination of certain statistical associations for CK20 including sensitivity.

In agreement with previous reports,[9,23,29,32,33] we find that neurofilament is a sensitive and specific marker for MCC. Neurofilament was expressed in a substantial fraction of cases regardless of MCPyV and CK20 status. Although we observed relatively lower sensitivity for neurofilament in VN-MCC, our sample size was too small for robust statistical comparison. Neurofilament expression was highly specific for MCC, with no expression detected in the vast majority of extracutaneous SmCC. Of 61 SmCC tumours evaluated, only two displayed focal neurofilament staining (one case of SCLC and one sinonasal SmCC). Unlike a previous study,[31] we did not observe neurofilament expression in cervical SmCC. A limitation of our study is that primary parotid SmCC cases were not available for study. An additional limitation is that our study cohort included few MCC tumours with squamous differentiation, in which context neurofilament has been reported to be less sensitive.[34]

Given the challenge of identifying small metastatic deposits of MCC in lymph nodes, immunohistochemistry of sentinel lymph node biopsies plays a critical role in accurate MCC staging. A previous report examining a small number of positive sentinel lymph nodes ($n = 5$) found that neurofilament had low sensitivity (20%) for detection of sentinel lymph node metastases.[19] In a larger cohort of lymph node metastases ($n = 16$), we find that neurofilament is useful for detection of metastatic MCC. Neurofilament is less sensitive than CK20, displays less intense staining than CK20 in most cases and may be sparsely or focally expressed, therefore our findings do not support the use of neurofilament in place of CK20 in sentinel lymph node evaluation for most cases of MCC. However, for cases with focal or absent CK20 expression in the primary tumour, neurofilament represents a highly specific and reasonably sensitive stain alongside cytokeratin cocktail for the evaluation of sentinel lymph node biopsies. Of note, in some cases neurofilament was effective in identifying lymph node metastases despite the apparent lack of expression in the primary tumour. A limitation of our study is that single-cell metastases were not examined, therefore we cannot comment on the sensitivity of neurofilament in this specific context.

In summary, given its superior specificity to CK20, neurofilament should be considered for suspected MCC cases in which additional confirmation of cutaneous origin is necessary. Neurofilament may be especially useful in cases of SmCC of unknown primary. However, rare cases of extracutaneous SmCC may display neurofilament expression. Neurofilament is frequently expressed in CK20-negative MCC, and is sensitive regardless of MCPyV status. Finally, neurofilament may also be useful in detection of sentinel lymph node deposits in cases of MCC with focal or absent CK20 expression.

Acknowledgements

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Conflicts of interest

The authors declare no relevant conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Case details for neurofilament (Nfil), cytokeratin 20 (CK20), and Merkel cell polyomavirus (MCPyV) staining.