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Neurofilament is Superior to Cytokeratin 20 in Supporting Cutaneous Origin for Neuroendocrine Carcinoma

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The authors declare no relevant conflicts of interest.

Abstract

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

29 MCC cases, and has unclear specificity. We evaluated immunohistochemistry for neurofilament and CK20 in
30 MCC compared with SmCC from other sites.

31

32 **Methods and Results:** We evaluated neurofilament expression in 55 MCC specimens from 39 unique
33 patients, including 9 CK20-negative MCC tumors. Neurofilament expression was observed in 42/55 (76.4%)
34 MCC cases, including 7/9 (77.8%) CK20-negative MCC cases. Neurofilament was expressed in 9/12 (75%)
35 Merkel cell polyomavirus-positive tumors and 5/10 (50%) virus-negative tumors. Compared to a standard
36 immunohistochemical panel (cytokeratin cocktail and CK20), neurofilament was 87.5% sensitive for detecting
37 SLN metastases. Neurofilament and CK20 expression was also assessed in 61 extracutaneous SmCC from 60
38 unique patients, with primary sites including lung (27), bladder (18), cervix (3), gastrointestinal tract (3),
39 sinonasal tract (2), and other sites (7). The specificity of neurofilament and CK20 for MCC versus non-
40 cutaneous SmCC was 96.7% and 59.0%, respectively.

41

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

46

47 **Keywords**

48 Merkel cell carcinoma, small cell carcinoma, neuroendocrine carcinoma, neurofilament, cytokeratin 20, Merkel
49 cell polyomavirus, sentinel lymph node

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53 **Introduction**

54 Merkel cell carcinoma (MCC) is a rare but aggressive cutaneous malignancy that arises primarily on sun-
55 damaged skin of elderly patients.[1] Metastatic disease to locoregional skin, regional lymph nodes, and distant
56 sites is a frequent occurrence. Estimated disease-specific mortality is 33-46%. Current evidence supports the
57 existence of two subtypes of MCC: virus-positive MCC (VP-MCC), associated with the oncogenic Merkel cell
58 polyomavirus (MCPyV); and virus-negative MCC (VN-MCC), with a high burden of UV-associated mutations.[2-5]

59 Standard management for MCC includes surgery, radiotherapy, and sentinel lymph node mapping.[6] As a small

60 blue cell tumor with close morphologic resemblance to certain other tumor types, immunohistochemistry has a

61 critical role in diagnosis and accurate staging of MCC.

62 Considering the aggressive potential of MCC, accurate diagnosis is essential to timely management. MCC may

63 be mistaken for other cutaneous tumors, and can be morphologically identical to metastasis from

64 extracutaneous small cell neuroendocrine carcinoma (SmCC) such as small cell lung carcinoma (SCLC). In

65 addition, MCC may present as a metastasis of unknown primary in lymph nodes or other extracutaneous sites,

66 raising the differential diagnosis of SmCC from a range of sites. Standard diagnostic immunohistochemistry for

67 MCC includes cytokeratin 20 (CK20), neuroendocrine markers, and often thyroid transcription factor 1 (TTF-1).

68 Neuroendocrine markers (including chromogranin A, synaptophysin, and/or CD56) are expressed in MCC, but

69 may also be expressed in other cutaneous carcinomas such as basal cell carcinoma,[1, 7, 8] and do not exclude

70 extracutaneous SmCC or other poorly differentiated metastases. TTF-1 expression is relatively specific for

71 SCLC in comparison to MCC, but is not expressed in approximately 15% of SCLC, and may be expressed in

72 unusual cases of MCC.[9-13] MCPyV has been shown to be relatively specific for MCC compared to other

73 cutaneous carcinomas and SCLC, but is less sensitive than other markers such as CK20,[1, 14-17] and is often

74 negative in CK20-negative MCC.[18]

75 Due to the high rates of lymph node metastasis for even small MCC tumors, sentinel lymph node biopsy is

76 recommended by the National Comprehensive Cancer Network for all cases.[6] As any size of metastatic

77 deposit is considered positive for staging purposes, immunohistochemistry (especially for CK20 and

78 cytokeratin cocktail) is routinely used to assist in detection of small metastases, including isolated tumor cells.[1,

79 [19] Cytokeratin cocktail is sensitive, although staining of background lymph node fibroblastic reticular cells may

80 present a challenge to evaluation for small metastases.[20, 21] CK20 staining in lymph nodes is relatively specific

81 for MCC; however, sensitivity may be limited due to low or absent expression in some tumors.[1] No single

82 neuroendocrine marker is consistently expressed in MCC, limiting routine use of any given neuroendocrine

83 marker in SLN evaluation.[1]

84 Given the limitations of other markers, CK20 is considered a key marker for MCC, with roles in diagnosis and

85 sentinel lymph node evaluation. In MCC, CK20 is classically expressed in a paranuclear dot-like pattern, with

86 or without cytoplasmic staining. Although CK20 is expressed in most MCC,[9-11, 13, 19, 22-30] staining may be focal

87 in some tumors, requiring careful interpretation. Approximately 5-10% of MCC cases completely lack

88 expression of CK20, requiring more extensive immunophenotyping and clinical correlation for diagnostic

89 confirmation. CK20 is considered to be specific for MCC relative to other cutaneous carcinomas.[1] Although

90 CK20 expression has been reported to favor MCC over most extracutaneous SmCCs, a minority of SCLC may

91 express CK20,[9, 10, 27, 30] and CK20 is frequently expressed in parotid and cervical SmCC.[13, 30, 31]

92 Neurofilament is an intermediate filament expressed in the majority of MCC, classically in a paranuclear dot-

93 like pattern similar to cytokeratins.[9, 23, 29, 32, 33] Limited reports suggest that neurofilament expression is specific

94 to MCC relative to SCLC.[23, 32] However, neurofilament expression has not been characterized in a spectrum of

95 extracutaneous SmCCs. Although one study of sentinel lymph node biopsies for MCC that included five lymph

96 node metastases found neurofilament to be less sensitive than CK20,[19] this finding has not been replicated in a

97 larger cohort. In addition, studies have been limited regarding the relationship of neurofilament expression to

98 CK20 expression and MCPyV status.[34]

99 To better define the diagnostic utility of neurofilament for the diagnosis and staging of MCC, we examined

100 neurofilament and CK20 immunohistochemical expression in a cohort of 116 neuroendocrine tumors, including

101 MCC and SmCC from diverse anatomic sites.

102

Materials and Methods

Case Cohort

105 The study was conducted following a protocol approved by the Institutional Review Board at the University of

106 Michigan (HUM00045834, approval date 8/11/2016). Cases of MCC and SmCC were identified using a

107 retrospective search of the pathology database and previously assembled study sets at the University of

108 Michigan. Cases were selected on the basis of tumor adequacy for staining. Cases for the CK20-negative

109 MCC cohort were required to have complete absence of CK20 expression (confirmed by repeat CK20

110 immunohistochemistry as described below). Hematoxylin and eosin (H&E)–stained sections were reviewed by

111 P.W.H. to confirm the diagnosis. The MCC cohort consisted of 55 specimens from 39 unique patients,

112 including 9 previously characterized CK20-negative MCC tumors,[18, 35] and a set of 16 matched primary tumor-

113 sentinel lymph node metastasis CK20-positive MCC pairs. All metastases were at least 1 mm in maximal

114 dimension. For 22 MCC cases from 14 unique patients, results of MCPyV immunohistochemistry previously

115 performed for other studies were available.[2, 35, 36] The SmCC cohort consisted of 61 tumors from 60 unique

116 patients, with primary sites including lung (27), bladder (18), cervix (3), gastrointestinal tract (3), sinonasal tract

117 (2), ovary (3), breast (1), prostate (1), thymus (1) and larynx (1). Five negative lymph nodes from non-MCC

118 cases served as negative controls.

Immunohistochemistry

120 Neurofilament (Cell Marque, Rocklin, CA, USA, mouse monoclonal clone 2F11, predilute) and CK20 (Cell

121 Marque, Confirm™ rabbit monoclonal antibody, 1:200 dilution) immunohistochemistry was performed using the

122 Ventana automated immunostainer (Ventana, Tucson, AZ) (5-7). Neurofilament staining was performed on all

123 tumors. CK20 staining was performed on all extracutaneous SmCC, all CK20-negative MCC, and a subset of

124 CK20-positive MCC. All parameters were scored independently by two dermatopathologists (P.W.H. and

125 D.R.F.) in a blinded manner, with any major discordance resolved by a third independent scorer (M.P.C.).

126 CK20 and neurofilament immunohistochemistry was scored as either negative or positive, with expression in a

127 minimum of five tumor cells necessary to consider a tumor positive. CK20 and neurofilament staining pattern

128   was assessed as paranuclear dot-like and/or cytoplasmic/membranous. Intensity of staining was scored (0-3).

129   Final intensity values were averaged from individual scores and placed in the following categories: negative

130   (0), weak (1-1.4), moderate (1.5-2.4) and strong (2.5 and above). Extent of staining (focal < 10%, intermediate

131   = 10-75%, diffuse >75%) was determined by consensus across independent scorers. Upon initial case review,

132   there was a substantial rate of interobserver variability in distinction of intermediate from diffuse extent, and of

133   moderate from strong intensity; therefore, these categories were grouped as intermediate/diffuse and

134   moderate/strong for purposes of statistical analysis. Specific scores for each case are shown in the

135   Supplementary Table.

136

137   **Statistical analysis**

138   To determine significant difference between variables, Fisher's exact test or analysis of variance was used for

139   categorical variables, and the Mann-Whitney U test or Student's t test was used for continuous variables.

140   Statistical significance was defined as a P-value less than 0.05. Analyses were performed using Graphpad

141   Prism 7 software (Graphpad Software Inc., La Jolla, CA, USA).

142

143   **Results**

144   **Neurofilament expression in MCC**

145   To evaluate the sensitivity of neurofilament expression for the diagnosis and staging of MCC, we assembled a

146   cohort of 55 MCC tumors, including primary-metastasis pairs and previously characterized CK20-negative

147   MCC tumors (Table 1, Supplementary Table) [18]. Neurofilament expression was observed in 42/55 (76.4%)

148   MCC cases, including 7/9 (77.8%) CK20-negative MCC cases (Figure 1A, B). Most cases displayed diffuse or

149   intermediate extent of expression across the tumor, with 7.3% of cases staining focally (Figure 1C). Intensity of

150   neurofilament staining was weak (34.5%) or moderate (41.8%). Staining pattern was consistently paranuclear

151   dot-like when present. The extent and intensity of neurofilament staining in CK20-negative cases was similar to

152   other MCC (Table 1, Figures 2, 3). In all 3 tumors (from 2 patients) in our cohort that displayed a component of

153   squamous differentiation (Supplementary Table, cases 17A, 17B, and 32), neurofilament was weakly

154   expressed at an intermediate percentage in the neuroendocrine component, and absent in the squamous

155   component. Among cases previously characterized for the presence of MCPyV, neurofilament was expressed

156   in 9/12 (75%) VP-MCC tumors and 5/10 (50%) VN-MCC tumors (Table 1, Supplementary Table). The intensity

157   of neurofilament expression was weak in most cases of VN-MCC that displayed expression (Table 1).

158

159   **Neurofilament expression in lymph node metastases**

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

161 87.5% and specificity of 100% for detecting lymph node metastases (Supplementary Table). The presence or

162 absence of neurofilament expression was consistent between the primary tumors and matched metastases in

163 10 of 16 pairs. Of the remaining pairs, 5 were negative for neurofilament expression in the primary tumor and

164 positive in the matched metastasis (with intermediate to diffuse expression in the metastasis), whereas 1 pair

165 was weakly positive for neurofilament in the primary tumor and negative in the matched metastasis

166 (Supplementary Table).

167 With the exception of neural structures, no background staining of lymph node elements by neurofilament was

168 observed. Although neurofilament expression has been reported in cutaneous melanocytic nevi [37], nodal nevi

169 lacked neurofilament expression in all cases examined (0/5). Therefore, neurofilament expression was highly

170 specific for MCC metastases.

171

172 **Neurofilament and CK20 staining in distinguishing MCC from non-cutaneous SmCC**

173 CK20 and neurofilament expression was evaluated in non-cutaneous SmCC to determine which

174 immunohistochemical marker is best able to distinguish MCC from non-cutaneous neuroendocrine carcinomas.

175 CK20 expression was observed in 25/61 (41.0%) non-cutaneous SmCC cases, resulting in a specificity of 59%

176 for distinction from MCC (Table 2, Figure 1D, Figures 2 and 3). CK20 staining was examined in one matched

177 pair of SCLC tumors, in which the primary tumor was negative for CK20, and a liver metastasis displayed focal

178 CK20 expression.

179 Our criteria for considering tumors CK20 positive were relatively permissive (expression in at least 5 cells, with

180 at least weak intensity staining), therefore we considered whether performance might be improved by more

181 stringent scoring criteria. Specificity for MCC was improved by requiring moderate/strong intensity of staining

182 (specificity 71.8%) or expression in >10% of tumor cells (specificity 85.2%); however, these more stringent

183 criteria also excluded 28.2% of MCC cases previously scored as positive by the original criteria. Requiring

184 tumors to display a component of paranuclear dot-like CK20 staining improved specificity slightly (to 65.5%)

185 and excluded relatively few MCC previously scored as positive (5.1%). Considering only tumors with positive

186 CK20 staining, expression in >10% of tumor cells was significantly associated with MCC relative to SmCC (p <

187 0.01), whereas staining intensity and the presence of paranuclear dots were not significantly associated with

188 cutaneous origin (p=0.56 and 0.21, respectively).

189 Neurofilament was much more frequently expressed in MCC compared to non-cutaneous SmCC (p <0.0001),

190 and was 96.7% specific for this distinction (Table 1, Figure 1A-D, Figure 3). Two non-cutaneous SmCC cases

191 were positive for neurofilament: a CK20-negative sinonasal SmCC with focal weak expression, and a SCLC

192 with focal expression of both neurofilament and CK20 (Supplementary Table). Both MCC and non-cutaneous

193 SmCC displayed paranuclear dot-like staining for neurofilament when positive.

194

Discussion:

MCC is an aggressive cutaneous carcinoma with high frequency of recurrence and 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 anatomic 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
anatomic 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 tumors 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 tumors evaluated, only 2 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 tumors
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 tumor, 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 apparent lack of expression in the primary tumor. 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.

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345

Tables

Table 1. Neurofilament expression in MCC and SmCC.

Tumor	n	Positive cases	Extent				Intensity		
			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%)

*Limited sampling of one tumor precluded definitive evaluation for extent of expression. GI: gastrointestinal.

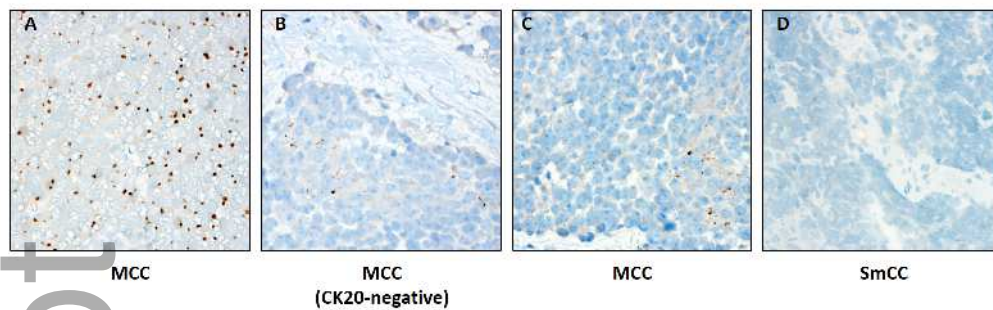Table 2. Cytokeratin 20 expression in MCC and SmCC.

Tumor	n	Positive cases	Extent			Intensity			Pattern		
			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%)

Figures

Figure 1. Representative cases of neurofilament expression in neuroendocrine carcinomas. (A) Diffuse dot-like neurofilament expression in a CK20-positive 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 SmCC. All images are at 400x.
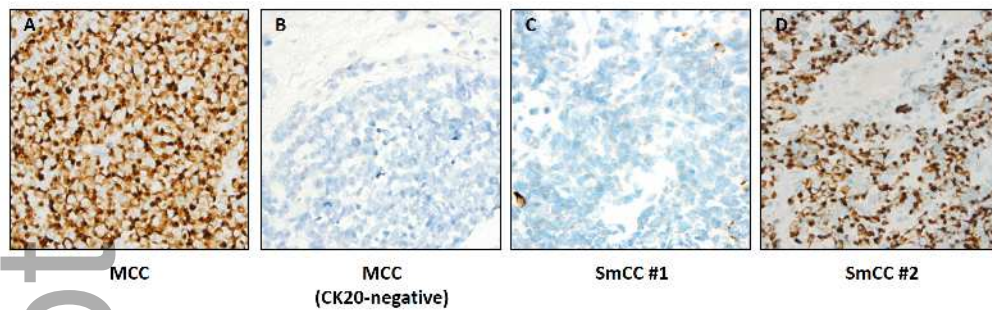
Figure 2. Representative cases of CK20 expression in neuroendocrine carcinomas. (A) Diffuse strong CK20 expression in MCC. (B) Absence of CK20 expression in CK20-negative MCC. (C) Representative example of focal CK20 expression in an extracutaneous SmCC. (D) Representative example of diffuse CK20 expression in an extracutaneous SmCC. All images are at 400x.

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) tumors. Figures include cases for which the given parameter could be scored.

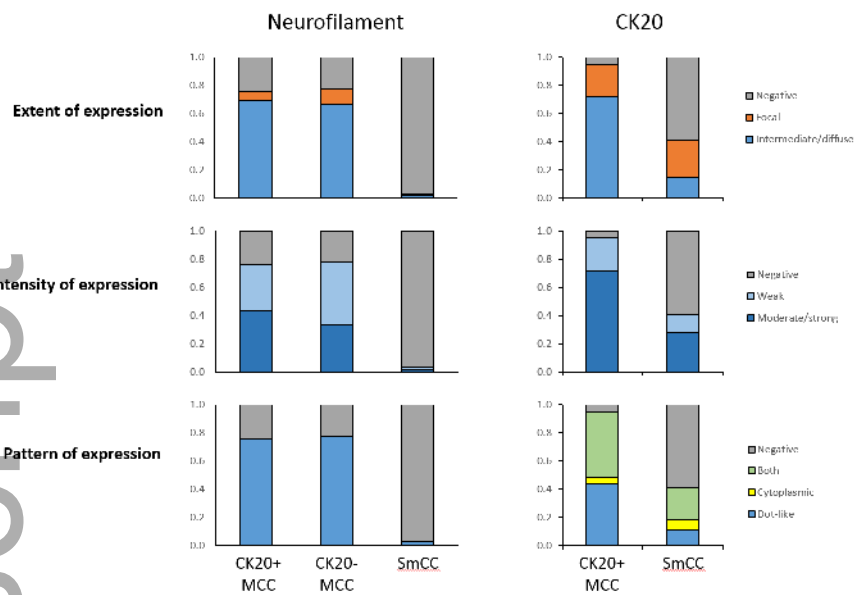| A	B	C	D
MCC	MCC (CK20-negative)	MCC	SmCC

his_13758_f1.tif

MCC   MCC (CK20-negative)   SmCC #1   SmCC #2

his_13758_f2.tif

Neurofilament   CK20

Extent of expression

Intensity of expression

Pattern of expression

his_13758_f3.tif