Simon Caroline (Orcid ID: 0000-0002-1572-5093)
Fullen Douglas (Orcid ID: 0000-0002-2524-4509)
Harms Paul (Orcid ID: 0000-0002-0802-2883)
Chan May (Orcid ID: 0000-0002-0650-1266)

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Caroline E. Talsma, MD; Min Wang, PhD; Aleodor A. Andea, MD; Douglas R. Fullen, MD; Paul W. Harms, MD, PhD; May P. Chan, MD

Department of Pathology, University of Michigan, Ann Arbor, MI, USA

Corresponding author:
May P. Chan, MD
University of Michigan
NCRC Building 35
2800 Plymouth Road
Ann Arbor, MI 48109
T: (734)764-4460
F: (734)763-4095

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Caroline E. Talsma, MD; Min Wang, PhD; Aleodor A. Andea, MD; Douglas R. Fullen, MD; Paul W. Harms, MD, PhD; May P. Chan, MD

Department of Pathology, University of Michigan, Ann Arbor, MI, USA

Corresponding author:
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University of Michigan
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Merkel cell carcinoma (MCC) is a rare aggressive skin cancer that mostly affects elderly Caucasian and immunocompromised patients.[1,2] It typically presents as a painless flesh colored papule or nodule on sun-exposed skin. The head and neck region is most frequently affected, followed by upper and lower extremities.[1] Most

cases follow an aggressive course with a high incidence of local recurrence, regional and distant metastases, and a high mortality rate.[1] It is now known that approximately 80% of MCCs harbor Merkel cell polyomavirus (MCPyV), whereas a smaller subset of cases are induced by ultraviolet (UV) radiation.[2]

Microscopically MCC has a basaloid appearance with nests and sheets of atypical blue cells containing scant cytoplasm and "powdery" nuclei.[2] Over 80% of MCCs express cytokeratin 20 (CK20) in a characteristic paranuclear dot-like pattern, a feature that is most helpful in confirming the diagnosis.[2] On the other hand, distinction of CK20-negative MCCs from other poorly differentiated carcinomas can be challenging. In particular, some cases of MCC may closely mimic human papillomavirus (HPV)-related non-keratinizing squamous cell carcinoma (SCC) given their basaloid appearance and, in some tumors, the presence of squamous metaplasia. HPV-related SCCs commonly arise in the oropharynx and often metastasize in a pattern similar to MCCs in the head and neck region.[3] Other differential diagnoses of MCC include metastatic high-grade neuroendocrine carcinomas from visceral organs, basal cell carcinoma, melanoma, and lymphoma.[2]

As a surrogate immunohistochemical marker for HPV-related SCC, p16 is typically expressed in over 70% of the carcinoma cells in a "block positivity" pattern.[3] Given the histopathologic similarities between HPV-related SCC and MCC, it would be

prudent to determine the utility of p16 in this differential diagnosis. To our knowledge, p16 expression in MCCs has been examined in two previous studies. Lassacher et al. found that 16 of 21 (76%) MCCs demonstrated nuclear p16 staining in >70% of the tumor cells, while the remaining (24%) cases showed less diffuse staining.[4] Cook et al. reported p16 immunoreactivity in all 11 MCC tumors and 6 of 7 MCC cell lines in their cohort.[5] Neither study related p16 expression to MCPyV status. As MCPyV-negative MCCs are less likely to express classic MCC markers such as CK20, neurofilament, and chromogranin, and more likely to express CK7 and TTF1,[2,6] expression for p16 in these tumors could present additional diagnostic challenge.

To confirm the above findings and to explore any association between p16 expression and MCPyV, we retrospectively identified 19 (9 primary and 10 metastatic) MCCs from 19 patients with known MCPyV status (12 positive and 7 negative, as demonstrated by large T antigen immunohistochemistry[7]), and performed p16 immunohistochemistry (clone E6H4, Roche) on all cases. Staining pattern (diffuse vs. patchy), intensity (strong vs. weak), and compartment (nuclear vs. cytoplasmic) were recorded. Fluorescence in-situ hybridization (FISH) using a 9p21 probe spanning the *CDKN2A* gene was performed when p16 staining was weak or negative.

Table 1 summarizes the clinical and pathologic characteristics of our cases. The majority of the patients had a primary MCC located in the head and neck area (58%) or extremity (37%); only one primary tumor was located on the trunk (5%). Of the 10 metastatic tumors examined in this study, only one was a distant metastasis; the rest were regional metastases. We observed diffuse and strong p16 staining in 12 of 12 (100%) MCPyV-positive cases, and in 6 of 7 (86%) MCPyV-negative cases. Strong staining was seen in both cytoplasmic and nuclear compartments of these 18 tumors (Figure 1A-F). One MCPyV-negative case (case 8) showed diffuse but weak cytoplasmic blush (Figure 1G-I). No statistically significant difference was found between primary and metastatic tumors, or based on MCPyV status. The one case with weak p16 staining did not show 9p21 loss by FISH.

The frequent p16 overexpression in MCCs is believed to be biologically compensatory in nature based on our current understanding of Rb pathway dysregulation in MCC. In MCPyV-positive MCC, the viral large T-antigen contains a Rb binding domain that can lead to phosphorylation and inactivation of Rb, a tumor suppressor protein, resulting in unchecked cell proliferation.[8] In MCPyV-negative MCCs which are characterized by high tumor mutational burden, *RB1* inactivating mutation represents one of the key drivers of tumorigenesis.[9] p16 functions as another tumor suppressor in the Rb pathway by inhibiting CDK4/6.[10] Consequently, inactivation of Rb in both MCC molecular subclasses is expected to trigger

compensatory activation of p16. This phenomenon is akin to that observed in HPV-related SCC, in which Rb is inactivated by viral oncoprotein E7, and p16 is overexpressed through a feedback mechanism.[11]

While the exact molecular mechanism behind the distinctively weak p16 staining in one of our cases is unclear, it is entirely possible that this tumor may be driven by an oncogenic pathway independent of Rb. Mutation in the p16-encoding *CDKN2A* gene is another possibility, as this has been reported in rare MCCs.[4] Deletion of this gene, however, has been ruled out by the negative FISH on our case. Lastly, promoter hypermethylation of the *CDKN2A* gene has been described in rare MCCs and may serve as another plausible explanation for the weak p16 staining in this case.[5]

In sum, p16 expression is strong and diffuse in the majority of MCCs, and is therefore not useful in distinguishing MCC and HPV-related SCC. Overexpression of p16 is likely a response to Rb inactivation irrespective of MCPyV status. Recognition of these findings will avoid misdiagnosis of MCC as HPV-related SCC based on p16 expression, and prompt additional immunohistochemical workup with CK20, MCPyV, and neuroendocrine markers[2,12] to clinch the diagnosis of MCC.

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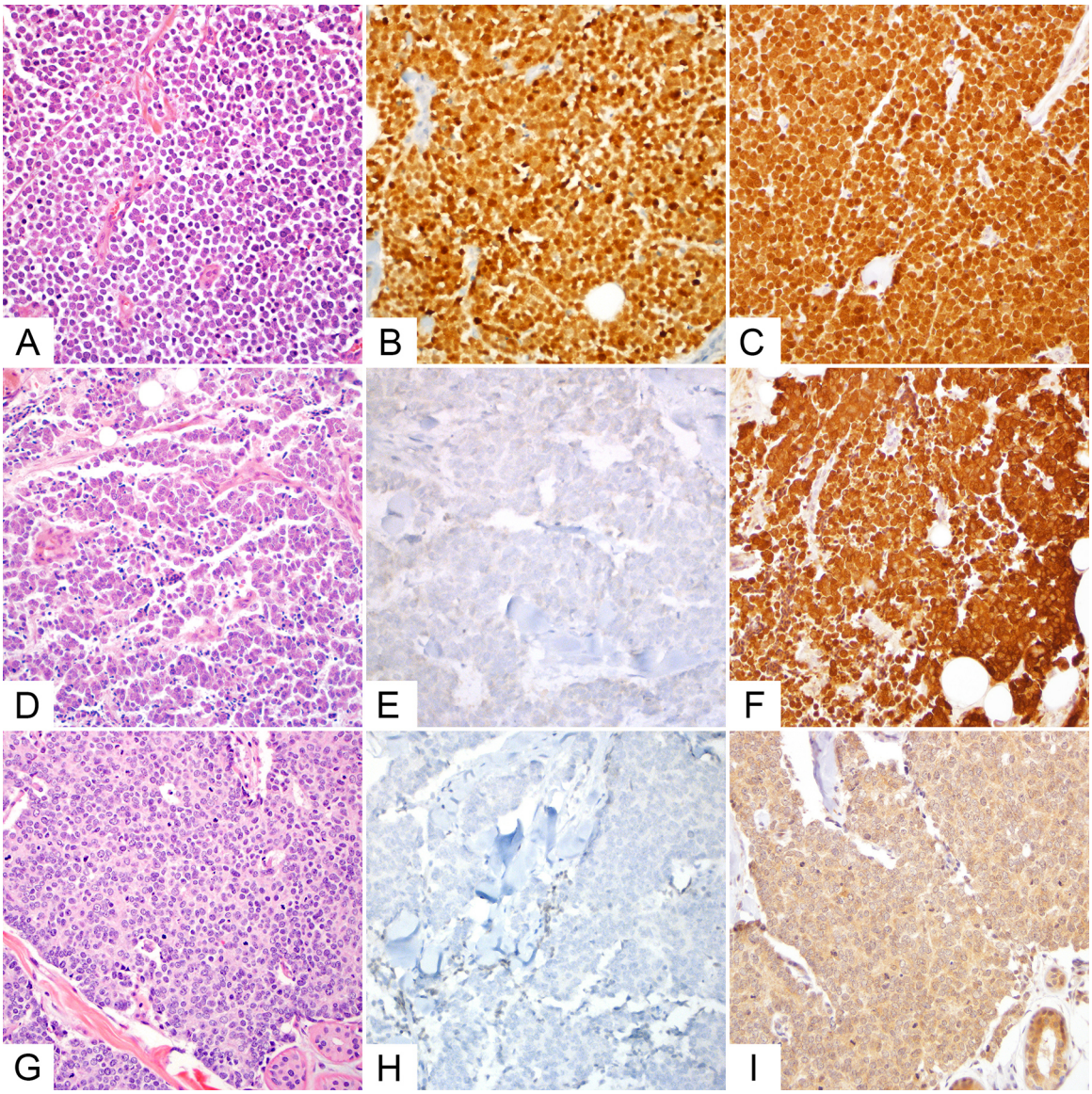

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Table 1. Clinical characteristics and p16 staining in the current cohort of Merkel cell carcinomas.

Case	Primary (P) vs. Metastatic (M)	MCPyV Status	Location of Primary Tumor	Regional (R) vs. Distant (D) Metastasis	p16 Staining			
					Strong	Diffuse	Nuclear	Cyto-plasmic
1	P	+	H&N	N/A	Y	Y	Y	Y
2	P	+	Extremity	N/A	Y	Y	Y	Y
3	P	+	Extremity	N/A	Y	Y	Y	Y
4	P	+	Trunk	N/A	Y	Y	Y	Y
5	P	+	Extremity	N/A	Y	Y	Y	Y
6	P	-	H&N	N/A	Y	Y	Y	Y
7	P	-	H&N	N/A	Y	Y	Y	Y
8	P	-	H&N	N/A	N	Y	Y	N
9	P	-	H&N	N/A	Y	Y	Y	Y
10	M	+	H&N	R	Y	Y	Y	Y
11	M	+	Extremity	R	Y	Y	Y	Y
12	M	+	Extremity	R	Y	Y	Y	Y
13	M	+	Extremity	R	Y	Y	Y	Y
14	M	+	H&N	R	Y	Y	Y	Y
15	M	+	Extremity	R	Y	Y	Y	Y
16	M	+	Extremity	R	Y	Y	Y	Y
17	M	-	Extremity	R	Y	Y	Y	Y
18	M	-	Extremity	D	Y	Y	Y	Y
19	M	-	Extremity	R	Y	Y	Y	Y

+, Positive; -; negative; D, distant; H&N, head and neck; M, metastatic; MCPyV, Merkel cell polyomavirus; N, no; N/A, not applicable; P, primary; R, regional; Y, yes.