Merkel cell carcinoma of lymph nodes without a skin primary tumor: a potential metastatic neoplasia associated with a brisk immune response

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To the Editor,

Merkel cell carcinoma (MCC) is a rare neuroendocrine cutaneous carcinoma frequently caused by Merkel cell polyomavirus (MCPyV). In 10% of cases, MCC presents as lymph-node metastasis (LNM) without a primary skin tumor (MCCWOP)[1]. We and others[1] confirmed that MCCWOP share a common phenotype with their cutaneous counterparts. However, whether MCCWOP constitutes an intranodal primitive neoplasia or a nodal metastasis from an occult or totally regressive skin MCC, remains unknown.

The metastastic process is related to epithelial–mesenchymal transition (EMT) characterized by a loss of epithelial markers such as E-cadherin and acquisition of a mesenchymal phenotype, with expression of N-cadherin or vimentin[2]. In addition, zinc finger E-box binding homeobox 1 (ZEB1)[3] is a crucial determinant of EMT. We hypothesized that investigating EMT markers in MCCWOP would help to determine whether they constitute a primary neoplasia, or a metastatic process. Expression of four EMT markers were evaluated by immunohistochemistry (**Supplementary Methods, Supplementary Figure 1**) in 60 cutaneous primary MCC, 18 LNM from cutaneous MCC and 15 MCCWOP. In the whole cohort (n=93), loss of E-cadherin, aberrant expression of N-cadherin and vimentin, expression of ZEB1 were observed in 91% (n=82), 88% (n=75), 6% (n=5) and 74.5% (n=61) of interpretable cases, respectively (**Supplementary Tables 1-2, Supplementary Figure 2**). Among the 78 MCC cases with an identified primary, only ZEB1 harbored a significant differential expression between primary tumors and LNM (p= 0.047) and was therefore considered as a surrogate of metastatic process in MCC. As such, 74% of the LNM from cutaneous MCC (n=11/15) but only 36% of primary tumors (n=19/52) showed high and diffuse expression of ZEB1 (score 2) (p=0.017). We found a similar pattern of ZEB1 (score 2) in 67% of MCCWOP cases (n=10/15),

(**Table 1, Figure 1)** suggesting that MCCWOP result from a metastatic process. Such scenario would therefore imply a complete regression of a skin primary tumor, as a result from an efficient anti-tumoral immune response[4]. We investigated this hypothesis by assessing intra-tumoral immune populations previously associated with better outcome and/or regression ($CD8^{4}$, $CD33^{brisk}/CD8^{+5}$) in the MCC cases from the same cohort (n=93) (**Table 1, Figure 1, Supplementary Table 1**). Among the 87 interpretable cases, 34 did not harbor CD8 lymphocytes (score 0, 39%), 45 displayed low infiltrates (score 1, 52%), 8 had brisk infiltrates (scores 2-5, 9%) and $CD33^{brisk}/CD8^{+}$ immune infiltrates were identified in 32 cases (42%). Among the 78 MCC cases with an identified skin primary, primary tumors more frequently harbored CD8 infiltrates (scores 1-5) (66% vs 31%, p=0.04) and $CD33^{brisk}/CD8^{high}$ infiltrates (48% vs 7%, p=0.005) than LNM (**Supplementary Table 1**). However, MCC cases with an identified primary were less frequently immune-infiltrated than MCCWOP. Indeed, CD8 and $CD33^{brisk}/CD8^{+}$ infiltrates were observed in 73% and 69% of MCCWOP (p=0.03 and 0.001 respectively) (**Table 1/Figure 1**), reflecting a brisker immune response in such cases. To conclude, this descriptive study favors the view of MCCWOP as a metastatic process, associated with a marked immune response that may account for the regression of a primary skin tumor.

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Abbreviation and acronyme list:

CD: Cluster of differentiation

EMT: Epithelial-mesenchymal transition

LNM: Lymph node metastasis

MCC: Merkel cell carcinoma

MCCWOP: Merkel cell carcinoma of lymph nodes without skin primary tumor

TMA: Tissue micro array

ZEB1: Zinc Finger E-Box Binding Homeobox 1

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Tables and figures legends:

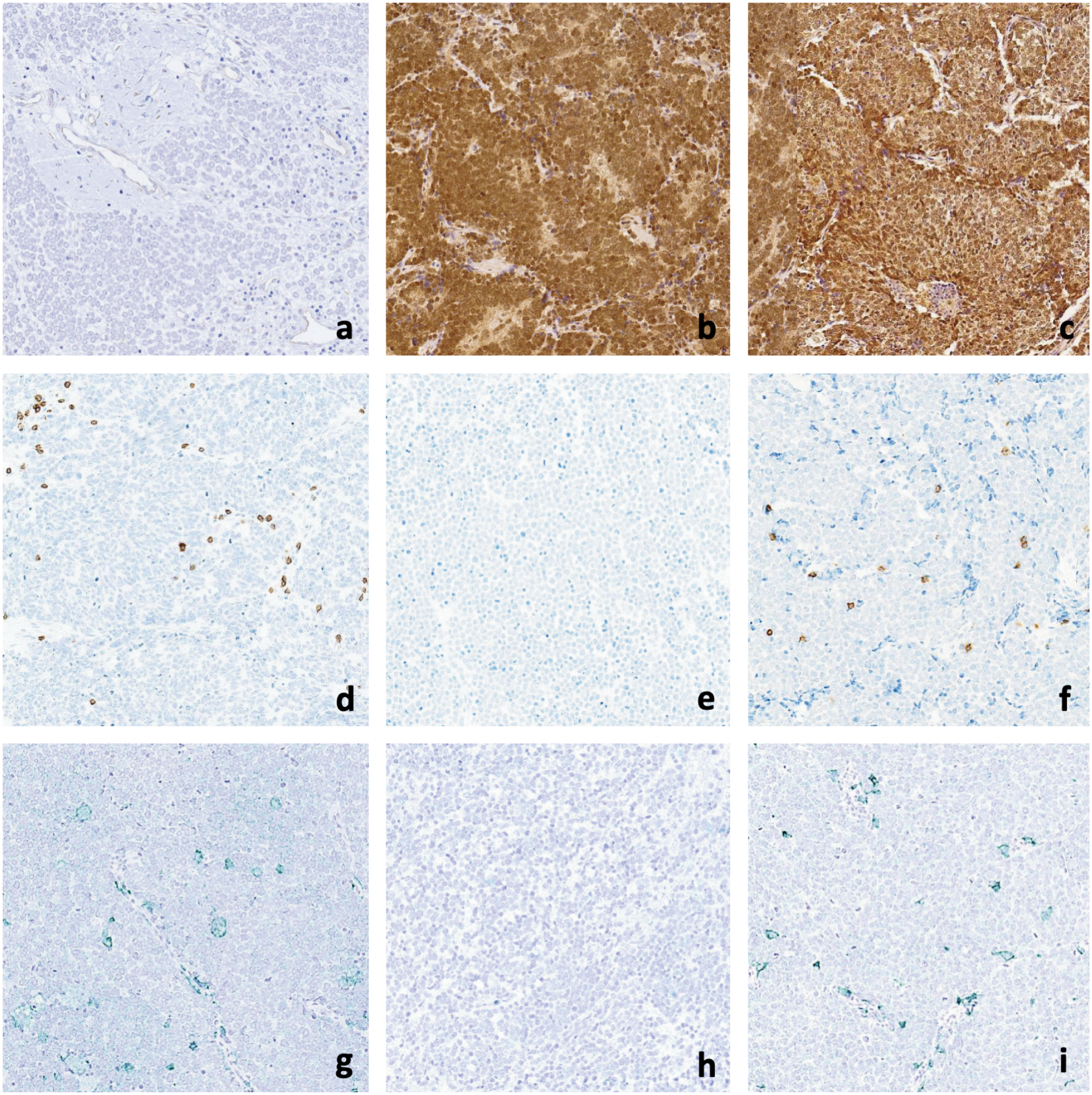
Table 1. Immunohistochemical detection of ZEB1 expression and immune infiltrates in primary cutaneous MCC, LNM from cutaneous MCC and MCCWOP.

Figure 1. Representative immunohistochemical stainings of ZEB1 (a,b,c), CD8 (d,e,f) and CD33 infiltration (g,h,i) in primary cutaneous tumor (a,d,g), metastasis of cutaneous MCC (b,e,h) and MCCWOP (c,f,i).

Table 1. Immunohistochemical detection of ZEB1 expression and immune infiltrates in primary cutaneous MCC, LNM from cutaneous MCC and MCCWOP.

Metastatic marker	All MCC cases (n=93)	Primary MCC (n=60)	LNM from cutaneous MCC (n=18)	MCCWOP (n=15)	p
ZEB1					**0.01**
Score 0-1	42 (51%)	33 (64%)	4 (26%)	5 (33%)	
Score 2	40 (49%)	19 (36%)	11 (74%)	10 (67%)	
Missing data	11	8	3	0	
Immune infiltrates	**All MCC cases (n=93)**	**Primary MCC (n=60)**	**LNM from cutaneous MCC (n=18)**	**MCCWOP (n=15)**	**p**
CD8 infiltrates					**0.03**
Absent	34	19 (34%)	11 (69%)	4 (27%)	
Present	53	37 (66%)	5 (31%)	11 (73%)	
Missing data	6	4	2	0	
$CD33^{brisk}/CD8^{+}$ infiltrates:					**0.001**
No	42	24 (52%)	14 (93%)	4 (31%)	
Yes	32	22 (48%)	1 (7%)	9 (69%)	
Missing data	19	14	3	2	

Score 0, lack of expression; 1, low staining of tumor cells or high staining of less than 50% of the tumor cells; 2, high staining of more than 50% of tumor cells.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