

# Merkel cell carcinoma of lymph node with unknown primary has a significantly lower association with Merkel cell polyomavirus than its cutaneous counterpart

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Rare cases of Merkel cell carcinoma have been encountered in lymph nodes with unknown extranodal primary, which exhibit similar morphologic and immunophenotypic features to those in primary cutaneous Merkel cell carcinomas. However, it is uncertain whether the nodal Merkel cell carcinoma is a primary tumor of the lymph node or represents a metastasis from an occult or regressed extranodal lesion. To establish an accurate diagnosis of the nodal Merkel cell carcinoma can be challenging because of significant morphologic mimics, including lymphoblastic lymphoma and metastatic small cell carcinoma. Moreover, there is no consensus for a diagnostic term, and many different terms have been used, which can be confusing and may not fully reflect the nature of nodal Merkel cell carcinoma. In this study, we investigated the detailed clinicopathologic features of 22 nodal Merkel cell carcinomas, with comparison to 763 primary cutaneous cases retrieved from the literature. Overall, the nodal and cutaneous Merkel cell carcinomas shared similar clinical presentations, morphologic spectrum, and immunophenotype; both were mostly seen in elderly male with a typical neuroendocrine morphology. Most of cases expressed CK20, synaptophysin, and chromogranin A; and PAX5 and TdT were also positive in majority of cases. However, nodal Merkel cell carcinomas had a significantly lower association with Merkel cell polyomavirus than cutaneous cases (31% vs 76%,  $P=0.001$ ). Therefore, these two entities may arise from overlapping but not identical biological pathways. We also recommend the use of the diagnostic term 'Merkel cell carcinoma of lymph node' to replace many other names used.

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Merkel cell carcinoma is a rare, high-grade cutaneous neuroendocrine neoplasm, which typically affects elderly white males in their 7th decade.<sup>1–4</sup> At the time of diagnosis, ~30% of cases have metastatic diseases, mostly to regional lymph nodes and skin of other sites.<sup>2</sup> Rare cases of high-grade neuroendocrine tumor have been encountered in the lymph nodes with unknown extranodal

primary, and these tumors were often described as 'Merkel cell carcinoma of lymph node with unknown primary' as they exhibit similar morphologic and immunophenotypic features to those in the skin Merkel cell carcinoma.<sup>5–14</sup> However, it is not clear whether the nodal Merkel cell carcinoma is a primary tumor of lymph node or represents a metastasis from an occult or regressed extranodal lesion. To establish an accurate diagnosis of nodal Merkel cell carcinoma can be challenging, and it has to be separated from other nodal metastatic neoplasms, such as small cell carcinoma and lymphoblastic lymphoma. Moreover, there is no consensus of diagnosis for this entity; a number of diagnoses have been rendered, including 'metastatic

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**Table 1** Immunohistochemical stains utilized in this study

Antibody	Clone	Dilution	Antigen retrieval	Manufacturer
Cytokeratin	AE1/AE3	1:200	FLEX TRS Low pH	DAKO
CK7	OV-TL 12/30	1:2000	FLEX TRS High pH	DAKO
CK20	Ks 20.8	1:50	FLEX TRS High pH	DAKO
CD3	Polyclonal	1:200	FLEX TRS High pH	DAKO
CD20	L26	1:400	FLEX TRS High pH	DAKO
CD45RB	2B11 + PD7/26	1:300	FLEX TRS High pH	DAKO
S100	Polyclonal	1:3000	None	DAKO
CD56	123C3	1:100	FLEX TRS High pH	DAKO
Chromogranin A	DAK-A3	1:1000	FLEX TRS High pH	DAKO
Synaptophysin	SP11	1:100	FLEX TRS High pH	Lab Vision
TTF1	8G7G3/1	1:75	FLEX TRS Low pH	DAKO
TdT	Polyclonal	1:80	FLEX TRS High pH	Supertech
MCPyV	CM2B4	1:500	FLEX TRS High pH	Santa Cruz
PAX5	24	1:150	FLEX TRS High pH	BD

Abbreviations: FLEX TRS High pH: DAKO EnVision™ FLEX Target Retrieval Solution, pH 9.0, 95 °C, 15 min; FLEX TRS Low pH: pH 6.0, 95 °, 15 min; DAKO: Dako North America, Inc., Carpinteria, CA; Lab Vision: Lab Vision Products/Thermo Fisher Scientific, Kalamazoo, MI; Supertech: Supertech Inc., Bethesda, MD; Santa Cruz: Santa Cruz Biotechnology, Inc., Santa Cruz, CA; BD: BD Biosciences, San Jose, CA; MCPyV: Merkel cell polyomavirus.

small cell carcinoma', 'small cell neuroendocrine carcinoma', 'metastatic neuroendocrine carcinoma', 'poorly differentiated carcinoma', and 'metastatic Merkel cell carcinoma of unknown primary'. These diagnoses can be confusing and may not fully reflect the nature of nodal Merkel cell carcinoma.

A new development over the past several years was the discovery of Merkel cell polyomavirus in ~80% of cutaneous Merkel cell carcinoma.<sup>15</sup> This virus is clonally integrated into the host genome, and is presumed to have an important role in the oncogenesis of cutaneous Merkel cell carcinoma. However, to date, the association of nodal Merkel cell carcinoma with Merkel cell polyomavirus has not been fully elucidated, and two recent studies showed controversial findings. One study demonstrated absence of Merkel cell polyomavirus in five of five primary parotid high-grade neuroendocrine carcinomas of Merkel cell type;<sup>16</sup> and another study detected presence of Merkel cell polyomavirus in all five nodal and one parotid gland Merkel cell carcinomas.<sup>17</sup>

To further explore the issues raised above, we collected 22 cases of nodal Merkel cell carcinoma with unknown extranodal primary. The detailed clinical presentations, morphologic features, immunohistochemical stains, and Merkel cell polyomavirus status were investigated. We also studied expression of PAX5 and TdT in our cases; both markers have been detected in the majority of cutaneous Merkel cell carcinomas.<sup>18–22</sup>

## Materials and methods

### Case Selection

This study was approved by the Institutional Review Board at the City of Hope National Medical Center. The pathology files in our department from 2000 to 2011 were searched to retrieve cases of nodal Merkel

cell carcinoma with an unknown extranodal primary. The cases to be accepted in our study met all of the following criteria: (1) we have carefully reviewed the detailed clinical history, image studies, and tissue biopsy diagnosis of the selected cases; and clinically, the patients did not have any cutaneous Merkel cell carcinoma or neuroendocrine tumor from other sites prior to and following the diagnosis, (2) cytologically, the tumor cells revealed neuroendocrine features with a high nucleus to cytoplasm ratio and fine chromatin, (3) immunohistochemically, the tumor cells were positive for CK20, and, for rare CK20 negative cases, reactivity for CK AE1/AE3 or CAM 5.2 was required, and (4) the tumor expressed neuroendocrine markers, including synaptophysin and/or chromogranin A. Eventually, 22 cases met all the criteria and were included in this study, including 19 cases from our institution and 3 from the outside institutions.

In addition, we attempted to compare the clinicopathologic features of our 22 nodal cases with those from the cutaneous Merkel cell carcinoma in the published data. We therefore performed an extensive literature search in the PubMed ([www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed)) with several sets of keywords, including 'Merkel cell carcinoma' in the 'Title' field, 'Merkel cell carcinoma' and 'immunohistochemical', and 'Merkel cell polyomavirus'. The original articles were reviewed to select cases with clinical and/or pathology studies. Eventually 763 cases of cutaneous Merkel cell carcinoma were retrieved from the English literature and included in our review.<sup>15,18–29</sup>

### Immunohistochemical Staining

For all 22 cases, a panel of antibodies was used for an initial diagnostic workup, including CD3, CD20, CD45, CD56, chromogranin A, CK AE1/AE3, CK7, CK20, S100, synaptophysin, and TTF1. A second

**Table 2** Clinical features of 22 cases of nodal Merkel cell carcinoma

Case no.	Sex/age	Lymph node location	Node size	Major clinical history	Treatment	Follow-up (months)	Outcome
M01	M/52	Right submandibular	1.0 cm	Skin BCC, schwannoma	S	8	Alive, tumor free
M02	M/52	Right cervical	1.2 cm	Adenosquamous carcinoma of lung	C, R, S	8	Alive
M03	M/92	Left cervical/intraparotid	2.2 cm		C, R, S	83	Alive
M04	M/63	Left cervical	4.1 cm	Left axillary and right inguinal node metastasis	C, R, S	14	Died
M05	F/66	Right axillary					
M06	M/81	Cervical/intraparotid	1.5 cm	SLL/CLL	S	48	Alive
M07	M/72	Left supraclavicular	14 cm	Colon cancer, urothelial carcinoma	R, S	7	Alive
M08	F/76	Left inguinal			R, S	8	Alive, tumor free
M09	M/77	Left cervical	1.1 cm	Brain metastasis of MCC	S	18	Died
M10	M/56	Left inguinal	3.6 cm		C	3	Alive, responsive
M11	M/62	Left cervical/intraparotid	1.3 cm	SLL/CLL, possible liver MCC metastasis	C, R, S	19	Alive, tumor free
M12	M/66	Right inguinal	15 cm				
M13	M/75	Right axillary	3.7 cm	Left laryngeal SCC Multiple poorly differentiated SCC of head/neck	C	2	Died
M14	M/64	Right axillary					
M15	F/59	Left axillary	2.7 cm	Progressive hilar, abdominal and inguinal nodes	C	9	Alive
M16	M/61	Left axillary	8.5 cm	SLL/CLL 5 years ago, concurrent with MCC	C, R, S	24	Alive
M17	M/48	Left cervical	27 cm	Metastatic renal cell carcinoma	S	140	Alive
M18	F/59	Right inguinal			C	2	Alive
M19	M/70	Right inguinal	6.5 cm		C	2	Alive
M20	M/50	Right inguinal	4.8 cm		C	3	Alive
M21	M/70	Left inguinal	4.5 cm		C	17	Alive
M22	M/64	Left inguinal					

Abbreviations: BCC: basal cell carcinoma; SLL/CLL: small lymphocytic lymphoma/chronic lymphocytic leukemia; MCC: Merkel cell carcinoma; SCC: squamous cell carcinoma; C: chemotherapy; R: radiotherapy; S: surgical dissection of regional lymph nodes.

panel of immunostains was performed in 13 cases with additional blocks or unstained slides, including PAX5, TdT, and Merkel cell polyomavirus large T antigen. The immunostains were performed on a Dako Autostainer with Envision (+) Detection Kit (Dako, Carpinteria, CA). The expression of PAX5, TdT, and Merkel cell polyomavirus large T antigen was graded as negative (–), weakly positive (+), or strongly positive (++) , whereas the expression of other antibodies was simply graded as negative (–) or positive (+). The antibody clones, dilutions, antigen retrieval methods, and manufactures were summarized in Table 1.

### Merkel Cell Polyomavirus DNA Detection by Polymerase Chain Reaction (PCR)

Thirteen of 22 cases had sufficient tumor cells in the paraffin blocks or unstained slides for PCR assays. Genomic DNA was extracted using 0.4 µg/µl proteinase K in Tris-EDTA buffer. The tissue was digested at 55 °C overnight and was then boiled for 10 min before used for PCR. Two sets of primers were designed to detect MCPyV: a LT3 primer pair with a 308 base-pair PCR product (forward: 5'-TTG TCT

CGC CAG CAT TGT AG-3' and reverse: 5'-ATA TAG GGG CCT CGT CAA CC-3') and a VP1 primer pair with a 351 base-pair PCR product (forward: 5'-TTT GCC AGC TTA CAG TGT GG-3' and reverse: 5'-TGG ATC TAG GCC CTG ATT TTT-3'), targeting the coding regions for viral large T antigen and viral capsid protein 1, respectively. Water was used as a PCR-negative control, instead of genomic DNA, and a case of metastatic Merkel cell carcinoma with known Merkel cell polyomavirus infection was used as a positive control. PCR for β-globin was used as an endogenous control for quality and load of genomic DNA (PCR product 268 base-pair, forward primer 5'-GAA GAG CCA AGG ACA GGT AC-3', and reverse primer 5'-CAA CTT CAT CCA CGT TCA CC-3'). The PCR assays were carried out on a GeneAmp® PCR System 9700 (Applied Biosystems, Carlsbad, CA). The amplification reactions for all target DNA regions were performed in a reaction volume of 30 µl with the following conditions: initial denaturing at 95 °C for 15 min, then denaturing at 95 °C for 30 s followed by annealing at 58 °C for 30 s and extension at 72 °C for 30 s, 45 cycles, and final extension for 30 min at 72 °C. The amplified products were electrophoresed in a 2% agarose gel.

## Results

### Clinical Information

For the 22 cases in our study, a number of diagnostic terms had been used by outside institutions, as discussed in the introduction section. In most of the cases, the tumor was considered as a metastatic carcinoma of unknown origin, particularly metastatic Merkel cell carcinoma. However, none of the 22 patients had any history of primary cutaneous Merkel cell carcinoma prior to the diagnosis. Extensive physical and radiological investigations also did not discover any extranodal primary or any signs of regressed lesion after the diagnosis. Nineteen patients were followed up closely, and none of them developed any cutaneous Merkel cell carcinoma with an average interval of 24.3 months, ranging from 2 to 140 months.

The major clinical features for the 22 patients were summarized in Table 2. There was a male predominance with a male-to-female ratio of 4.5:1 (18 males and 4 females). The mean age was 65.2 years, ranging from 48 to 92 years. The tumors often involved the head and neck lymph nodes (nine cases, including cervical, intraparotid, submandibular, and supraclavicular), inguinal (eight), and axillary (five). The greatest dimension of lymph nodes was available in 17 patients, with a mean size of 6.0 cm. Particularly in the inguinal region, the lymph nodes were often bulky with a mean size of 10.8 cm (eight cases, ranging from 4.5 to 27 cm). Eight patients also presented with other aggressive or systemic tumors, excluding localized basal cell carcinoma, which included pulmonary adenocarcinoma in case 3, colon cancer and urothelial carcinoma in case 9, laryngeal squamous cell carcinoma in case 13, multiple poorly differentiated head/neck squamous cell carcinomas in case 14, and metastatic renal cell carcinoma in case 22. Interestingly, three patients (cases 6, 11, and 16) also had chronic lymphocytic leukemia/small lymphocytic lymphoma prior to the diagnosis of nodal Merkel cell carcinoma. In most of the cases, nodal Merkel cell carcinoma was usually confined to the regional lymph nodes without involvement of extranodal organs despite the bulky lesions. In cases 5 and 15, the tumors disseminated into other regional lymph nodes without extranodal involvement in the fashion similar to that of lymphomas. The patient in case 10 developed brain metastasis from Merkel cell carcinoma, and the patient in case 11 showed possible liver metastasis, but the diagnosis was not confirmed by tissue biopsy.

Five patients received regional lymph node dissection in combination with local radiotherapy and chemotherapy, and other treatment plans included radiotherapy and surgery (2 patients), surgery only (4), and chemotherapy only (6). Of the 19 patients with follow-up, most had good response to the treatments and remained stable and tumor-free. Two

patients died of metastatic Merkel cell carcinoma to multiple lymph node regions or to the brain, and 2 other patients died of aggressive squamous cell carcinoma in the head/neck region.

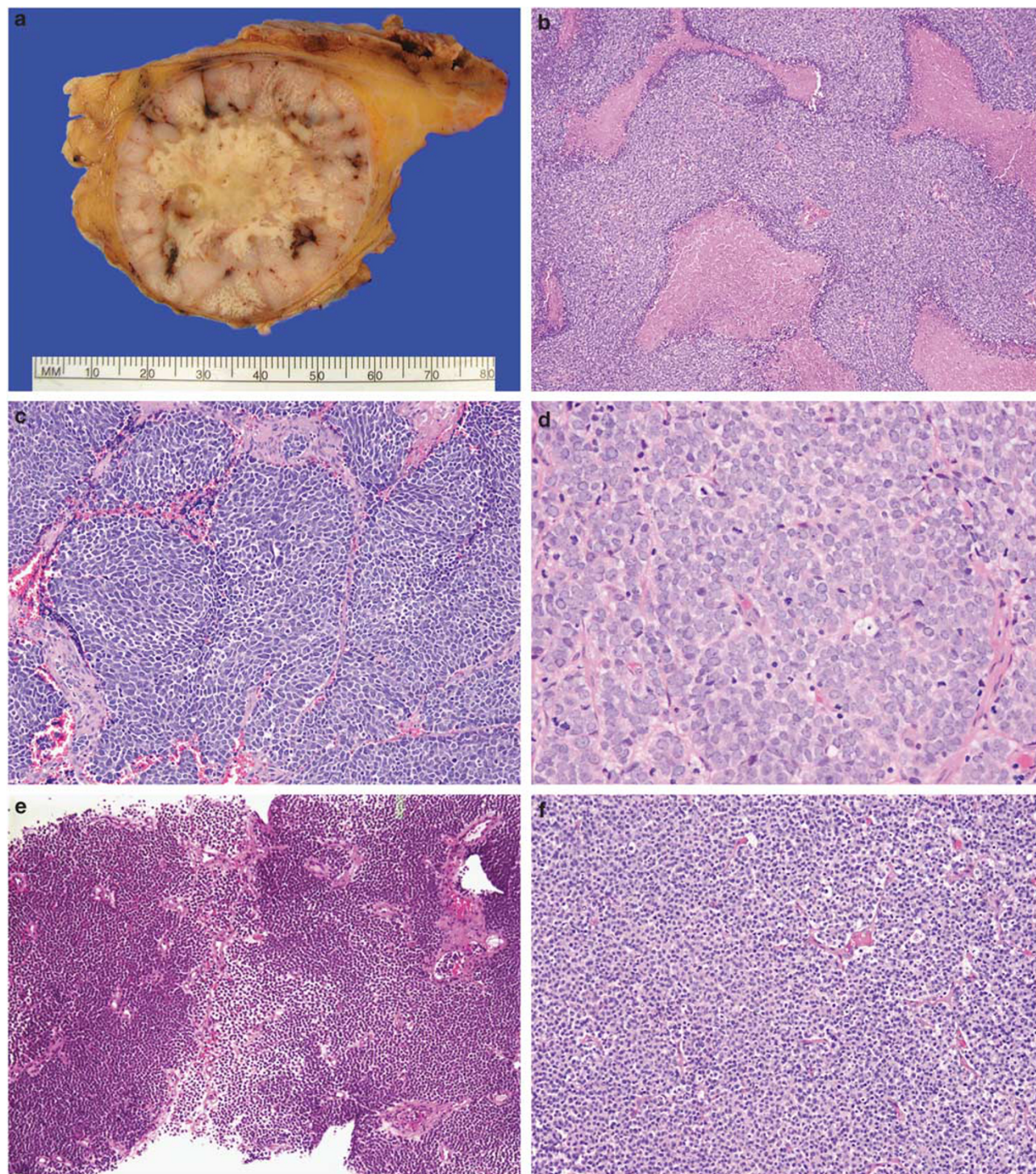
### Gross and Histopathologic Features

Grossly, the lymph nodes in most cases were markedly enlarged with entire replacement by the tumor, and large areas of degeneration and necrosis were often present, particularly in the center of the specimen (Figure 1a). Microscopically, the 22 cases of nodal Merkel cell carcinoma displayed a broad morphologic spectrum. Architecturally, there were several identifiable patterns: (1) sheets of growth pattern with geographic necrosis (Figure 1b), (2) solid nests, cords, and organoid arrangements (Figure 1c), (3) occasionally solid nests divided by delicate fibrovascular septa (Figure 1d), and (4) rarely diffuse infiltration, closely mimicking a hematologic neoplasm (Figures 1e and f). Cytologically, the tumor cells in most cases were medium to large in size and had a high nucleus to cytoplasm ratio with scant amounts of cytoplasm. The nuclei were round to oval and had characteristic fine, 'salt-and-pepper' chromatin with small or inconspicuous nucleoli (Figure 1c). In a few cases, the tumor cells revealed powdery or watery chromatin and small nucleoli, a finding which was unique to Merkel cell carcinoma (Figure 1d). One case closely resembled lymphoblastic lymphoma, which was composed of diffuse, monotonous small cells with scant cytoplasm and round nuclei with dense but fine chromatin (Figure 1e). The tumor cells in two cases closely mimicked lymphoma of mature lymphocytes, with a diffuse infiltrative pattern, condensed chromatin, visible nucleoli, and moderate amount of cytoplasm, in contrast to the majority of other cases (Figure 1f).

Despite the significant morphologic variations, the tumors in our study generally shared typically neuroendocrine features, which were superimposable to those seen in cutaneous Merkel cell carcinoma. However, none of our 22 cases showed any squamous differentiation or differentiation to skin adnexal structures, which can be occasionally seen in cutaneous Merkel cell carcinomas. One of the three cases with history of chronic lymphocytic leukemia/small lymphocytic lymphoma showed concurrent Merkel cell carcinoma and chronic lymphocytic leukemia/small lymphocytic lymphoma in the same lymph node.

### Immunohistochemistry

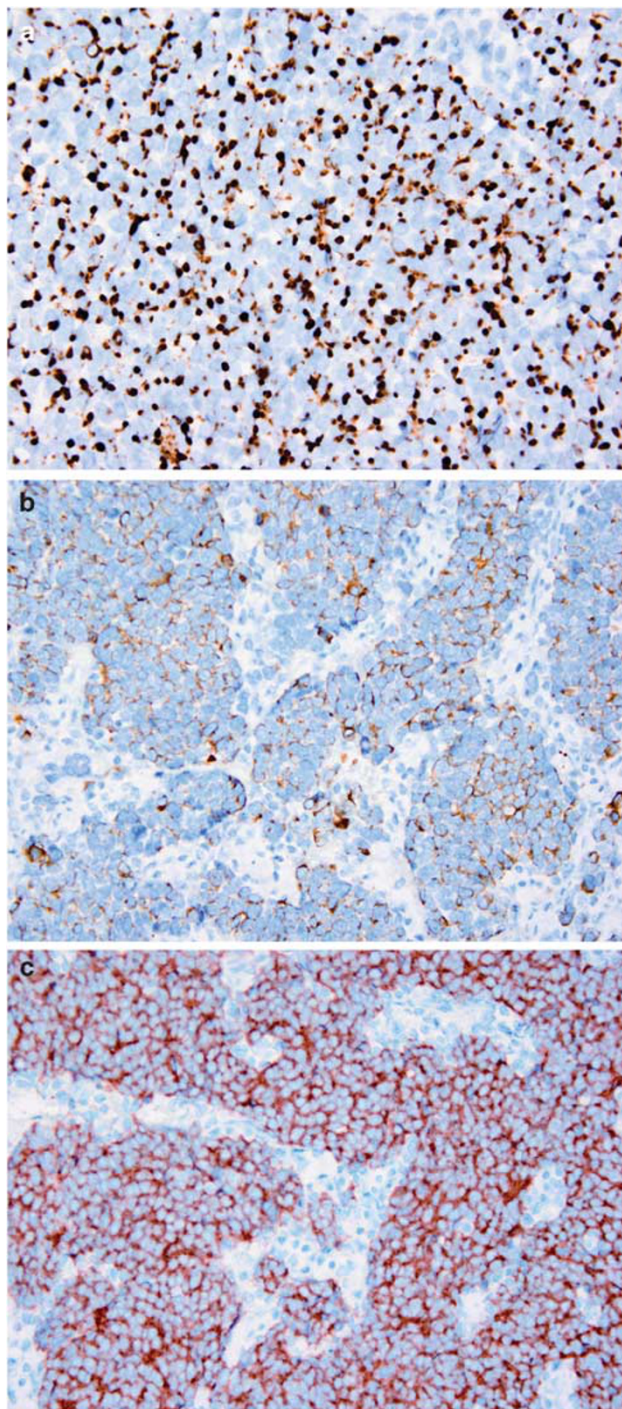
CK AE1/AE3 was stained in 19 cases, all of which (100%) showed diffuse and strong positivity. CK20 was positive in 20 of 22 cases (91%) with characteristic perinuclear 'dot-like' staining in most (Figure 2a). Three of 22 cases (14%) expressed



**Figure 1** Gross image and morphologic features of nodal Merkel cell carcinoma. **(a)** A markedly enlarged lymph node with complete replacement by tumor and central necrosis. **(b)** Sheets of growth pattern and geographic necrosis (Hematoxylin & eosin, 4 ×). **(c)** Nested and organoid arrangements; and the tumor cells have round to oval nuclei with fine chromatin and inconspicuous nucleoli (Hematoxylin & eosin, 10 ×). **(d)** Nested growth pattern with delicate fibrovascular septa; and the tumor cells exhibit powdery or watery chromatin, which is diagnostic for Merkel cell carcinoma (Hematoxylin & eosin, 20 ×). **(e)** Diffuse infiltration by small, monotonous tumor cells, resembling lymphoblastic lymphoma (Hematoxylin & eosin, 4 ×). **(f)** Diffuse infiltration by large tumor cells, mimicking a large cell lymphoma (Hematoxylin & eosin, 10 ×).

CK7, with two cases co-expressing CK20. One case (case 3) only expressed CK AE1/AE3 but neither CK7 nor CK20. Synaptophysin was positive in 19 of

19 cases (100%) stained, whereas chromogranin was detected in 13 of 19 cases (68%) (Figures 2b and c). TTF1 immunostain was performed in 21 cases, none



**Figure 2** Immunohistochemical staining of nodal Merkel cell carcinoma (40 $\times$ ). Tumor cells are positive for CK20 with characteristic perinuclear 'dot-like' staining (a). Tumor cells also express synaptophysin (b) and chromogranin (c) but not TTF1.

of which showed any reactivity. All of the cases were negative for selected lymphoid markers, including CD3 (0/8), CD20 (0/10), and CD45 (0/8).

Immunostains for TdT, PAX5, and Merkel cell polyomavirus large T antigen were also performed in the cases with additional tissue available. Ten of 13 (77%) cases were TdT positive (6 cases with strong

staining and 4 weak). The TdT staining pattern was mostly heterogeneous even in the cases with strong positivity (Figure 3a), with strong and diffuse reactivity in only one case (Figure 3b). PAX5 was positive in 8 of 13 cases (62%, 2 moderate and 6 weak) (Figure 3c). The Merkel cell polyomavirus large T antigen was detected in 3 of 13 cases (23%; cases 10, 12, and 13) using the antibody CM2B4, with diffuse and strong nuclear staining (Figure 3d).

#### Detection of Merkel Cell Polyomavirus DNA

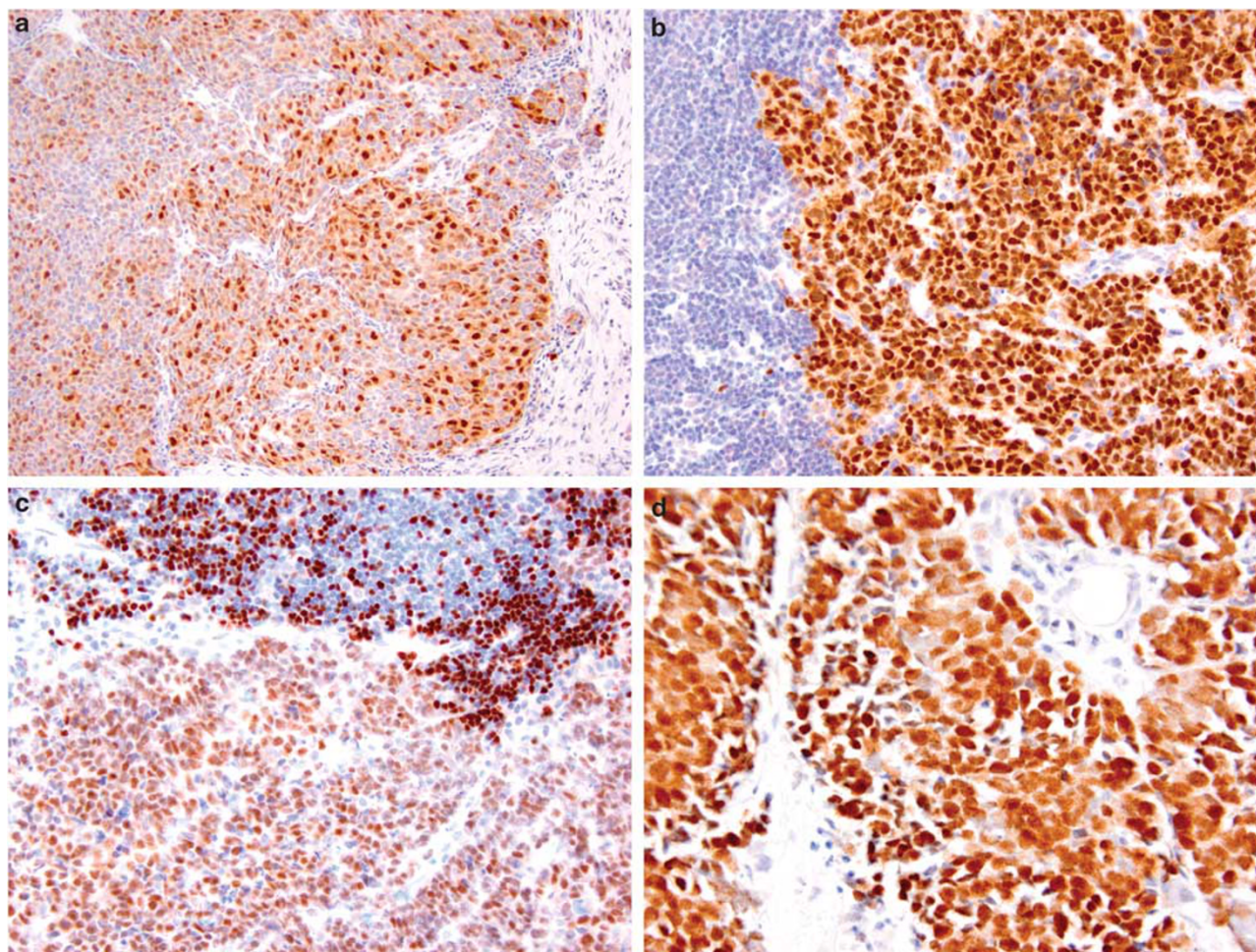
PCR assays were performed in 13 cases to detect the presence of Merkel cell polyomavirus. Two sets of primer pairs, LT3 and VP1, were used to cover the coding regions for Merkel cell polyomavirus large T antigen and viral capsid protein 1, respectively. The PCR assays with the LT3 primer pair detected viral DNA in four cases (cases 8, 10, 12, and 13), whereas three cases (cases 8, 10, and 13) were positive for viral DNA using the VP1 primer pair (Figure 4).

## Discussion

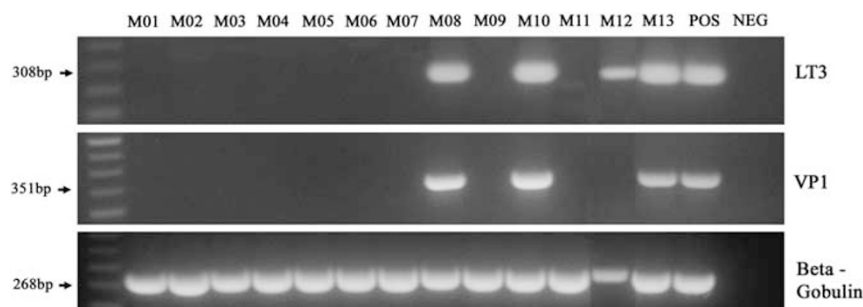
### Nodal Merkel Cell Carcinoma Shows Similar Clinical Features and Nearly Identical Morphology and Immunophenotype to Its Cutaneous Counterpart

The clinicopathologic features of our 22 nodal cases were compared with the 763 cases of primary cutaneous Merkel cell carcinoma from the English literature (Tables 3 and 4). Clinically, both entities mostly affected elderly males. Eight of our 22 patients (36%) also had other aggressive or systemic tumors, which is close to that in cutaneous Merkel cell carcinoma (27%).<sup>30–32</sup> Histologically, our cases generally displayed similar morphologic spectrum to the cutaneous Merkel cell carcinoma; however, none of our cases were associated with any non-neuroendocrine differentiation, including squamous cell, basal cell or skin adnexal structure, which can be occasionally seen in cutaneous Merkel cell carcinomas.<sup>33</sup> The skin and nodal Merkel cell carcinomas also showed a nearly identical immunoprofile; most of cases expressed CK20, synaptophysin, chromogranin A, PAX5, and TdT, whereas very few expressed CK7 and TTF1. The only significant finding was that the nodal Merkel cell carcinoma had a significantly lower association with Merkel cell polyomavirus than the cutaneous Merkel cell carcinoma (31% vs 76%,  $P=0.001$ ).

In our study, the patients generally had good response to the treatment and most of them remained tumor-free. One recent study revealed that the nodal Merkel cell carcinoma with an unknown extranodal primary had a lower recurrence rate and a less-aggressive clinical course, compared with the cutaneous Merkel cell carcinoma with nodal metastasis, with a median overall survival of ~104 months in the nodal cases.<sup>34</sup> Another more



**Figure 3** Immunohistochemical staining of nodal Merkel cell carcinoma. (a) An example of strong positivity for TdT but with heterogeneous staining (20 ×). (b) A case with strong and homogenous positivity for TdT (20 ×). (c) A case with moderate staining for PAX5, and the background reactive B cells showing strong positivity (20 ×). (d) An example with strong and nuclear reactivity for Merkel cell polyomavirus large T antigen (40 ×).



**Figure 4** PCR detection of Merkel cell polyoma virus DNA. Four of 13 cases of nodal MCC show the presence of viral DNA using a LT3 primer pair, and the viral DNA is also detected in three cases with a VP1 primer pair.

recently published study showed that the overall 2-year survival of nodal Merkel cell carcinoma patients was significantly improved vs matched stage IIIB cutaneous Merkel cell carcinoma (76.9–36.4%).<sup>35</sup>

#### Possible Histogenesis of Nodal Merkel Cell Carcinoma

Currently, it is still not certain whether the ‘nodal Merkel cell carcinoma’ is truly a primary tumor of

lymph node or not. Nineteen of our 22 patients were followed up closely, and none of them developed any cutaneous Merkel cell carcinoma within an average interval of 24.3 months. It is hard to imagine that the occult primary tumors would have remained undetectable after such a long period of time, given that Merkel cell carcinoma is a very aggressive and fast-growing tumor. Regression of cutaneous Merkel cell carcinoma and nodal

**Table 3** Summary of the results of cutaneous Merkel cell carcinoma collected from the literature

Reference no.	Male	Female	Mean age	CK7	CK20	SYN	CGA	TTF1	PAX5	TdT	MCPyV
19	25	15	78.3	2/40	35/40	39/40	29/40	1/40		28/40	
20	5	10	70	3/15	14/15	13/15	11/15	0/15		8/15	
23	4	9	65.7	4/13	13/13	10/13	13/13	0/13			
24	6	14	78		15/20	19/20	20/20	0/20			
25	253	159	71								
18	13	13	72.5		26/26	25/26	21/25	3/25	23/26	19/26	
21									24/34		
22									29/31		
15											8/10
26	14	19									21/33
27					32/36						42/53
28											91/114
29	15	12	78		27/27						17/27
Total	335	251	71.7	9/68	162/177	106/114	96/113	4/113	76/91	55/81	179/237

Abbreviations: CGA: chromogranin A; SYN: synaptophysin; MCPyV: Merkel cell polyomavirus.

**Table 4** Comparison of the clinicopathologic features of nodal MCC with cutaneous MCC

	Nodal MCC		Cutaneous MCC		P-value
Gender (M:F)	18:4	(4.5:1)	335:251	(1.33:1)	
Mean age (years)	65.2	(N=22)	71.7	(N=526)	
CK7	3/22	14%	9/68	13%	1.00
CK20	20/22	91%	162/177	92%	1.00
Synaptophysin	19/19	100%	106/114	93%	0.60
Chromogranin A	13/19	68%	96/113	85%	0.10
TTF1	0/21	0%	4/114	3.5%	1.00
PAX5	8/13	62%	76/91	84%	0.12
TdT	10/13	77%	55/81	68%	0.75
MCPyV	4/13	31%	179/237	76%	0.001

Abbreviation: MCPyV, Merkel cell polyomavirus.

metastasis has rarely been reported, mostly after either surgical excision or needle biopsy of the primary lesion; and it was thought to be due to the procedures, which stimulated cell-mediated immune clearance of the residual tumor cells.<sup>36–44</sup> It is difficult to envision the spontaneous resolution of cutaneous Merkel cell carcinoma without prior medical intervention in all of our cases; moreover, it is very unlikely that all the primary tumors would have regressed without leaving any visible remnants. Therefore, we speculate that some of our nodal cases may represent primary nodal neoplasms.

Others have proposed that the 'primary' nodal Merkel cell carcinoma could be the result of malignant transformation from pre-existing intranodal epithelial nests or pluripotent epidermal stem cells. Development of cutaneous Merkel cell carcinoma may be related to ultraviolet light irradiation and immunosuppression.<sup>45–48</sup> In our study, 8 of 22 (36%) patients had a history of other aggressive or systemic tumors prior to the diagnosis of nodal Merkel cell carcinoma, and 3 of them had chronic lymphocytic leukemia/small lymphocytic lymphoma. Approximately 27% of patients with cutaneous Merkel cell carcinoma had other invasive non-Merkel cell

carcinoma malignancies, with chronic lymphocytic leukemia/small lymphocytic lymphoma the most common one, accounting for 45% of all tumors.<sup>30–32</sup>

The discovery of Merkel cell polyomavirus in cutaneous Merkel cell carcinoma may also at least partially account for the infectious etiology in the pathogenesis of Merkel cell carcinoma.<sup>15</sup> It has been proven to function in the Merkel cell carcinoma oncogenesis through integration into the host genomic DNA.<sup>15</sup> However, we only detected Merkel cell polyomavirus in 4 of 13 cases (31%), which is significantly lower than that in the cutaneous cases. The lower expression of Merkel cell polyomavirus in the nodal Merkel cell carcinoma is not likely due to antigen loss during 'metastasis'; studies have shown the same frequency and pattern of Merkel cell polyomavirus large T-antigen expression in the paired nodal metastases and corresponding primary cutaneous tumors.<sup>29</sup> Recently, one study demonstrated absence of Merkel cell polyomavirus in five cases of primary parotid high-grade neuroendocrine carcinoma of Merkel cell type.<sup>16</sup> In contrast, another study revealed Merkel cell polyomavirus in all five nodal and one intraparotid Merkel cell carcinomas with PCR assays alone. In 2012, Rodig *et al.*<sup>17</sup> was able to detect the presence of Merkel cell polyomavirus in nearly all cutaneous Merkel cell carcinomas using a novel monoclonal antibody (Ab3) and quantitative PCR.<sup>49</sup> The increased sensitivity of detection is likely related to shorter amplicons of PCR reactions, more primer sets, and utilization of quantitative PCR, which is more sensitive than the agarose-gel-based detection method.

### Differential Diagnosis of Nodal Merkel Cell Carcinoma

To establish an accurate diagnosis of nodal Merkel cell carcinoma can be challenging due to lack of cutaneous presentation and presence of a broad



morphologic spectrum. The application of needle core biopsies often constrains pathologists with limited amounts of tissue. A unique morphologic feature of nodal Merkel cell carcinoma is the powdery and watery chromatin, which is virtually diagnostic of Merkel cell carcinoma. A number of metastatic tumors must also be considered in the differential diagnosis, including metastatic neuroendocrine carcinoma from the lung or genitourinary tract, small cell melanoma, B-cell lymphoblastic lymphoma, and mature B-cell lymphoma. Nodal Merkel cell carcinoma often has morphologic features indistinguishable from pulmonary small cell carcinoma. However, pulmonary small cell carcinoma typically presents with a mass lesion in the lung, and the tumor cells are positive for TTF1 but not CK20 and Merkel cell polyomavirus. Small cell carcinoma from the urinary bladder and prostate usually does not express CK20, but TTF1 can be positive in 52–83% of cases.<sup>50–52</sup> The distinction between nodal Merkel cell carcinoma and small cell melanoma is usually straightforward as melanoma cells express S100 and other melanocytic markers (HMB45, MITF, and MART1).

The distinction between nodal Merkel cell carcinoma and hematologic malignancies is not a problem in most cases as Merkel cell carcinoma often forms cohesive solid nests. However, it can be difficult in occasional cases with a diffuse growth pattern. The small cell variant of nodal Merkel cell carcinoma closely resembles B-cell lymphoblastic lymphoma (Figure 1e). Significantly, most cases of Merkel cell carcinoma express PAX5 and TdT, which could be a pitfall together with the absence of CD20. However, both PAX5 and TdT in Merkel cell carcinomas usually show heterogeneous stains, in contrast to the strong and diffuse pattern in B-cell lymphoblastic lymphoma. On occasion, nodal Merkel cell carcinoma may show nonclassic morphologic findings with a diffuse growth pattern, simulating the appearance of a mature large cell lymphoma. Interestingly, nodal Merkel cell carcinoma may disseminate to different regional lymph nodes (eg, axillary and inguinal) in the fashion similar to that of lymphomas.

Therefore, for any tumor in the lymph node with a neuroendocrine appearance, a careful review of the patient's medical records and communication with the referral physician are necessary to eliminate the possibility of a metastasis from a cutaneous Merkel cell carcinoma. An initial battery of immunostains should include pan-CK, CK7, CK20, TTF1, chromogranin A, synaptophysin, and S100 to rule out a metastatic neuroendocrine carcinoma or melanoma. In some cases, lymphoid markers (CD45, CD3, and CD20) may be necessary to rule out a lymphoma. If PAX5 and TdT are used, the stains should be interpreted cautiously in the context of other immunostains. After excluding the possibility of metastasis from a primary extranodal tumor, we recommend diagnosis of 'Merkel cell carcinoma of

lymph node' or 'high-grade neuroendocrine carcinoma of lymph node, favoring Merkel cell carcinoma'. A comment in the final report is necessary to briefly explain the nature of this tumor and to recommend systemic clinical workup and close follow-up to rule out metastasis.

## Conclusions

In summary, our findings showed that the nodal and cutaneous Merkel cell carcinomas shared similar clinical presentations (excluding the location, obviously), morphologic spectrum, and immunophenotype. However, nodal Merkel cell carcinoma had a significantly lower association with Merkel cell polyomavirus; therefore, these two entities may arise from overlapping but not identical biological pathways. However, the exact etiology of the nodal Merkel cell carcinoma is still unclear. We also recommend use of the diagnostic term 'Merkel cell carcinoma of lymph node' to replace many other names used.

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## Disclosure/conflict of interest

The authors declare no conflict of interest.

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