Expression of TdT in Merkel Cell Carcinoma and Small Cell Lung Carcinoma

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Key Words: Merkel cell carcinoma; Small cell lung carcinoma; Pulmonary carcinoid tumor; Immunohistochemistry; Terminal deoxynucleotidyl transferase; TdT

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Upon completion of this activity you will be able to:
- list clinicopathologic findings seen in patients with Merkel cell carcinoma (MCC).
- list the similarities and differences between MCC and small cell lung carcinoma (SCLC).
- list the typical immunophenotype in MCC and SCLC.
- discuss the role of terminal deoxynucleotidyl transferase in the diagnosis of MCC and in its differential diagnosis with SCLC, lymphoblastic lymphoma, and melanoma.

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Questions appear on p 974. Exam is located at www.ascp.org/ajcpcme.

Abstract

Merkel cell carcinoma (MCC) is an uncommon tumor with indistinct clinical features. The differential diagnosis includes small cell lung carcinoma (SCLC). We characterized the expression of terminal deoxynucleotidyl transferase (TdT) and a panel of immunohistochemical markers in 40 MCC, 30 SCLC, and 6 pulmonary carcinoid tumor (PCT) cases. We used antibodies against TdT, thyroid transcription factor (TTF)-1, cytokeratins (CKs) 7 and 20, chromogranin, and synaptophysin. Immunostaining was recorded semiquantitatively. Of 40 MCC cases, 28 (70%) were positive for TdT, showing, on average, more than 25% of tumor cells reactive with moderate nuclear staining intensity. TTF-1 (1 [3%]), CK7 (2 [5%]), CK20 (35 [88%]), chromogranin (29 [73%]), and synaptophysin (39 [98%]) were expressed in the MCCs. Of the 5 CK20– MCC cases, 4 were positive for TdT. SCLC showed expression of TTF-1 (23/30 [77%]), CK7 (22/30 [73%]), chromogranin (16/30 [53%]), and synaptophysin (22 [73%]) and no CK20 (0%) expression. Of 30 SCLC cases, 2 (7%) were positive for TdT. TdT may be beneficial in rare cases of CK20– MCC and may assist in distinguishing between MCC and SCLC. There is significant immunohistochemical variability and overlap between these 2 tumors.

Merkel cell carcinoma (MCC) is a rare, aggressive, primary neuroendocrine carcinoma of the skin that has characteristic morphologic and immunophenotypic features. The incidence of MCCs is on the rise and has tripled since 1986.[1,2] MCCs are usually found on sun-exposed sites, such as the head and neck region and upper extremities, but they can occur almost anywhere. UV radiation and immunosuppression have been implicated in the pathogenesis of MCCs, with recent reports demonstrating a 13-fold increased risk for MCC in patients with HIV infection.[3-5] The majority of MCCs are found in elderly people, with a median age at diagnosis of 69 years.[1]

MCCs have variable manifestations, but they most commonly appear as solitary purple to red nodules or plaques, especially in the early stages of the lesion.[6,7] Morphologically, MCCs are composed of small round blue cells, with minimal cytoplasm, within the dermis and/or subcutis **Image 1**.[8] Cytologically, the tumor cells have pale or dense hyperchromatic nuclei with a finely granular chromatin pattern. Tumor cells can often show nuclear molding and associated crush artifact. The differential diagnosis includes other tumors with small round blue cell morphologic features, such as melanoma and lymphoma, but more important, small cell lung carcinoma (SCLC). SCLC may have nearly identical histopathologic features and aggressive clinical behavior. Once a diagnosis of MCC has been made, it is recommended that the patient be screened to exclude metastatic SCLC.[9]

Immunohistochemical analysis is valuable in the evaluation and differentiation of MCC and SCLC. MCCs are reactive with antibodies to cytokeratins (CKs), such as low-molecular-weight CK7 and CK20, which stains in

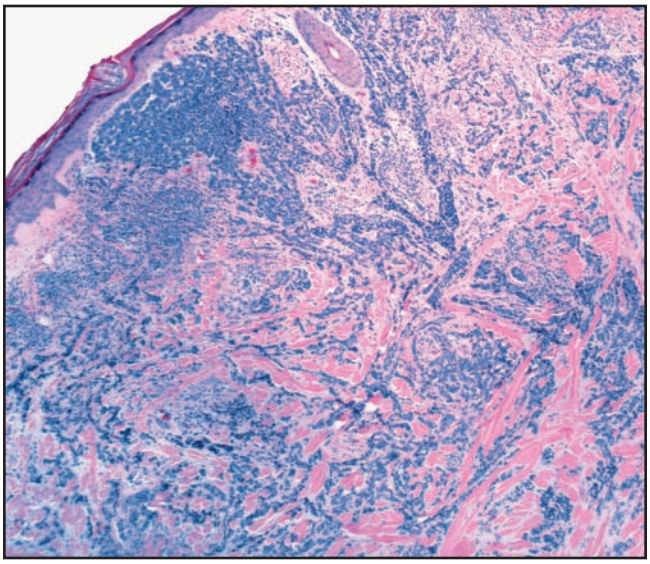

Image 1 Conventional Merkel cell carcinoma showing features of a tumor consisting of small round blue cells with minimal cytoplasm infiltrating the dermis (H&E, ×10).

a characteristic paranuclear dot-like pattern.[10,11] Similar to SCLCs, most MCCs are also positive for the neuroendocrine markers chromogranin, synaptophysin, and CD56. In contrast with MCCs, SCLCs demonstrate high expression of thyroid transcription factor (TTF)-1 and CK7.[12-16] Recently, nuclear reactivity with terminal deoxynucleotidyl transferase (TdT) has been described in MCCs.[17,18]

TdT is a DNA polymerase found in mammals that catalyzes the elongation of nucleotide chains. Its actions are greatest in developing early B cells and T cells during immunoglobulin and T-cell receptor rearrangements, respectively. Lymphoblastic lymphoma/leukemia and acute myeloid leukemia, as well as medulloblastoma, Ewing sarcoma, and pediatric rhabdomyosarcoma, have nuclear expression of TdT.[19-21] Studies by Sur et al[17] and Buresh et al[18] demonstrated that TdT was identified in 8 of 15 and 19 of 26 MCCs with variable but often moderate to strong nuclear staining intensity in a significant percentage of neoplastic cells.

TdT expression in SCLC remains unknown, and its expression might be useful in distinguishing MCC from SCLC. The aim of our study was to evaluate a group of cases of MCC, SCLC, and pulmonary carcinoid tumors (PCT) with the use of immunohistochemical markers for TdT, TTF-1, CK7, CK20, chromogranin, and synaptophysin.

Materials and Methods

The study was approved by the Sunnybrook Health Sciences Centre Ethics Review Board, Toronto, Canada. All patients were seen at or sought consultations from physicians at Sunnybrook Health Sciences Centre. We studied 40 cases of MCC, 30 cases of SCLC, and 6 cases of PCT accessioned during the 1995-2009 period in the Department of Anatomic Pathology, Sunnybrook Health Sciences Centre. Pertinent demographic and clinical data were retrieved from the electronic medical records.

Widely accepted criteria on H&E-stained sections in conjunction with immunohistochemical studies were used to make the diagnosis, and only clear-cut cases were selected. Cases were reviewed by 2 pathologists (Z.G. and S.J.R.) and 1 dermatopathologist (W.H.) who selected a block containing representative tumor for immunohistochemical studies. These studies were performed on formalin-fixed, paraffin-embedded tissue sections using a panel of antibodies raised against TdT, TTF-1, CK7, CK20, chromogranin, and synaptophysin. Immunostaining was performed on a DAKO Autostainer (DAKO, Carpinteria, CA) using a polymer detection system. The primary antibodies that were used, including dilutions and antigen-retrieval methods, are listed in **Table 1**.

The stained slides were assessed for TdT reactivity as follows: reactivity in fewer than 1% of tumor cells was scored as negative, reactivity in 1% to 25% of cells as +, reactivity in 26% to 50% of cells as 2+, and reactivity in more than 50% of cells as 3+. Nuclear staining was evaluated by using a 3-tiered scale (weak, moderate, or strong).

Table 1
Antibodies With Source, Dilution, and Antigen-Retrieval Method

Antibody	Clone	Source	Dilution	Antigen Retrieval
TdT	Polyclonal	DAKO, Carpinteria, CA	1:25	HIER, pH 6.0
TTF-1	8G7G3/1	NeoMarkers, Fremont, CA	1:400	HIER, pH 6.0
CK7	BC1	Biocare Medical, Concord, CA	1:50	HIER, pH 6.0
CK20	KS20.8	Novocastra, Newcastle upon Tyne, England	1:200	Pepsin, 10 min
Chromogranin	Polyclonal	DAKO	1:400	Pepsin, 10 min
Synaptophysin	27G12	Vector Labs, Burlingame, CA	1:200	HIER, pH 6.0

CK, cytokeratin; HIER, heat-induced epitope retrieval; TdT, terminal deoxynucleotidyl transferase; TTF-1, thyroid transcription factor 1.

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Two pathologists reviewed and scored all cases (Z.G. and S.J.R.).

All original slides were reviewed to confirm the diagnoses. The majority of cases and immunohistochemical markers had easily identifiable internal positive control samples (epidermis and sweat gland epithelium). There were no significant differences in reactivity or distribution of staining by anatomic site.

Note was taken of all clinical information available from patient charts, including imaging studies, up until the time of our study. Patients with a definite diagnosis of MCC were thought to include cases that had negative imaging studies and no evidence of a primary lung lesion at the time of diagnosis or the presence of regional nodal metastases. All SCLC cases had supportive clinical and imaging findings.

Results

Clinical Data

A total of 40 MCCs, 30 SCLCs, and 6 PCTs were selected and analyzed. Of the 40 MCC patients, 25 (63%) were men and 15 (38%) were women, ranging in age from 59 to 95 years (mean, 78.3 years). Of the MCC tumors, 12 (30%) were from the head and neck, 3 (8%) from the trunk, 6 (15%) from the upper extremity, and 17 (43%) from the lower extremity; 2 (5%) manifested as metastasis (lymph node and liver). **Table 2** shows the clinical data. Of the 30 patients with SCLC, 17 (57%) were men and 13 (43%) were women, ranging in age from 54 to 87 years (mean, 70.2 years). Of the 30 SCLC cases, 23 cases were primary and 7 occurred as metastasis. Of the 6 patients with PCT, 2

Table 2
Characteristics and Immunohistochemical Results for TdT and CK20 Staining in 40 Merkel Cell Carcinoma Cases*

Case No./Sex/Age (y)	Tumor Site	CK20	TdT Reactivity	TdT Intensity
1/M/74	Upper leg	–	3+	Moderate
2/M/76	Arm	+	+	Weak
3/M/79	Wrist	+	Negative	—
4/M/63	Face (ear)	+	2+	Moderate
5/F/80	Groin	–	Negative	—
6/M/59	Shoulder	+	Negative	—
7/M/90	Upper leg	+	2+	Moderate
8/M/76	Lower leg	+	2+	Moderate
9/F/66	Lower leg	+	+	Weak
10/F/65	Neck	+	Negative	—
11/F/85	Upper leg	+	+	Weak
12/F/81	Face (cheek)	+	+	Strong
13/M/76	Upper leg	–	2+	Moderate
14/F/80	Lower leg	+	+	Weak
15/F/95	Finger	+	2+	Moderate
16/M/74	Metastasis, liver	+	Negative	—
17/M/81	Face (cheek)	+	+	Weak
18/M/83	Upper leg	+	3+	Strong
19/F/82	Ankle	–	2+	Moderate
20/M/80	Face (cheek)	+	Negative	—
21/M/79	Face (eyelid)	+	2+	Moderate
22/M/78	Lower leg	+	Negative	—
23/F/64	Buttock	+	2+	Strong
24/F/85	Chest	+	+	Weak
25/M/80	Shoulder	+	+	Moderate
26/M/77	Face (forehead)	+	+	Moderate
27/M/80	Face (eyelid)	+	+	Weak
28/M/90	Upper leg	+	+	Weak
29/M/73	Lower leg	+	2+	Moderate
30/F/89	Face (forehead)	+	Negative	—
31/F/71	Lower leg	+	2+	Moderate
32/F/89	Face (cheek)	+	2+	Moderate
33/F/87	Arm	+	2+	Strong
34/M/74	Lower leg	+	2+	Moderate
35/M/87	Lower leg	+	Negative	—
36/M/91	Face (lip)	+	Negative	—
37/M/78	Face (nose)	+	Negative	—
38/M/78	Metastasis, node	–	2+	Moderate
39/F/67	Lower leg	+	Negative	—
40/M/71	Upper leg	+	2+	Moderate

CK20, cytokeratin 20; TdT, terminal deoxynucleotidyl transferase.
* For CK20, +, positive; –, negative; for TdT, negative, <1% tumor cells reactive; +, 1%-25% tumor cells reactive; 2+, 26%-50% tumor cells reactive; 3+, >50% tumor cells reactive.

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Table 3
Characteristics and Immunohistochemical Staining Results for 30 SCLC and 6 PCT Cases

Case No./Sex/Age (y)	Tumor Site	Imaging	TdT	TTF-1	CK7	CK20	Chromogranin	Synaptophysin
SCLC								
1/F/74	RLL	+	–	+	–	–	+	+
2/M/83	LUL	+	–	–	+	–	–	–
3/M/71	LLL	+	–	+	–	–	+	+
4/F/77	LMB	+	–	–	+	–	–	+
5/F/54	RLM	+	+	–	–	–	+	+
6/M/64	LLL	+	+	+	+	–	+	+
7/M/59	RMB	+	–	–	+	–	+	+
8/F/66	RML	+	–	+	+	–	–	–
9/M/66	RUL	+	–	+	+	–	+	+
10/M/76	Metastases, brain	+	–	+	+	–	–	–
11/M/57	Metastases, brain	+	–	+	+	–	–	–
12/F/77	RUL	+	–	+	–	–	+	+
13/F/65	Metastases, liver	+	–	+	+	–	+	+
14/M/57	LLL	+	–	+	+	–	+	+
15/M/83	Metastases, skin	+	–	+	+	–	–	+
16/M/69	Metastases, adrenal	+	–	+	+	–	+	+
17/F/69	RUL	+	–	+	+	–	–	–
18/F/65	LLL	+	–	+	+	–	–	+
19/F/62	LUL	+	–	+	–	–	+	+
20/F/69	Metastases, LN	+	–	+	+	–	+	+
21/M/78	RUL	+	–	–	–	–	+	+
22/M/61	LLL	+	–	+	–	–	–	+
23/F/87	RUL	+	–	+	+	–	–	+
24/M/59	LUL	+	–	+	+	–	–	–
25/F/73	LUL	+	–	+	+	–	–	+
26/M/81	RUL	+	–	+	–	–	+	+
27/M/87	RLL	+	–	–	+	–	+	+
28/M/69	RUL	+	–	+	+	–	–	+
29/F/67	Metastases, LN	+	–	+	+	–	–	–
30/M/81	LUL	+	–	–	+	–	+	–
PCT								
1/M/75	RLL	+	–	–	–	–	+	+
2/F/92	Carina	+	–	+	+	–	+	+
3/M/54	RUL	+	–	+	–	–	–	+
4/F/53	RLL	+	–	+	–	–	+	+
5/F/70	RUL	+	–	–	–	–	+	+
6/F/70	RML	+	–	+	–	–	+	+

CK, cytokeratin; LLL, lung, left lower lobe; LMB, lung, left main bronchus; LN, lymph node; LUL, lung, left upper lobe; PCT, pulmonary carcinoid tumor; RLL, lung, right lower lobe; RLM, right lung mass; RMB, lung, right main bronchus; RML, lung, right middle lobe; RUL, lung, right upper lobe; SCLC, small cell lung carcinoma; TdT, terminal deoxynucleotidyl transferase; TTF-1, thyroid transcription factor 1; +, positive; –, negative.

(33%) were men and 4 (67%) were women, ranging in age from 53 to 92 years (mean, 69.0 years). Five cases were from the right lung, and the remaining case was from the carina. **Table 3** shows the clinical data.

Table 4
Immunohistochemical Staining of MCC, SCLC, and PCT*

Antibody	MCC (n = 40)	SCLC (n = 30)	PCT (n = 6)
TdT	28 (70)	2 (7)	0 (0)
TTF-1	1 (3)	23 (77)	4 (67)
CK7	2 (5)	22 (73)	1 (17)
CK20	35 (88)	0 (0)	0 (0)
Chromogranin	29 (73)	16 (53)	5 (83)
Synaptophysin	39 (98)	22 (73)	6 (100)

CK, cytokeratin; MCC, Merkel cell carcinoma; PCT, pulmonary carcinoid tumor; SCLC, small cell lung carcinoma; TdT, terminal deoxynucleotidyl transferase; TTF-1, thyroid transcription factor 1.
* Data are given as number (percentage).

Immunohistochemical Studies

The immunohistochemical data for all cases studied are summarized in Tables 2 and 3 and **Table 4**. The evaluation of immunohistochemical studies by method was based on the overall expression of the antibodies in the tumor. In all cases, reactivity was diffuse, demonstrating a mixed population of positive and negative cells with no variability seen by region of the tumor.

Terminal Deoxynucleotidyl Transferase

Of 40 MCC cases, 28 (70%) were positive for TdT with nuclear staining (Tables 2 and 4) **Image 2** and **Image 3**. Of the 40 cases, 16 (40%) demonstrated moderate TdT staining intensity, whereas 8 (20%) showed weak and 4 (10%) showed strong nuclear TdT staining intensity. In 12 cases, there was no positive staining for TdT. In 15 (38%) of 40 MCC cases, 25% to 50% of tumor cells were reactive to TdT. Reactivity to TdT in more than 50% of tumor cells was seen in only 2 (5%)

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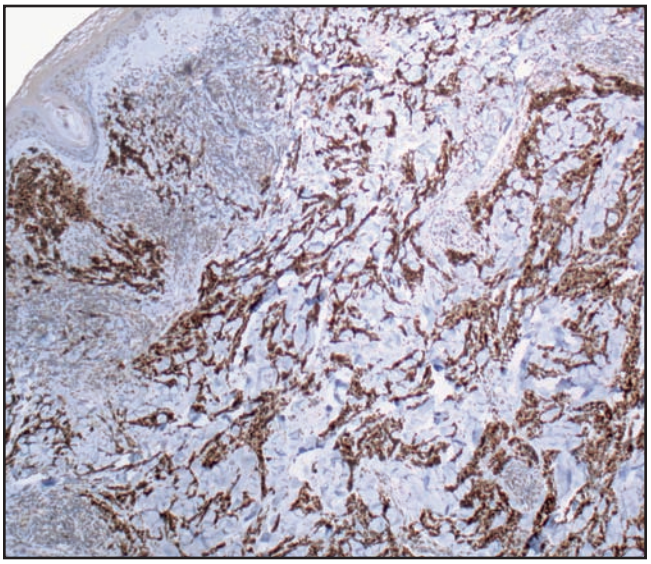

Image 2 Terminal deoxynucleotidyl transferase immunostain in Merkel cell carcinoma showing positivity in more than 50% of tumor cells (×10).

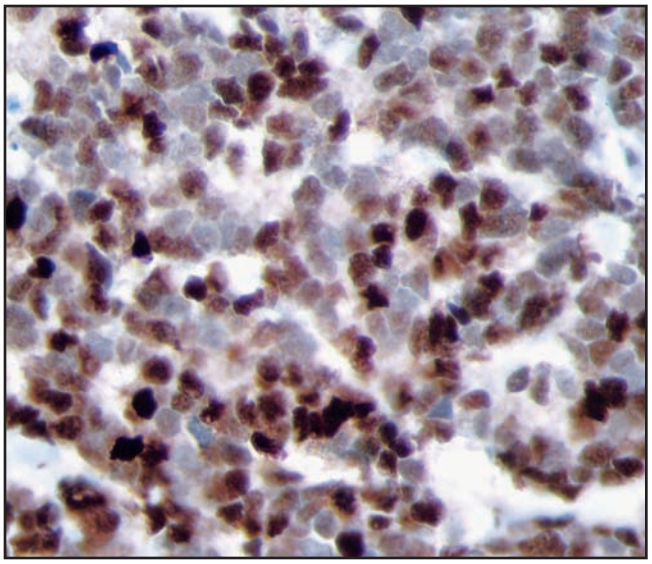

Image 3 Terminal deoxynucleotidyl transferase immunostain in Merkel cell carcinoma showing moderate nuclear staining (×40).

of 40 cases, whereas 11 (28%) of 40 had reactivity in fewer than 25% of tumor cells.

Of 30 SCLC cases, 2 (7%) were positive for TdT **Image 4**. Both cases showed moderate nuclear TdT staining intensity in fewer than 25% of tumor cells (Table 3). Immunohistochemical studies showed no positive staining for TdT in PCTs (0/6).

Thyroid Transcription Factor 1

Of the 40 MCC cases, 1 (3%) was positive for TTF-1. The positive MCC case showed weak TTF-1 nuclear staining intensity, with fewer than 25% of tumor cells reactive (Table 4). Moderate to strong nuclear staining for TTF-1 was seen in 23 (77%) of 30 SCLC cases and 4 (67%) of 6 PCT cases (Table 3). More than 50% of tumor cells were reactive in all cases.

Cytokeratins 7 and 20

Of 40 MCC cases, 2 (5%) were positive for CK7, with moderate cytoplasmic staining in 25% to 50% of tumor cells. In 35 (88%) of 40 MCC cases, there was positivity for CK20 with typical paranuclear dot-like and membranous positivity **Image 5**. More than 50% of tumor cells were positive, and the staining intensity was strong for CK20 in all positive MCC cases (Tables 2 and 4).

Of 30 SCLC cases, 22 (73%) were positive for CK7, showing strong cytoplasmic staining intensity and reactivity in more than 50% of tumor cells. None of the SCLC or PCT cases was positive for CK20. Only 1 (17%) of 6 PCTs was positive for CK7. Staining for CK7 was cytoplasmic and moderate in intensity, with 25% to 50% of tumor cells reactive (Tables 3 and 4).

Chromogranin and Synaptophysin

Of the 40 MCC cases, 29 (73%) and 39 (98%) were positive for chromogranin and synaptophysin, respectively (Table 4). Staining for chromogranin was positive in 16 (53%) of 30 SCLC cases and 5 (83%) of 6 PCT cases.

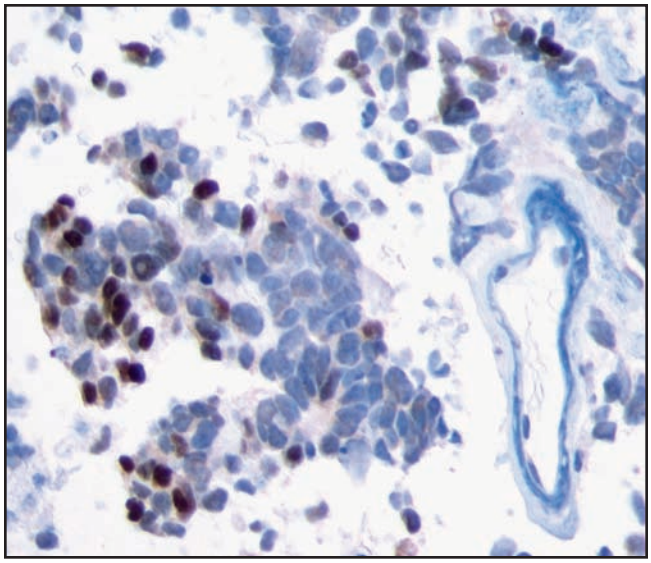

Image 4 Terminal deoxynucleotidyl transferase immunostain in small cell lung carcinoma showing moderate nuclear staining in fewer than 25% of tumor cells (×40).

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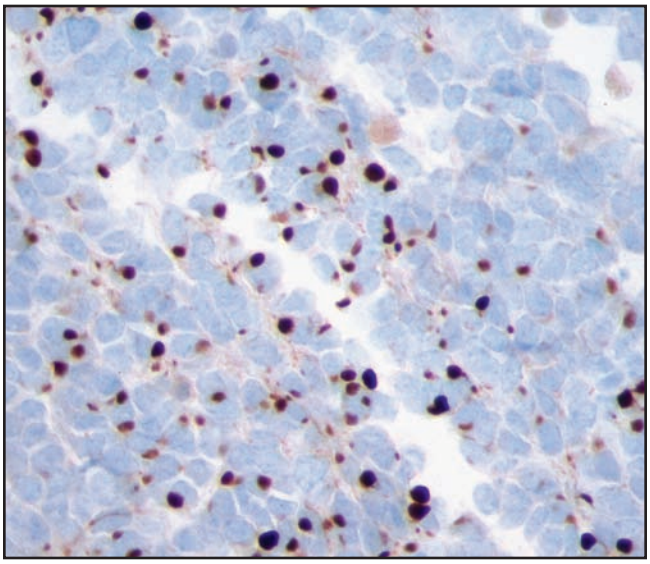

Image 5 Immunostain for cytokeratin 20 in Merkel cell carcinoma showing typical paranuclear dot-like positivity (×40).

Staining for synaptophysin was positive in 22 (73%) of 30 SCLC cases and all PCTs (Tables 3 and 4). The chromogranin and synaptophysin markers showed strong cytoplasmic staining intensity with more than 50% of tumor cells positive in all MCC, SCLC, and PCT positive cases.

Discussion

MCC is an uncommon skin tumor that tends to affect the elderly population, with a reported tripling in its incidence during the last 20 years.[1,2] MCCs have been associated with sun damage and immunosuppression and are aggressive tumors with a poor prognosis.[3-5] Diagnosis of MCCs can be difficult because many other tumors can appear histologically similar. MCCs are composed of small round blue cells forming sheets with hyperchromatic nuclei, inconspicuous nucleoli, and minimal cytoplasm. The differential diagnosis includes other small round blue cell tumors, such as basal cell carcinoma, lymphoma, melanoma, cutaneous Ewing sarcoma, and metastatic SCLC. Numerous panels of immunohistochemical stains are essential in excluding these MCC imitators; however, SCLC is much more difficult because both tumors have some overlapping immunohistochemical and histologic features.[9-16] Furthermore, similar to MCCs, SCLCs are very aggressive neuroendocrine cancers with a high mortality. Numerous studies have demonstrated that MCCs and SCLCs show comparable genetic alterations.[22-24] Differentiating MCC from SCLC is difficult but crucial for appropriate patient management and prognosis.

Typically, MCCs are positive for CK20 and negative for TTF-1, whereas SCLCs are positive usually for CK7 and TTF-1.[10-15,25-27] However, many exceptions exist, and these patterns are often variable with overlap between MCCs and SCLCs.[13] Up to 20% of SCLCs may be negative for TTF-1, and TTF-1 usually does not stain MCCs, but rare cases have been reported.[28] Moreover, many reports have demonstrated that SCLCs can be positive for CK20 and some MCCs may be positive for CK7, in addition to CK20.[29,30] A few markers can help clear this confusion. One such marker is neurofilament.[31] Neurofilament is positive in MCCs but is consistently negative in SCLCs. Unfortunately, neurofilament is not always expressed in MCCs.

Recently, Feng et al[28] revealed that a novel polyomavirus, Merkel cell polyomavirus (MCV), is clonally integrated into the genome of MCCs. The role of MCV in the pathogenesis of MCC is unknown. Many groups have studied MCV as a specific marker for MCC. Two recent studies identified MCV as a specific marker for MCCs in histologically similar tumors, including SCLCs. Through a polymerase chain reaction–based approach, Duncavage et al[32] demonstrated that MCV DNA was absent in 32 pulmonary neuroendocrine carcinomas tested. A study by Busam et al[33] used a specific antibody (CM2B4) to a predicted antigenic epitope on the MCV T antigen and showed that all pulmonary neuroendocrine carcinomas (0/26) failed to react, whereas the majority of MCCs (10/15) demonstrated positive staining. In contrast, through the use of a polymerase chain reaction specific for MCV, Helmbold et al[34] demonstrated the presence of MCV in 7 (39%) of 18 SCLCs.

TdT is a nuclear protein that seems to be expressed in a large proportion of MCCs. The results of our study on a relatively large number of MCC cases are similar to previous observations regarding the frequent expression and nuclear reactivity of TdT in MCC.[21,22] In the present study, 70% of MCC cases exhibited TdT positivity, with most cases demonstrating more than 25% of tumor cells reactive for TdT with moderate to strong nuclear intensity. It is interesting that 2 of the 30 SCLCs demonstrated TdT positivity, with fewer than 25% of tumor cells reactive and with moderate staining intensity. We further confirm the variable and often overlapping immunohistochemical results between MCCs and SCLCs because all markers, including TdT but with the exception of CK20, were positive in both tumors. In 1 MCC case (3%), there was positivity for TTF-1, and 2 cases (5%) were also positive for CK7, while 77% of SCLCs were positive for TTF-1 and 73% of SCLCs were also positive for CK7. Synaptophysin and chromogranin were commonly expressed in both MCC and SCLC.

CK20 is considered to be the most specific and sensitive marker for MCC. The majority of MCC cases in our study were positive for CK20 (35/40 [88%]), demonstrating typical paranuclear dot-like and membranous positivity. Although

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CK20 has been reported to be occasionally positive in SCLCs,[30] in our study, all 30 cases were negative. It is interesting that of the 5 MCC cases that were negative for CK20, 4 (80%) were positive for TdT. All 4 CK20–/TdT+ cases demonstrated moderate TdT nuclear staining intensity, with more than 25% of tumor cells reactive.

Although histologic and clinical correlation is important, TdT may be used as a tertiary stain in the diagnosis of MCC and to assist in its distinction from metastatic SCLC. The use of 3 markers, CK20, TTF-1, and TdT, in the differential diagnosis and in all suspected cases of MCC is a far more powerful and assuring tool than relying on 2 antibodies, of which only 1 antibody result would commonly be present in either case. We also have demonstrated that TdT may be beneficial in rare cases of CK20– MCC. Moreover, whether TdT is present in other small cell carcinomas arising from other sites is yet to be determined.

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