

MERKEL CELL CARCINOMA VERSUS METASTATIC SMALL CELL PRIMARY BRONCHOGENIC CARCINOMA

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Abstract

Introduction: Merkel cell carcinoma (MCC) of the skin is a rare, aggressive, malignant neuroendocrine neoplasm. The tumor classically demonstrates positive immunohistochemistry (IHC) staining for chromogranin A (ChrA), cytokeratin 20 (CK20), neuron specific enolase (NSE) and/or achaete-acute complex-like 1 (MASH1). The newly identified Merkel cell polyomavirus (MCPyV) has been found to be associated with most MCC cases. The primary histologic differential diagnoses of cutaneous MCC is small cell primary bronchogenic carcinoma (SCLC); moreover, both are of neuroendocrine origin. SCLC accounts for approximately 10-15% of all primary lung cancer cases; this histologic subtype is a distinct entity with biological and oncological features distinct from non-small cell lung cancer (NSCLC). In contradistinction to MCC, SCLC is classically IHC positive for cytokeratin 7 (CK7) and transcription factor (TTF-1). Similar to SCLC, MCC cell lines may be classified into two different biochemical subgroups designated as Classic and Variant.

In our review and case report, we aim to emphasize the importance of a multidisciplinary approach to the approach to this difficult differential diagnosis. We also aim to comment about features of the cells of origin of MCC and SCLC; to summarize the microscopic features of both tumors; and to review their respective epidemiologic, clinical, prognostic and treatment features. We want to emphasize the initial workup study of the differential diagnosis patient, including evaluating clinical lymph nodes, a clinical history of any respiratory abnormality, and chest radiogram. If a diagnosis of primary cutaneous MCC is confirmed, classic treatment includes excision of the primary tumor with wide margins, excision of a sentinel lymph node, and computed tomography, positron emission tomography and/or Fluorine-18-fluorodeoxyglucose positron emission tomography scan studies.

Key words: Merkel cell carcinoma; thyroid transcription factor; somatostatin; IMP3; small cell lung carcinoma (SCLC)

Abbreviations and acronyms: Merkel cell carcinoma (MCC), thyroid transcription factor 1 (TTF-1), immunohistochemistry (IHC), neuron specific enolase (NSE), small cell lung carcinoma (SCLC), chromogranin A (CgA), insulin-like growth factor II mRNA binding protein 3 (IMP3), neurofilament protein (NF), cytokeratin 7 (CK7), cytokeratin 20 (CK20), surveillance, epidemiology, and end results (SEER), non-small cell lung cancer (NSCLC), computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI), Merkel cell polyoma virus (MCPyV).

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Introduction

Merkel cell carcinoma (MCC) of the skin is an aggressive but rare malignant neuroendocrine tumor. This tumor has a high rate of recurrence and metastasis the main histopathological differential diagnosis is cell lung carcinoma (SCLC). Most MCC are usually positive to cytokeratin 20 (CK20) stain as well as may show some neuroendocrine differentiation, expressing either neuron specific enolase

(NSE), chromogranin A, or both [1-2]. MCC small-cell lung carcinoma (SCLC), and both are of neuroendocrine origin. Comparable to SCLC, MCC cell lines are classified into two different biochemical subgroups designated as 'Classic' and 'Variant' [3]. MCC mostly affects elderly people and occurs predominantly on the sun-exposed areas of the skin, suggesting ultraviolet exposure (UV) exposure in its etiology and need to be differentiating with melanoma [5-7].

Merkel cells

Merkel cells are neuroendocrine in origin, expressing markers such as neuron-specific enolase (NSE) and bombesin. These cells are located in the basement membrane zone (BMZ) at the dermal epidermal junction, where they function as mechanoreceptors [8-11]. Ken Hashimoto, M.D., emeritus professor at Wayne State University in Detroit, Michigan, USA made great contributions to our understanding of Merkel cells. Dr Hashimoto showed that the Merkel cells could readily be identified via electron microscopy because of their characteristic round, dense cytoplasmic granules, their association with Schwann cells, and their smooth periphery surrounded by a basal lamina [8-11]. Dr Hashimoto further showed that Merkel cells were distributed in the nail matrix, nail bed and the skin of the fingers, including hairless portions. In primitive mesenchyme, the Merkel cells were surrounded by a sheath of Schwann cells or cells similar to Schwann cells. Non-myelinated terminals of axons (neurites) within such Schwann or Schwann-like cells were often in contact with the Merkel cells [11]. Dr Hashimoto also described dense cytoplasmic granules surrounded by a unit membrane, titled Merkel cell granules; these granules were found in variable numbers, and tended to concentrate toward one side of the cell cytoplasm. In selected sections, well developed areas of the Golgi apparatus were noted [8-11]. From the periphery of the Merkel cell, spine-like processes projected into surrounding Schwann cells. Very fine filaments (2-4 nm) originating from the plasma membrane formed a bundle and filled each spine. The proximal end of the bundle fanned out into the peripheral cytoplasm at the root of the spine. Schwann cell-Merkel cell junctions often produced desmosome-like plasma membrane thickenings on both cells [8-11]. The Merkel cells tended to cluster in certain areas of the epidermis, usually in the skin basement membrane zone. Clear cells were also noted among the grouped Merkel cells, which did not contain typical Merkel cell granules [8-11]. The identity of these cells was not certain. The epidermal Merkel cells were connected to the adjacent keratinocytes via desmosome-like mesenchymal Merkel cells; they projected little spines into the surrounding keratinocytes. These spines contained bundles of fine filaments, comparable to those seen in traditional mesenchymal Merkel cells. When two Merkel cells came into contact, desmosome-like densities were developed on the plasma membrane of every apposed cell. The distribution of the Merkel cell granules was polarized in the direction of adjacent basal lamina, and the Golgi apparatus was typically found near the nucleus in the opposite side cytoplasm of the cell [8-11]. Neurites, if found, were present outside the basal lamina and adjacent to the accumulated granules [8-11]. The neurites could surround the basal and lateral borders of the Merkel cell, but in these preparations no specialized junctional structures were detected between the Merkel cell and the neurites. Most Merkel cells were not in direct contact with the basal lamina to any significant extent; in contrast, they were contacted either by neurites or processes of basal cells [8-11]. In exceptional instances, a large area of the basal surface of the Merkel cell was exposed to the basal lamina. In such an area, half desmosome-like structures were present both in the basal plasma membrane of the Merkel cell and the basal lamina itself. Anchoring fibrils were attached to the dense areas of the basal lamina. In the skin of the finger, eccrine glands were descending from

the epidermis. Within the mass of epithelial cells forming the eccrine gland anlagen, Merkel cells were frequently observed [8-11].

Organelles of Merkel cells

Merkel cell granules are apparently packaged in Golgi body-derived small vesicles [8-11]. Between the unit membrane and the dense core substance of each granule, an electron-lucid halo could be appreciated. Moreover, granules with a similar halo were often seen in the Golgi body area and probably represented immature granules. As the dense core substance filled the vesicles, they moved toward one side of the cell, frequently the opposing side relative to the Golgi body [8-11]. In many mature granules, additional spaces delimited by unit membrane were also completely filled with the dense substance. The electron density of each granule varied, and the size of each granule also varied (80-200 nm); however, the granule density and size did not correlate. Multivesiculated bodies, and large dense bodies with delimiting unit membranes (compatible with lysosomes) were also found, numbering a few in each cell [8-11]. Some lysosomal dense bodies contained half-digested melanosomes, which in turn appeared comparable to large melanosomal complexes of keratinocytes. Melanosomes were also seen in isolation within some epidermal Merkel cells. Interestingly, some epidermal Merkel cell cytoplasmic vacuoles also contained what appeared to be Merkel cell granules. Such vacuoles were thus interpreted as autophagosomes [8-11]. In some cells, bundles of tonofilaments separated Merkel cell granules into groups. Free ribosomes were less numerous in Merkel cells than in keratinocytes. In some Merkel cells, glycogen particles were seen [8-11]. The Merkel cell nucleus was slightly indented, but not significantly compared to other types of cells. Within the Merkel cells, a nucleolus was often absent. Finally, filament-filled, short peripheral spines on the Merkel cells were noted, and likely represent a unique structure for Merkel cells. No similar organelle has been found in keratinocytes, melanocytes or Langerhans cells; dendrites of these cells lack a filamentous core structure, and are long and slender. The functional significance of the Merkel cell spines is not clear; they may 1) assist cellular locomotion via flagella-like movement, 2) assist in cellular stability by engaging with neighboring keratinocytes, or 3) represent a defensive, pressure sensitive extension of the cell when Merkel cells are mechanically constricted between surrounding keratinocytes [8-11].

Possible neuroectodermic origin of Merkel cells

Dr. Hashimoto's studies also suggest that the Merkel cells originate from the neuroectoderm, next migrate into the skin with the growth of peripheral nerves, and finally settle into the basal keratinocytic layer of the epidermis [9-11]. The 1) large number of mesenchymal Merkel cells observed, as well as 2) the rarity of dermal Merkel cells in adult human skin seem to support this concept [12]. The Merkel cell migration hypothesis was previously proposed by Breathnach & Robins; moreover, Breathnach, Lyne and Hollis reported that in very young sheep embryo skin, no Merkel cells were found in the epidermis. In more mature embryo skins (57-144 days gestation) Merkel cells were identified in the epidermis [13,14].

Both melanocyte and chromaffin cells originate from the neural crest; the melanocyte finally settles in the epidermis. However, it is not clear whether all mesenchymal Merkel cells eventually migrate to the epidermis; some may remain intact in the postnatal dermis, and some Merkel cell granules may be autophagocytosed in the dermis and/or epidermis [9-11]. The absence of Merkel cells in adult eccrine glands and hair follicles suggests that such autodigestion of granules may actually occur at a certain stage of fetal skin development, as the same mechanism has been suggested for the disappearance of melanocytes from selected areas of the human hair outer root sheath [8-11]. Dr Hashimoto suggested that the epidermal entry of Merkel cells could occur in a consistent sequence. First, the Merkel cell would be stripped of its Schwann sheath, in its superior portion at the mesenchymal-epidermal tissue junction. Next, the Merkel cell would penetrate the epidermal basal lamina, and make contact with basal layer keratinocytes. Desmosomes would then be formed between the Merkel cells and keratinocytes. The interrupted basal lamina would then establish continuity with the basal lamina of the Schwann cell that accompanied the Merkel cell to the point of epidermal entry. As the epidermal basal keratinocytes migrate upward within the epidermis, Merkel cells connected to them would be pulled up into the epidermal basal layer [8-11]. Neurites would, in turn, follow each Merkel cell. The counterbalanced kinetic forces between such upward traction and opposing forces provided by both 1) desmosomal junctions between the Merkel cells and adjoined basal layer keratinocytes and 2) half-desmosomes with the basal lamina would determine the final anatomic position of each Merkel cell [8-11]. Notably, when nerve endings of a sensory nerve attempt to enter the epidermis as free nerve endings, they also shed their Schwann sheath, and the epithelial basal lamina fuses with that of the Schwann cell [15]. It has also been noted that if the Merkel cell granules were equivalent to synaptic vesicles of cholinergic pre-synapses, one would expect to see a discharge of some of the granules into the extracellular space; however, this phenomenon has not been documented. Moreover, no specialized junctions such as synaptic complexes have been found between Merkel cells and adjacent neurites. The 1) absence of plasma membrane fusion of the Merkel cell granule and 2) absence of specialized junctions between Merkel cells and intra-epidermal sensory nerve endings has been confirmed in adult skin and mucous membrane Merkel cells [9,14]. On the other hand, the concentration of the granules in the cytoplasm in apposition to adjacent neurites takes place as soon as the Merkel cell enters the epidermis, suggesting that these granules are indeed a source of stimuli for the neurites themselves [9].

Epidemiology of MCC

MCC is a rare cutaneous neoplasm. Studies revealed an increase in incidence from 0.15 to 0.44 cases for every 100,000 inhabitants between 1986 and 2001. Around 50% of the patients eventually develop metastatic disease, predilecting the liver, bones and brain [16-17]. The etiology of MCC is still unknown. In a recent large Danish study, it was also reported that the incidence of MCC is rising [18]. The authors reviewed the medical records of 51 patients diagnosed with MCC from 1995 until 2006 in eastern Denmark. The nationwide incidence of MCC was also

determined from the Danish Cancer Registry for the period 1986-2003 [18]. The authors found that 14/51 of the MCC patients developed recurrence, and 37/51 (73%) died during the study period. The mean clinical follow-up period was 13 months (range 1-122) [18]. Moreover, a group of 153 total 1986-2003 MCC patients were identified in the Danish Cancer Registry, and the incidence rate had increased 5.4 fold over this 18 year period. The prevalence was highest in people over age 65; the authors suggested treatment with curative intent should include excision of the primary tumor with wide margins, excision of a sentinel lymph node (SLN), computed tomography (CT) or positron emission tomography (PET) of the thorax and abdomen, and adjuvant radiotherapy to the surgical bed. The authors also recommended that in the case of advanced disease, systemic palliative chemotherapy should remain an option [18].

Etiology of MCC

The skin of the head and neck is a common site for MCC, classically presenting in fair complected, elderly patients [3]. Radical surgical excision with pathological verification of complete removal of the tumor is the recommended treatment. Early MCC can be cured by surgery with or without postoperative radiation therapy, whereas advanced MCC is currently considered to be incurable. In 2008, a new polyoma virus sequence was detected in the genome of MCC tumors [18,19]. Merkel cell polyoma virus (MCPyV) appears to be the first example of a human oncogenic polyoma virus. Specific mutations in the viral genome and its clonal integration to the tumour genome represent strong evidence against MCPyV being a passenger virus that secondarily infects MCC tumors. MCPyV genomes are clonally integrated into tumor tissue in approximately 85% of all MCC cases [18]. All integrated viral genomes recovered from MCC tissue or MCC cell lines harbor signature mutations in the early gene transcript encoding for the large T-Antigen (LT-Ag). These mutations selectively abrogate the ability of LT-Ag to support viral replication while still maintaining its Rb-binding activity, suggesting a continuous requirement for LT-Ag mediated cell cycle deregulation during MCC pathogenesis [19]. The growing incidence and recognition of MCC in elderly and/or immunosuppressed individuals suggests that these two factors (advanced age and immunosuppression) represent likely links to the etiology of MCC [20,21].

Clinical features, demographics and survival rates of MCC

MCC characteristically develops rapidly and asymptotically over months. Most MCCs are located on sun-exposed areas. The clinician needs to be aware of the asymptomatic, nontender, rapidly expanding nature of these tumors, especially in patients 1) over fifty years of age with 2) a lesion in sun-exposed area and 3) any past history of immunosuppressive therapy. About 50% of MCCs present on the head and neck; 40% present on the extremities and the remainder on the trunk and genitalia. MCC rarely arises on sun-protected areas such as the oral and genital mucosae; in these cases, the tumor is characterized by a particularly poor prognosis. It usually presents as solitary, firm, flesh-colored to red nodule with a smooth, shiny surface, and occasionally with telangiectasias.

MCC is an aggressive neoplasm. Its 5-year disease-specific survival rate approximates 60% [22]. Although MCC is still regarded as a rare tumor entity, its incidence is significantly increasing. In this regard, the American Cancer Society estimated almost 1500 new cases in the United States in 2008 [23]. The newly identified MCPyV has been found associated with most MCC cases. Nevertheless, the precise molecular pathogenesis of MCC and its link to MCPyV is not yet fully understood. In a large study, one group of authors performed a surveillance study utilizing data from the U.S. National Cancer Institute SEER (Surveillance, Epidemiology, and End Results) Program from 1973 to 2006. The authors analyzed the demographics and survival characteristics of MCC [23]. The authors reported that SEER had documented 3870 new cases of MCC during this period. The incidence was higher in men (2380 cases, 61.5%) than in women (1490 cases, 38.5%). Most patients were Caucasian (94.9%) between 60 and 85 years of age. MCC was rare in African Americans. The most common clinical site of presentation was the head and neck [23]. The salivary glands, nasal cavity, lip, lymph nodes, vulva, vagina and esophagus were the most common extracutaneous sites. The 10-year relative survival rate was higher in women than men (64.8% vs. 50.5%, $p < 0.001$). Patients 50-69 years had the highest 10-year relative survival rate (59.6%). Stage of disease was the best predictor of survival [23]. The authors reported that MCC arises predominantly in the skin of head and neck, in Caucasian men over 70 years of age. Age did not predict survival; however, gender, anatomic site and tumor size revealed clear differences [23].

Histopathology of MCC

MCC usually appears as a dermal tumor nodule, that frequently also invades the subcutaneous adipose tissue. Under hematoxylin and eosin (H&E) examination, the tumor cells are small, round cells with basophilic nuclei and minimal cytoplasm (Fig. 1). Mitoses are frequent, and apoptotic tumor cells are frequently present. The papillary dermis and adnexa are often spared. Three histologic subtypes of MCC have been recognized: 1) the small cell variant, histologically indistinguishable from bronchogenic small cell carcinoma, 2) the intermediate variant, featuring vesicular, basophilic nuclei with prominent nucleoli and high mitotic activity, and 3) the trabecular variant, featuring cords of tumor cells. Overall, uniform, poorly cohesive cells containing cytoplasmic argyrophilic granules and round to oval nuclei with indented cell membranes are common histologic features of MCC. In the latest data, the trabecular form is considered the best differentiated with a better prognosis, while the small cell form is considered relatively undifferentiated with a worse prognosis. However, comprehensive data are not available and mixed and transitional histologic forms are frequently encountered; thus, no definitive histologic-prognostic association exists. A tumor size ≤ 2 cm, female gender, primary tumor localized in the upper limb, and pathologically proven negative lymph nodes are factors highly significant for a favorable prognosis and have been incorporated into the new staging system for MCC [25-27].

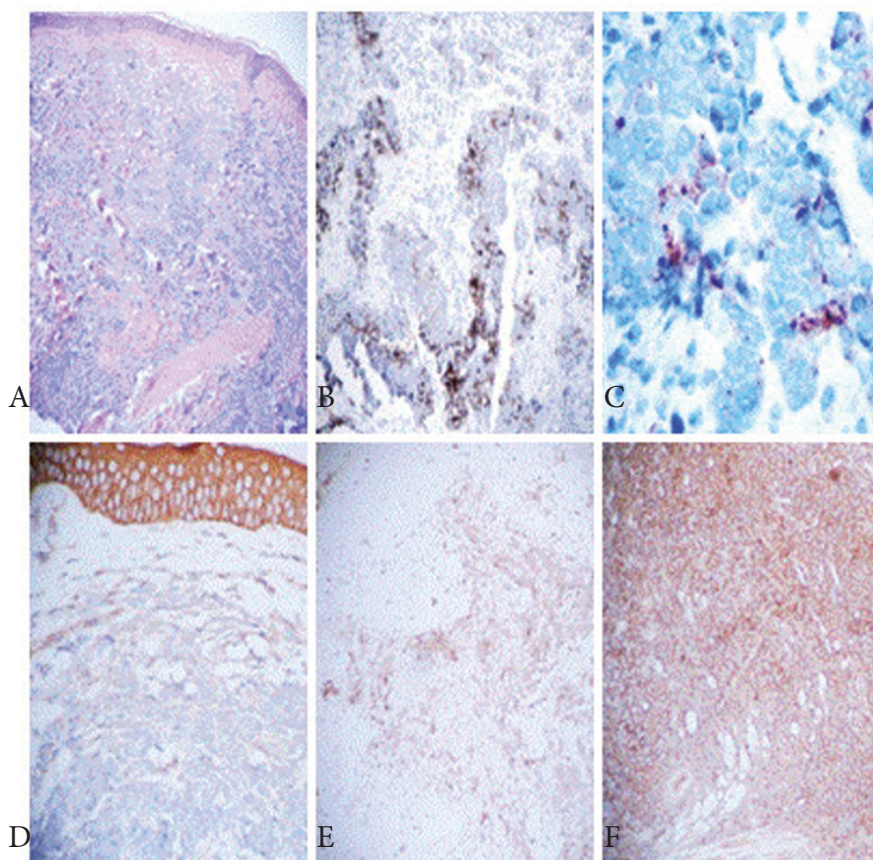


Figure 1. a. H&E staining reveals cords and sheets of dark blue tumor cells, infiltrating the dermis. b. Focal areas of the tumor demonstrated positive IHC staining for Ber-EP4 (red arrow, brown staining). c. Only focal areas of the tumor were paranuclear dot IHC positive for CK20 (red arrow, brown staining). d. IHC IMP3 positive staining in the epidermis above the tumor, and in focal areas of the tumor (red arrows, brown staining). e. Focal tumoral IHC positive staining for somatostatin (red arrow, brown staining). f. Diffusely positive IHC staining on the tumor cells for Chromogranin A (red arrow, brown staining).

Differential diagnosis of MCC

The histologic differential diagnosis of MCC includes SCLC, basal cell carcinoma, amelanotic malignant melanoma, malignant lymphoma, carcinoid tumor and atypical fibroxanthoma [24, 29].

Efficacy in identifying microscopically positive SLNs and radiologic imaging

Sentinel lymph node biopsy (SLN) enables the identification of occult nodal metastases; it is believed that up to 20% of primary MCCs have metastases to proximal lymph node chains [29,30]. A recent study in patients with MCC demonstrated significant positive (8/16) and false negative (8/16) results in SLN. Thus, the authors concluded that given the high rate of SLN positivity, SLN should play a role in the management of MCC. Given the risk of false negative SLN, close observation of regional nodal basins is warranted in patients who have presented a negative SLN. Additional studies are required to investigate the impact of SLN on survival. The role of lymphoscintigraphy in MCC also warrants further studies [30]. Another large study assessed the use of SLN in conjunctival and eyelid MCC tumor patients, and addressed SLN in therapeutic management as recommended by a multidisciplinary consensus committee [31]. The authors performed a single center, prospective, nonrandomized clinical study between January 2008 and January 2010. Seventeen patients were included: 4 (2 conjunctival and 2 eyelid) melanomas, 4 eyelid MCCs, 8 (2 conjunctival, 2 eyelid, 2 eyelid/conjunctival, and 2 corneal/conjunctival) squamous cell carcinomas, and 1 eyelid meibomian gland carcinoma. Preoperative lymphoscintigraphy was performed one day before surgery to label lymph node(s) [31]. A surgical biopsy was then performed along with an extemporaneous pathological examination; these procedures were followed by a secondary complete lymph node dissection, performed only in instances of positive histology. The authors found that in all cases, one or more SLN were identified (3-13). Two biopsies (1 MCC and 1 squamous cell carcinoma) revealed neoplastic invasion of the SLN, and led to complete cervical node dissection. Adjunct regional treatment was indicated for 1 melanoma, 4 MCCs, and 2 squamous cell carcinomas. One false negative result was noted in the group of squamous cell carcinomas after 6 months, and it was treated. No relapse or death events were observed for the remaining 16 patients. The mean overall follow-up period was 18.2 months [30]. The authors concluded that as in previous studies, SLN biopsy for eyelid and conjunctival tumors is both safe and effective in identifying microscopically positive SLNs. The SLN procedure may also revive interest in the study of cervicofacial lymphatic drainage. The investigation was to be expanded and extended to other medical teams [31]. The study was of special interest in MCC since the majority of MCC cases present in the head or neck. In another study of 240 patients with primary MCC evaluated between 1981 and 2008, 99 had diagnostic imaging at initial presentation with biopsy proven primary cutaneous MCC, and had histopathologic nodal evaluation within 4 weeks of the initial scan [32]. The authors showed that computed tomography (n= 69) demonstrated a sensitivity of 47%, a specificity of 97%, a positive predictive value of 94%, and a negative predictive

value of 68% in detecting nodal basin involvement. Fluorine-18-fluorodeoxyglucose positron emission tomography scanning (n= 33) demonstrated a sensitivity of 83%, a specificity of 95%, a positive predictive value of 91%, and a negative predictive value of 91% in detecting nodal basin involvement. [32].

Immunohistochemical (IHC) staining on MCCs

The “small round blue cell” histologic pattern of MCC must be differentiated from several other tumors, such as small cell bronchogenic carcinoma, carcinoid tumor, malignant lymphoma, and small cell malignant melanoma. Therefore, immunohistochemical (IHC) stains are required to confirm the diagnosis. MCCs are positive for selected epithelial and neuroendocrine markers, but are negative for hematolymphoid and melanocytic markers [33-47]. Table I shows characteristic IHC staining patterns for these entities. Positive staining for anti-Cytokeratin 20 (CK20) and neuron specific enolase (NSE) are quite specific for MCC. CK20 staining usually shows a paranuclear dot-like pattern, present in 97% of all MCCs stained with this antibody. This highly sensitive staining feature is very important in histopathologic analysis, to distinguish MCCs from other small round blue cell tumors. Thyroid transcription factor-1 (TTF-1) is usually expressed in small-cell bronchogenic carcinoma, but is consistently absent in MCC. TTF-1 belongs to a family of homeodomain transcription factors, and is selectively expressed in thyroid, lung and diencephalon derived tumors. TTF-1 has been further identified as a transcriptional regulator of thyroid-specific genes. Leukocyte common antigen/CD45 (LCA) is negative in MCC, but classically positive in malignant lymphoma [32-47]. SCLC is usually Cytokeratin 7(CK7) positive, but this marker is negative in MCC. Neurofilament protein (NFP) is usually positive in MCC, and consistently negative in SCLC. The differentiation between MCC and malignant melanoma is based on the negativity of the latter for CK 20 and its positivity for S-100 and HMB-45; MCC is classically negative for these markers[32-47]. Further, the tumor cells of MCC display additional antigens in varying frequency and intensity; these include Chromogranin A (CgA), synaptophysin, tenascin-C and CD56/NCAM. Finally, achaete-scute complex-like 1 (MASH1) is important in the development of the brain and the neuroendocrine system including pulmonary neuroendocrine cells. A recent study using a cDNA array identified MASH1 as one of the best gene markers to differentiate SCLC from MCC [46].

Treatment of MCCs

A wide local removal of the tumor is required, as well as removal of any positive and/or suspected lymph nodes in proximity to the MCC. MCC is a radiosensitive tumor. Radiotherapy has an important role in its treatment; chemotherapy may also be utilized in the treatment of MCC [48]. Even following aggressive surgical and radiation treatment MCC has a high rate of locoregional recurrence, including in early stage disease [50]. Recently, guidelines for the diagnosis and treatment of MCC were reported by the Cutaneous Oncology Group of the French Society of Dermatology [50]

Tumor	CK20	CK7	NSE	NF	TTF-1	S100	MASH1	LCA/ CD45	Mart-1/ Melan A	CgA
MCC	+	–	+	+	–	–	+	–	–	+
SCLC	–	+	+	–	+	–	–	–	–	+
Melanoma	–	–	–	–	–	+	–	–	+	–
Lymphoma				–	–		–	+	–	–

Table I. Comparison of immunohistochemistry staining in MCC with that of histologic differential diagnoses

Metastasis of MCCs

In addition to classic sites such as lymph nodes, liver, bone and brain, MCC can also metastasize to sites such the leptomeninges, intraspinal areas (epidural and intradural), pancreas, small bowel mesentery, gingiva, kidneys and other sites [51-55].

Case Report

A 76 year old female presented for a routine dermatologic examination; an asymptomatic, erythematous papule was observed on the left back. The patient's medications included potassium chloride, fexofenadine, Janumet® for diabetes mellitus, meloxicam for osteoarthritis, Cozaar®, miodipine, metoprolol for high blood pressure and Crestor® for high cholesterol. The patient's past medical history included arthritis, skin cancer, type 2 diabetes and elevated blood pressure. Her surgical history included a hysterectomy in 1980, removal of an ovarian cyst in 1984, knee surgery in 1993 and rotator cuff surgery in 2003. The patient's mother had a clinical history of melanoma. A skin biopsy of the erythematous papule was obtained; hematoxylin and eosin (H&E) staining and immunohistochemistry staining was performed.

Methods

Our H & E staining and IHC studies were performed as previously reported [56-60]. For IHC, we utilized the following antibodies: monoclonal mouse anti-human TTF-1, insulin-like growth factor II mRNA binding protein 3 (IMP3), ribosomal protein S6-pS240/phosphorylation site specific, serotonin, survivin, synaptophysin, bromodeoxyuridine, tissue inhibitor of metalloproteinases 1 (TIMP-1), CK20, NSE, neurofilament protein, cyclo-oxygenase enzyme 2 (COX-2), Cyclin D1, CD56/NCAM, Chromogranin A (CgA), calretinin, epithelial membrane antigen (EMA/CD227), Ber-EP4, pancytokeratin (clone AE1/AE3), proliferating cell nuclear antigen (PCNA), MIC2 and Ewing's sarcoma marker (CD99). We also utilized a polyclonal rabbit anti-human antibody to somatostatin. All antibodies were obtained from Dako (Carpinteria, California, USA). Our study was IRB exempt because we utilized archival samples without specific patient identifiers.

Results

Microscopic examination of the H&E sections demonstrated a malignant neoplasm comprised of small round blue cells infiltrating through the dermal collagen and subcutaneous adipose tissues. Specifically, no epidermal

involvement was observed. The tumor was present as trabecular cords, and nests; areas of crush artifact and zonal necrosis were noted. However, no Azzopardi phenomenon was appreciated within the tumor. Individual tumor cells displayed round, regular nuclei with fine, dispersed chromatin, indistinct chromocenters and minimal amphophilic cytoplasm. Frequent tumor cell mitotic figures were seen, and numerous apoptotic cells are also observed within the tumor. The neoplastic process extended to the deep specimen borders in the sections examined. On IHC analysis, the tumor cells also displayed focally positive, membranous and cytoplasmic staining with the CgA special stain. Minimal „paranuclear dot” cytoplasmic staining was noted on review of the CK20 special stain. The following stains were completely or predominantly negative on the tumor cells: ribosomal S6-pS240, COX-2, Cyclin D1, neurofilament, calretinin, synaptophysin, CD99, TIMP1, serotonin and NSE. The following stains were positive in punctate, focal tumoral areas: CD56/NCAM, EMA/CD227, survivin, bromodeoxyuridine, Ber-EP4, PCNA, TTF-1, IMP3, Cytokeratin AE1/AE3 and somatostatin.

Discussion

Our case demonstrates selected IHC positive staining favoring a diagnosis of metastatic primary bronchogenic small cell carcinoma, including positivity to TTF-1 and IMP3; in addition, we noted minimal tumoral staining to somatostatin, CK20, NF and NSE. Multiple authors have demonstrated that differential IHC staining may assist in distinguishing MCC and SCLC. Previously documented differential IHC staining in this context includes CK7, CK20, NSE, CgA, synaptophysin, NF, TTF-1, CD56/NCAM, S-100 protein, vimentin, c-erb B-2 oncoprotein, and CD117/c-kit antigen [32-47]. Thus, our findings demonstrate the complexity of this differential diagnosis workup. The tumoral clinical presentation and H&E findings favored a diagnosis of primary MCC; however, our IHC staining favored a diagnosis of metastatic SCLC, and unfortunately our patient was lost to followup. Interestingly, we found limited tumoral IHC positivity to IMP3 (insulin-like growth factor II mRNA binding protein 3), a 580 amino acid oncofetal RNA binding protein containing four K homology domains. IMP3 is normally expressed in early embryonic tissues, and may also be expressed in a proportion of non-small cell lung carcinomas and pancreatic adenocarcinomas. Further, K homology domain-containing proteins may be overexpressed in high-grade neuroendocrine lung carcinomas and extrapulmonary small cell carcinomas [62-63].

Conclusions

Merkel cell carcinoma is a rare neuroendocrine tumor of the skin. Epidemiological factors strongly associated with this tumor include patient age over 65 years, fair complected skin, chronic sun exposure and immune suppression. The primary differential diagnosis of MCC is metastatic primary bronchogenic small cell carcinoma. Many challenges remain regarding the diagnosis and treatment of MCC. In order to provide clinical practice guidelines for the diagnosis and treatment of MCC, there is a need for further prospective multicenter evaluation its staging and treatment, including updating classifications for TNM staging [64]. Despite aggressive surgical and radiation treatment, MCC has a high rate of locoregional recurrence, even in early stage disease. Thus, SNL biopsy is useful for the staging and management of MCC patients. Finally, further research is needed to identify better clinical prognostic markers for this disorder.

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