

Immunohistochemical evaluation of canine and feline Merkel cell tumors—a report of two cases

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Abstract: Merkel cell tumors, a type of cutaneous round-cell tumors, are rarely observed in dogs and cats. This tumor type originates from cutaneous neuroendocrine cells. In the present study, the morphology of canine and feline Merkel cell tumors and their immunoreexpression of neuron-specific enolase, cytokeratin, chromogranin A, Ki67, and metallothionein were evaluated using histopathological and immunohistochemical methods. The canine Merkel cell tumor displayed a diffuse morphological pattern, whereas the feline tumor displayed a solid morphological pattern. Both tumors were positive for neuron-specific enolase and cytokeratin, but negative for chromogranin A. The Ki67 indexes in the canine and feline tumors were 7% and 25%, respectively. The expression of metallothionein was minimal in both tumors. In conclusion, this paper describes the detailed histopathological and immunohistochemical features of uncommon canine and feline Merkel cell tumors, including metallothionein expression, which has never been studied in such tumors previously.

Key words: Merkel cell tumor, dog, cat, immunohistochemistry

1. Introduction

Merkel cell tumors (MCTs) are a rare neuroendocrine neoplasm. This tumor type arises from Merkel cells of the skin, the origin of which is not fully understood. There are hypotheses that suggest a crest or epidermal origin (1–3). It is hypothesized that these cells have somatosensory, chemosensory, secretory, trophic, or inductive functions in the development of peripheral nerves and epidermal structures (2). Tumors arising from Merkel cells can be benign or malignant. In humans, MCTs are a form of the highly aggressive Merkel cell carcinoma (MCC) and are classified into 3 subtypes: trabecular (classic pattern), intermediate (solid pattern), and small cell (diffuse pattern) (1). MCTs are rarely diagnosed in dogs and cats; therefore, little is known about MCT pathogenesis, breed predilections, and etiology in these species. Canine MCTs are considered to be a benign neoplasm (3,4), whereas feline MCTs are considered to be malignant, similar to their human equivalent (4). The tumor is usually in the form of an intradermal, unencapsulated mass extending into the subcutis. The neoplastic cells are round, resembling other cutaneous round-cell tumors such as plasmacytoma, mast-cell tumors, histiocytoma, transmissible venereal tumors, balloon-cell melanoma, and nonepitheliotropic lymphoma.

Epidermal and adnexal invasions are not observed (3). Due to the nonspecific morphology and rarity of MCTs in dogs and cats, these tumors are often misdiagnosed by routine hematoxylin and eosin (H&E) staining. Therefore, additional immunohistochemical experiments should be conducted to properly diagnose MCTs (3). The neoplastic cells of MCTs in animals and MCC in humans typically express neuroendocrine markers, such as neuron-specific enolase (NSE), chromogranin A (CGA), synaptophysin (SYP), protein gene product 9.5 (PGP 9.5), substance P (SP), vasoactive intestinal polypeptide (VIP), insulin, gastrin, and epithelial marker cytokeratin (AE1/AE3) (3–6). However, CGA expression is occasionally absent in MCC (4).

The metallothioneins (MTs) are a family of cysteine-rich proteins located in the cytoplasm and nucleus during the S phase of the cell cycle (7). The key functions of these proteins are neutralization of free radicals, immunomodulation, and apoptosis inhibition. There are 2 forms of MTs, varying by metal occupancy: metal-free (apo-MT) and metal-bound (holo-MT) (8). Holo-MT has anticarcinogenic activity because it protects DNA from damaging agents, whereas apo-MT is correlated with tumor growth and progression. The interaction of apo-

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MT sulfhydryl groups with p53 leads to p53 inactivation and initiation of neoplastic cell proliferation (9,10). Overexpression of MT in human tumors, and particularly in breast, ovarian, uterine, and prostate carcinomas, is related to high proliferative activity of neoplastic cells and to therapeutic resistance. However, downregulation of MT expression in hepatocellular carcinomas is associated with poor prognosis (11). In certain tumors, i.e. primary pulmonary carcinoma, MT expression is positively correlated with the expression of the cellular proliferation marker Ki67 (12,13). In dogs, the expression of MT has been studied in apocrine gland tumors, mammary gland tumors, melanotic tumors, and primary pulmonary carcinomas. In cats, studies have been limited to melanotic tumors and primary pulmonary carcinomas. The expression of MT in both MCTs and human MCC has not been previously evaluated.

The aim of the present study was to examine the immunoreexpression of NSE, CGA, AE1/AE3, MT, and Ki67 in 2 cases of MCTs (canine and feline).

2. Case history

The tumors in both species presented as an intradermal mass located in the buccal area. The canine tumor was obtained from a 5-year-old intact mongrel bitch, and the feline tumor was obtained from a 13-year-old neutered European shorthair male. Both tumors were surgically excised, sampled, immediately fixed in 10% buffered formalin, and routinely processed. Histological diagnosis was based on H&E and Mallory trichrome staining (staining kits from Bio-Optica, Milan, Italy). Sections destined for immunohistochemistry underwent antigen retrieval before staining. The immunohistochemical examination was performed using primary antibodies, visualization systems, and a chromogen, as summarized in the Table. The slides were counterstained with Mayer's hematoxylin. As positive controls, both canine and

feline tissue sections were processed together with the evaluated slides (NSE–brain cortex; CGA–gastric mucosa; AE1/AE3–skin; MT–mammary gland; Ki67–tonsil). As negative controls, the primary antibody was either replaced with mouse IgG2a (DAKO, Glostrup, Denmark) at the appropriate dilution (for NSE, Ki67, MT, and AE1/AE3) or omitted (for CGA). Brown precipitate was regarded as an indicator of a positive reaction. The slides were evaluated by light microscopy (BX52, Olympus, Tokyo, Japan) using Cell^B (Olympus) software. The immunoreactivities of NSE, CGA, AE1/AE3, and MT were evaluated using digital microphotography and were described as absent (-), minimal (10%–30%), moderate (30%–70%), or high (70%–100%) in a high-power field (HPF) (400×). The mitotic figures were counted in 10 randomly chosen areas of each slide (HPF 400×). Ki67 immunoreexpression was evaluated quantitatively in 10 randomly chosen areas of each slide (HPF 400×). The Ki67 index was expressed as a percentage of positively stained cells.

3. Results and discussion

In the dog, the tumor was unencapsulated, located in a deep area of the skin, and did not exhibit invasion into the skin or the adnexal structures. The cells were small (Figure 1) and surrounded by thick fibrovascular stroma, as confirmed by Mallory trichrome staining (inset of Figure 1). The nuclei were hyperchromatic, with an average of 7 per 10 HPFs of mitotic figures (400×). The cytoplasm was eosinophilic and sparse. The neoplastic cells had positive, high (approximately 80% of the neoplastic cells) intracytoplasmic granular or diffuse expression of NSE (Figure 2) and high (90% of the neoplastic cells) perinuclear expression of AE1/AE3 (Figure 3). CGA expression was absent (Figure 4). Cytoplasmic and membranous MT expression was minimal (approximately 15% of the cells) (Figure 5). The Ki67 index was 7% (Figure 6).

Table. Primary antibodies used for the particular methods of antigen retrieval and visualization.

Primary antibody	Clone	Dilution	Source	Antigen retrieval	Visualization system
Neuron-specific enolase (NSE)	Monoclonal mouse antihuman BBS/NC/VI-H14	1:100	DAKO, Denmark	2 × 5 min* TrisEDTA buffer pH 9	EnVision + System-HRP, Mouse (DAB)‡
Cytokeratin (AE1/AE3)	Monoclonal mouse antihuman AE1/AE3	1:50	DAKO, Denmark	2 × 5 min* TrisEDTA buffer pH 9	EnVision + System-HRP, Mouse (DAB)‡
Metallothionein (MT)	Monoclonal mouse antimetallothionein clone E9	1:50	DAKO, Denmark	2 × 5 min* TrisEDTA buffer pH 9	EnVision + System-HRP, Mouse (DAB)‡
Chromogranin A (CGA)	Polyclonal rabbit antihuman	Ready-to-use	DAKO, Denmark	2 × 5 min* TrisEDTA buffer pH 9	Impress Universal Reagent Anti-Mouse/Rabbit Ig Peroxidase§
Ki67	Monoclonal mouse antihuman MIB-1	1:75	DAKO, Denmark	2 × 5 min* TrisEDTA buffer pH 9	EnVision + System-HRP, Mouse (DAB)‡

*Antigen retrieval was conducted in a microwave oven at 650 W; ‡DAKO, Glostrup, Denmark; §Vector Laboratories Inc., Burlingame, CA, USA.

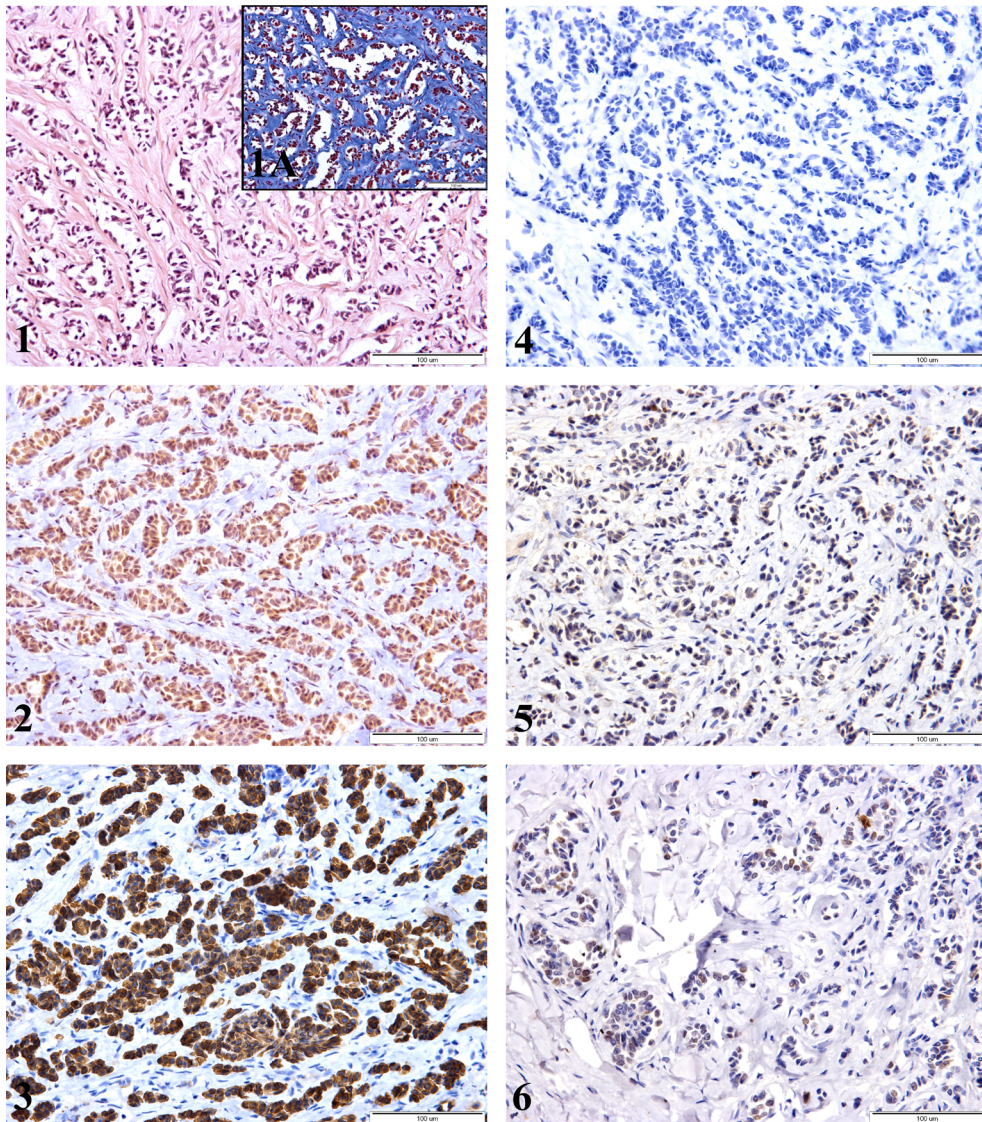


Figure 1. Merkel cell tumor in dog skin. Clusters of small neoplastic cells surrounded by abundant fibrovascular stroma – small cell subtype (H&E). Inset: Collagen fibers surrounding clusters of neoplastic cells (Mallory trichrome).

Figure 2. Merkel cell tumor in dog skin. Positive immunolabeling of neoplastic cells to NSE, DAB.

Figure 3. Merkel cell tumor in dog skin. Positive immunolabeling of neoplastic cells to AE1/AE3, DAB.

Figure 4. Merkel cell tumor in dog skin. No CGA expression in neoplastic cells, DAB.

Figure 5. Merkel cell tumor in dog skin. Cytoplasmic and membranous expression of MT in neoplastic cells, DAB.

Figure 6. Merkel cell tumor in dog skin. Expression of Ki67 in neoplastic cells, DAB.

In the cat, the tumor was unencapsulated, located in a deep area of the skin, and did not show epidermal or follicular invasion. The tumor was composed of monomorphic round cells that were arranged in sheets (Figure 7) and separated by delicate fibrovascular stroma (confirmed by Mallory trichrome staining, inset of Figure 7). The centrally located nuclei were round to oval in shape, with evenly dispersed chromatin and a sharply defined nuclear membrane. The number of mitotic figures was 56 per 10 HPFs (400×).

The cytoplasm was pale, eosinophilic, and moderate. The neoplastic cells showed high (approximately 90% of the neoplastic cells) cytoplasmic granular or diffuse expression of NSE (Figure 8), high (approximately 90% of the neoplastic cells) cytoplasmic perinuclear expression of AE1/AE3 (Figure 9), and no CGA expression (Figure 10). Cytoplasmic and nuclear expression of MT (Figure 11) was minimal and was observed in approximately 30% of the neoplastic cells. The Ki67 index was 25% (Figure 12).

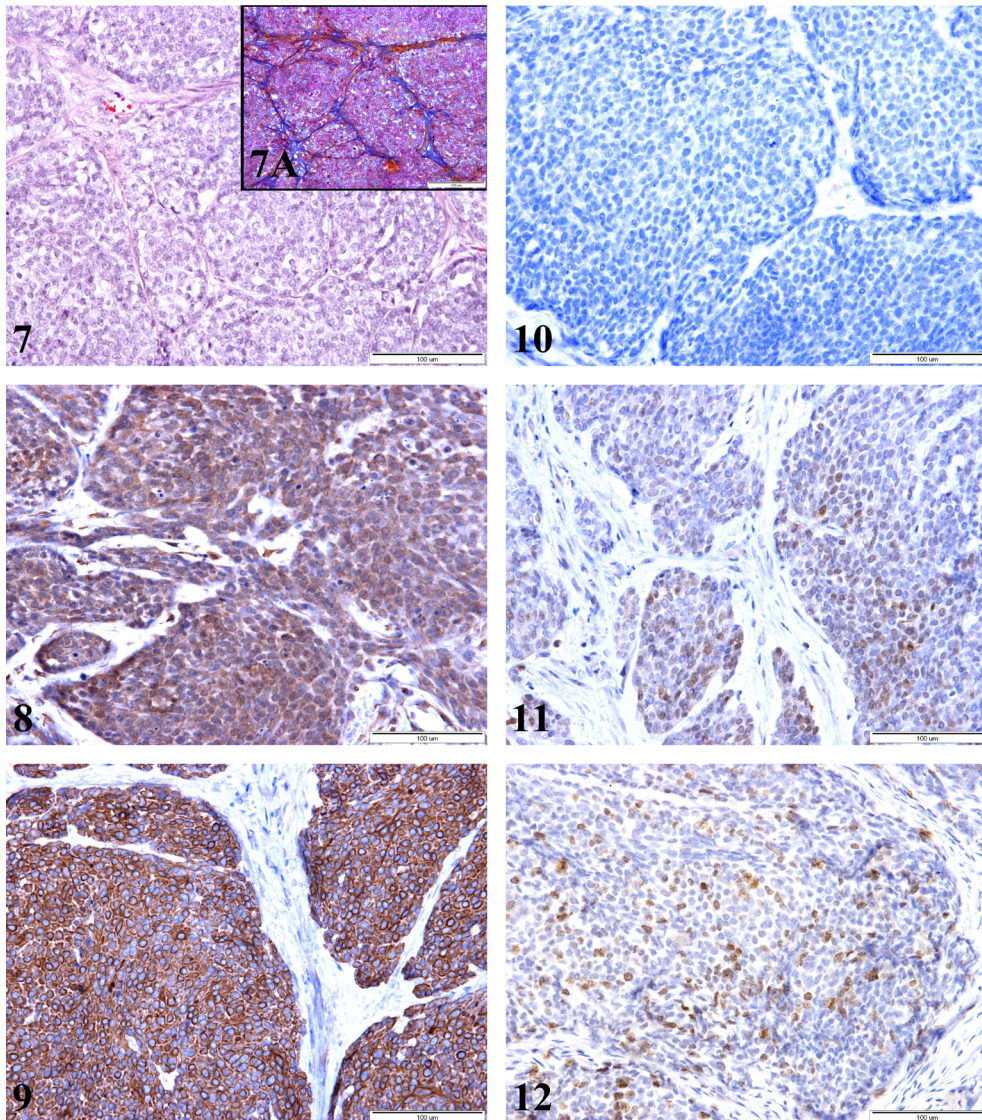


Figure 7. Merkel cell tumor in cat skin. Round neoplastic cells divided by thin fibrovascular stroma – intermediate subtype (HE). Inset: Delicate collagen fibers surrounding packets of neoplastic cells (Mallory trichrome).

Figure 8. Merkel cell tumor in cat skin. Positive immunolabeling of neoplastic cells to NSE, DAB.

Figure 9. Merkel cell tumor in cat skin. Positive immunolabeling of neoplastic cells to AE1/AE3, DAB.

Figure 10. Merkel cell tumor in cat skin. No CGA expression in neoplastic cells, DAB.

Figure 11. Merkel cell tumor in cat skin. Cytoplasmic and nuclear expression of MT in neoplastic cells, DAB.

Figure 12. Merkel cell tumor in cat skin. Expression of Ki67 in neoplastic cells, DAB.

The results of the present study suggest that canine MCTs correspond to the diffuse pattern (small-cell subtype) of human MCC, whereas feline MCTs correspond to the solid pattern (intermediate subtype). In both cases, the immunoreactivities of the neoplastic cells indicated positivity for NSE and AE1/AE3, which suggests a neuroectodermal origin of the cells. Similar immunolabeling was also observed by other authors (4–

6,14,15). In the present study, the neoplastic cells were CGA negative in both the canine and the feline MCTs, which corresponds to CGA-negative cases of human MCC (15).

Both the canine and the feline MCTs had minimal MT expression. In human large-cell neuroendocrine carcinoma of the lung, the expression of MT is also minimal or even absent (16). A complete lack of MT

expression is associated with amplified spontaneous and mutagenic DNA damage (10). High MT expression in canine apocrine gland tumors as well as in canine and feline melanomas is positively associated with tumor grade. Similar associations have been observed in many human tumors, e.g., cutaneous melanoma, adenoid cystic carcinoma of the salivary gland, and breast carcinoma (i.e. invasive carcinoma of no special type, invasive lobular carcinoma, tubular carcinoma, cribriform carcinoma, mucinous carcinoma, carcinoma with medullary features, and invasive papillary carcinoma) (9). However, only 25% of canine cutaneous melanomas and only 6% of feline cutaneous melanomas show expression of MT (10). Given that holo-MT protects DNA from damage and that apo-MT is related to tumor growth and progression, future research on the role of both MT forms in the pathogenesis of canine and feline MCTs will be necessary (9,10).

Mitotic activity and the Ki67 index were low in the canine MCT, whereas in the feline MCT mitotic activity was high and the Ki67 index was moderate. High mitotic activity and a high Ki67 index are indicative of malignancy

in many cutaneous neoplasms (17,18). In neuroendocrine tumors, the Ki67 and mitotic indexes have been considered to be malignancy indicators, although Ki67 is a more precise malignancy marker (19). These results suggest that the growth and progression of the evaluated feline MCT were probably more dynamic than those of the evaluated canine MCT. However, mitotic activity or the Ki67 index alone is not sufficient to determine the malignancy of a tumor. The major malignancy criterion is the metastatic potential of the neoplastic cells (20). Unfortunately, follow-up information was not available in the present study.

In this report, the histopathological and immunohistochemical findings of unusual MCTs were described in a dog and a cat. In the presented cases, the canine and feline MCTs showed high NSE and AE1/AE3 expression, indicating neuroectodermal origin. The lack of CGA expression in both evaluated tumors suggests that this marker cannot be used as a diagnostic marker in canine and feline MCTs. To the best of our knowledge, this is the first immunohistochemical study to evaluate MT expression in canine and feline MCTs.

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