Primary Malignant Peripheral Nerve Sheath Tumor with Eosinophilic Cytoplasmic Globules Arising from the Greater Omentum in a Dog

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ABSTRACT. A 10-year-old Golden Retriever dog had a solitary tumor mass arising from the greater omentum. Histologically, the tumor showed varying cellularity and patterns of cellular arrangement. In dense cellular areas, spindle-shaped cells were arranged in interlacing bundles. The sparse cellular area was characterized by loosely arranged fusiform cells. The neoplastic cells frequently contained PAS-positive eosinophilic globules in the cytoplasm, and mitotic figures were frequently observed. The tumor cells were positive to vimentin, S-100 protein, glial fibrillary acidic protein, myelin basic protein, neuron-specific enolase and myoglobin. The present tumor was diagnosed as a malignant peripheral nerve sheath tumor (MPNST) with eosinophilic cytoplasmic globules arising from the greater omentum.

To our knowledge, this may be the first case of primary omental MPNST in domestic animals.

KEY WORDS: canine, greater omentum, malignant peripheral nerve sheath tumor.

Primary omental tumors are extremely rare in animals. A search of the literature in English over the past 20 years revealed no reports of only two tumors (lipoma and liposarcoma) in 2 dogs [3, 8]. Moreover, only a few cases of a primary tumor of the peripheral nerves arising from the greater omentum have been reported in humans [2, 11], but none in domestic animals.

Malignant tumors arising from peripheral nerves or displaying differentiation along the lines of the various elements of the nerve sheath are collectively referred to as malignant peripheral nerve sheath tumors (MPNSTs) [24]. Human MPNSTs occasionally show histologic evidence of focally divergent differentiation to rhabdomyosarcoma, osteosarcoma, chondrosarcoma, angiosarcoma, epithelial elements, or a combination thereof [9, 24]. Similar representations such as epithelioid type [10], cartilaginous and osteogenic [1], glandular epithelial differentiation [20] or their complexes [12] have been found in canine MPNSTs. Furthermore, cases of atypical cell morphology such as tumor cells with eosinophilic cytoplasmic globules have also occurred in dogs and one goat [6, 15, 21]. Our report describes a canine MPNST with cytoplasmic globules arising from the greater omentum.

A 10-year-old neutered-male Golden Retriever was referred with a 1-week history of anorexia without vomiting or diarrhea. Ultrasonography showed a large tumor, solitary, well-defined and extrahepatic mass close to the liver with a smooth surface and non-homogeneous echo levels. Computed tomography (CT) detected a large mass in the left upper abdomen (Fig. 1).

The mass was surgically resected and, upon gross examination, was found to have arisen from the greater omentum and to reveal a smooth, dark whitish red surface with edematous areas (Fig. 2). The mass measured 17 × 11 × 8 cm in diameter and weighed 1,500 g. The cut surface showed solid tissue with hemorrhaging, necrosis and multiple cysts containing a dark red fluid. The tumor did not invade or adhere to other organs, and no metastasis or ascites was observed.

The resected tissue was fixed in 10% formalin and embedded in paraffin wax. Sections were cut at 5 µm and stained with haematoxylin and eosin (HE) and periodic acid-Schiff (PAS) with or without diastase digestion. For
the immunohistochemistry, the following primary antibodies were used: anti-porcine vimentin polyclonal antibody (DakoCytomation, Glostrup, Denmark), anti-cow S-100 polyclonal antibody (Dako), anti-cow glial fibrillary acidic protein (GFAP) polyclonal antibody (Dako), anti-human myelin basic protein (MBP) polyclonal antibody (Dako), anti-human neuron-specific enolase (NSE) monoclonal antibody (Dako), anti-human myoglobin polyclonal antibody (Dako), anti-α-smooth muscle actin monoclonal antibody (Dako), anti-human factor VIII-related antigen polyclonal antibody (Dako), anti-cytokeratin monoclonal antibody (Dako), anti-human lysozyme polyclonal antibody (Dako), anti-human myeloid/histiocyte antigen (Dako), and anti-human Ki-67 antigen monoclonal antibody (Dako). For each reagent, a section of an appropriate normal dog tissue sample was used as a positive control. Tissue sections for the detection of S-100, GFAP, MBP, NSE, myoglobin, α-smooth muscle actin, cytokeratin, and Ki-67 antigen were immersed in 0.01 M citrate buffer (pH 6.0) and autoclaved for 15 min at 121°C to retrieve antigens. Tissue sections for the detection of vimentin were immersed in 0.01 M citrate buffer (pH 6.0) and microwaved at 750 W for 10 min to retrieve antigens. Tissue sections for assays of lysozyme, myeloid/histiocyte antigen and factor VIII-related antigen were incubated at room temperature for 15 min in proteinase K solution (Dako) for antigen retrieval. All sections were dehydrated, rehydrated, rinsed with 0.05 M tris-buffered saline containing Tween (TBST; pH 7.6), treated with 1% hydrogen peroxide, and then rinsed again with TBST. Slides were incubated with primary antibody for 30 min at room temperature and, after rinsing with TBST, were treated with Simple Stain MAX-PO (Multi) (Nichirei, Tokyo, Japan) for 30 min at room temperature. They were then rinsed with TBST before being treated with a 3,3′-diaminobenzidine solution containing 0.01% hydrogen peroxide to facilitate a peroxidase colour reaction. After a further wash with TBST, the slides were counterstained with Mayer's haematoxylin.

Histologically, the tumor consisted predominantly of anaplastic spindle-shaped cells. The cellularity and pattern of the neoplastic cellular arrangement varied (Fig. 3). In the dense cellular areas, spindle-shaped cells were often arranged in interlacing bundles (Fig. 4a) or storiform patterns, with occasional whorl formation. The sparse cellular area was characterized by loosely arranged spindle-shaped cells with occasionally wavy shaped nuclei (arrow). Bar=30 µm. The mitotic
index (3.98 ± 1.07) was calculated by determining the mean number of mitoses counted in 10 fields microscopically observed with a × 400 magnification. The cells revealed spheroid eosinophilic globules of various sizes in their cytoplasm (Fig. 5). The globules were PAS-positive and diastase-resistant. Necrotic foci and infiltrates of various numbers of lymphocytes, plasma cells and neutrophils were common.

Immunohistochemically, most of the neoplastic cells reacted moderately to vimentin, S-100 protein, GFAP (Fig. 6), NSE, MBP and myoglobin (Fig. 7), but were negative for cytokeratin, α-smooth muscle actin, factor VIII-related antigen, cytokeratin, lysozyme and myeloid/histiocytic antigen. Similar immunohistostaining patterns were seen in the neoplastic cells with cytoplasmic eosinophilic globules. The proliferation index, measured as the percentage of Ki-67-positive cells in five high-power fields (a total of approximately 1,000 cells), was 78%.

Based on both histologic and immunohistochemical features, the present case was diagnosed as a malignant peripheral nerve sheath tumor with eosinophilic cytoplasmic granules arising from the greater omentum. The histological characteristics of the tumor described here were similar to those of the canine MPNSTs with eosinophilic cytoplasmic granule reported by Kuwamura et al. [15]. In dogs, palisading patterns of nuclear orientation are very uncommon, and verocay bodies formed by stacked parallel rows of palisading nuclei are extremely rare compared to their occurrence in human schwannomas [14].

Immunohistochemically, antibodies against S-100 protein, GFAP, MBP, and NSE have been widely used to suggest a neural origin of cell types [4, 16]. In the present case, most of the neoplastic cells were reactive to S-100 protein, GFAP, MBP, NSE and vimentin, suggesting a neuroectodermal lineage. Positive reactions with antibodies for S-100 protein and MBP have also been reported in 50–90% and 40%, respectively, of human MPNSTs [24].

The globule-possessing cells are different from infiltrating macrophages involved in erythrophagocytosis, because these cells were negative for lysozyme and myeloid/histioytic antigen, which has been used for the detection of macrophages [16]. Furthermore, ingested erythrocytes did not react to PAS. The presence of PAS-positive, diastase-resistant granules is characteristic of granular cell tumors (GCTs) in human and animals [7, 18]. It is generally accepted that granular cell tumors are derived from peripheral nerve components [24]. Thus, these granular cells most likely indicate the divergent differentiation of canine MPNSTs.

In the present case, most of the neoplastic cells were moderately positive for myoglobin. In previous study, 64% of canine MPNSTs showed a positive reaction to the skeletal muscle marker, myoglobin [6, 19]. Although MPNSTs with rhabdomyoblastic differentiation are a relatively rare type of human MPNTs [24], they are unlikely to be rare in dogs. Human MPNSTs with rhabdomyoblastic difference-
tion have a worse prognosis than classic MPNSTs [22], with a 5-year survival rate of only 12% based on a literature review by Brooks et al. [5]. To our knowledge, a prognosis of canine MPNSTs with rhabdomyoblastic differentiation has not been reported to date.

In human PNSTs, Ki-67 indices were reported to be 5–65% in MPNTs; in contrast, indices for BPNSTs were typically less than 5% [13]. Ki-67 antigen immunoreactivity correlated strongly with MPNTs versus BPNSTs as well as p53 expression [13]. In the present case, the vigorous Ki-67 antigen reactivity of neoplastic cells suggested that they were highly malignant. In MPNSTs of domestic animals, there are few data on Ki-67 expression, but the percentage of Ki-67-positive cells in five high-power resolution fields (about 1,000 cells) was found to be 40–50% in the metastases of a dog with MPNST [10]. Although a high Ki-67 labelling index (>25%) was correlated with a reduced survival rate [23] in humans, such a correlation is unclear in dogs. Neoplastic cells in the present case were highly malignant, though no metastasis was observed. Few metastases may be characteristic of primary omental MPNST in a dog.

To our knowledge, primary MPNSTs arising from the greater omentum have not been reported in animals. This is the first report describing the histopathological and immunohistochemical features of this phenomenon.

REFERENCES