NOTE *Pathology*

Multiple Osseous Metastases of a Carotid Body Tumor in a Dog

Mina OKAJIMA[1)], Akinori SHIMADA[1)]*, Takehito MORITA[1)], Masako YOSHIKAWA[2)] and Koji NISHIDA[3)]

[1)]Department of Veterinary Pathology, Tottori University, Tottori 680–8553, [2)]Alice Veterinary Clinic, 1–21–5 Shimokosaka, Higashiosaka-shi, Osaka 577–0803 and [3)]Medical Center Nishida Veterinary Clinic, 5–10–11 Ueda, Matsubara-shi, Osaka 580–0016, Japan

(Received 1 May 2006/Accepted 17 November 2006)

ABSTRACT. Metastasis of malignant carotid body tumor to multiple bones was detected in a 13-year-old female Siberian husky dog. Radiographs exhibited an abnormal mass in the retropharyngeal site and osteolytic lesions in the vertebral bodies, spinous process, tibia, and ribs. At necropsy, multiple masses were observed in the bones as well as at the dorsal area of the retropharynx. Histologically, the tumor cells, arranged in sheets and clusters, had eosinophilic finely granular cytoplasm. Immunohistochemistry showed the tumor cells were positive for neuron-specific enolase and synaptophysin. Electron microscopy demonstrated a number of dense membrane-bound granules in the cytoplasm of the tumor cells. Based on these findings, this case was diagnosed as multiple bone metastases of a malignant carotid body tumor. Spinal cord damage induced by the tumor mass was the cause of the hind limb paralysis of the present dog.
KEY WORDS: canine, carotid body tumor, osseous metastases.

J. Vet. Med. Sci. 69(3): 297–299, 2007

Chemoreceptors including carotid body and aortic body play a role in regulation of oxygen, carbon, and hydrogen ion content of the blood [7]. Carotid body lies dorsal to the bifurcation of the common carotid artery [5] and carotid body tumors arise at the cranial area of the artery [2]. Carotid body tumors in dogs tend to be more malignant than aortic body tumors, and metastases are present in approximately 30% of the reported cases [3]. To the best of our knowledge, osseous metastases of carotid body tumors in dogs have not been reported in the literature. In this report, we describe a malignant carotid body tumor with multiple osseous metastases in a dog.

A 13-year-old female Siberian husky dog was brought to the Medical Center Nishida Veterinary Clinic with a history of pain and lameness of the right hind limbs. During a period of approximately 3 months, clinical neurological abnormalities such as knuckling, astasia and loss of deep pain of both sides of hind limbs were progressed. Biochemistry and hematology showed aspartate aminotransferase 118 U/*l*, alkarine phosphatase 586 U/*l*, lactate dehydrogenase 514 U/*l*, lipase 299 U/*l*, C reactive protein 13 mg/d*l*. Radiographs exhibited an abnormal mass in the retropharyngeal site (Fig. 1) and osteolytic lesions in the vertebral bodies and spinous processes of 8th and 9th thoracic vertebrae (Fig. 2), proximal part of right tibia, and 2nd and 3rd ribs. Because of a poor prognosis, the dog was euthanatized by the administration of overdose of pentbarbital.

At necropsy, a mass (4.4 × 4.0 × 3.2 cm) encapsulated by a thin fibrous connective tissue was observed attaching to the dorsal area of the retropharynx. On cut surface, the mass was hemorrhagic with abundant vascularity and consisted of white and red foci. Multiple white nodular masses were detected in the 8th thoracic vertebra, right 3rd, left 2nd, 3rd and 5th ribs, and right tibia. Osteolytic changes were dominant in the 8th thoracic vertebral body, where there was an extensive growth of the nodular masses to the vertebral foramen, resulting in compression of the spinal cord at the 8th thoracic vertebra. The 8th thoracic spinal cord segment was pale in color and solid on palpation. Similar multiple nodules were also observed in the lung, liver, hilar lymph node and ovary.

Tissues from the mass and variety of organs including the brain, spinal cord, liver, spleen, lung, kidney, hilar lymph node and bone were collected and fixed by immersion in 10% neutral buffered formalin. These tissues were then dehydrated, embedded in paraffin wax, sectioned at 4 μm, and stained with hematoxylin and eosin (HE). Selected sections of the masses were stained with Grimelius' silver stain. Immunohistochemistry was also carried out on the selected sections of the masses with the use of primary antibodies against neuron-specific enolase (NSE) (mouse monoclonal, Dako, diluted at 1: 50), synaptophysin (mouse monoclonal, Progen, diluted at 1: 100) and calcitonin (rabbit polyclonal, Dako, diluted at 1: 100). After treatment with 0.1% trypsin solution at 37°C for 30 min and blocking endogeneous peroxidase activity with 3% H_2O_2 in phosphate buffered saline (PBS), sections were incubated with a primary antibody overnight at 4°C and then sequentially incubated with biotinylated goat anti mouse IgG (Dako) (1: 400) for 1 hr at room temperature, and with peroxidase-conjugated streptavidin (Dako) for 30 min. The sections were washed 3 times for 10 min each in PBS and then developed with 0.02% 3,3,-diaminobenzidine tetrahydrochloride and H_2O_2 (DAB-H_2O_2). The sections were counterstained with hematoxylin. Electron microscopy was carried out using samples (1–2 mm^3 cubes) from the formalin fixed retropharyngeal mass. The samples were rinsed in 0.1 M phosphate buffer (pH=7.4), post fixed for 1 hr in 1% osmium tetroxide, dehydrated in the graded alcohol, and embedded in Epon 815 epoxy resin

* CORRESPONDENCE TO: SHIMADA, A., Department of Veterinary Pathology, Tottori University, Minami 4–101, Koyama, Tottori 680–8553, Japan.

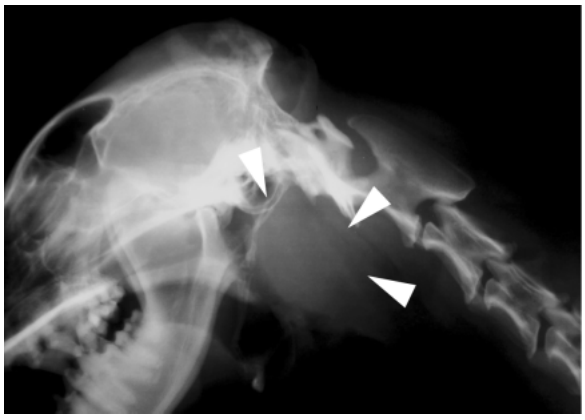

Fig. 1. Left lateral head radiograph demonstrating an abnormal mass (arrowheads) in the retropharyngeal site.

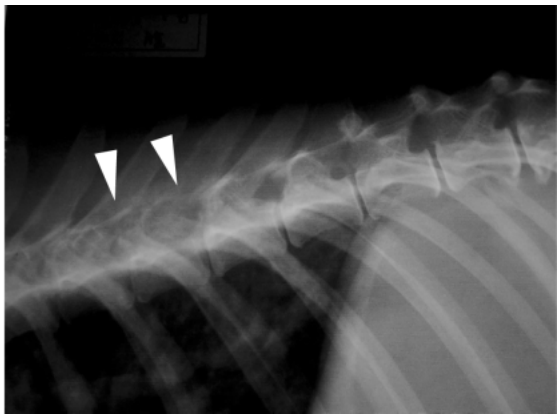

Fig. 2. Left lateral thoracic radiograph demonstrating osteolytic lesions in the vertebral bodies and spinous process of 8th and 9th thoracic vertebrae (arrow heads).

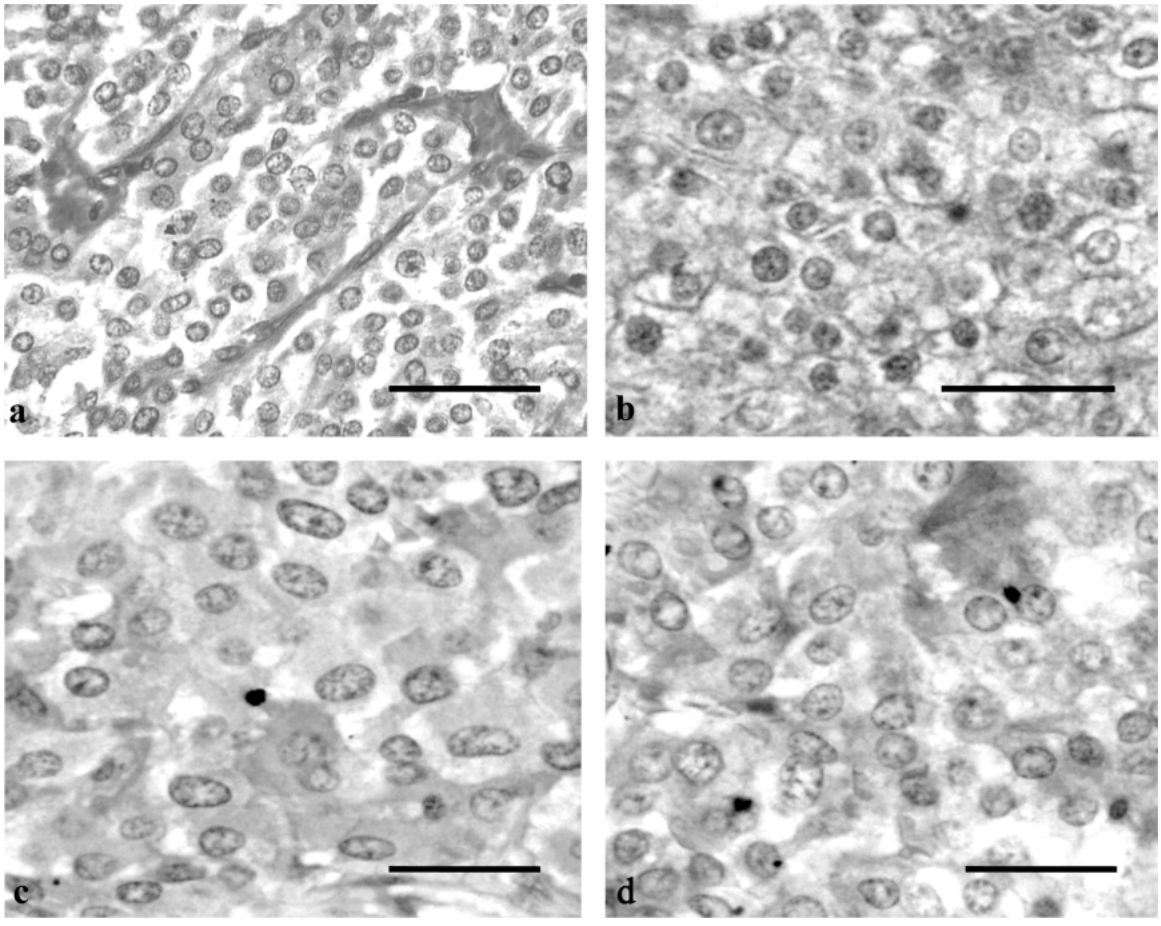

Fig. 3a. Cluster of neoplastic cells containing intracytoplasmic fine granules. Carotid body tumor in the retropharyngeal mass. Hematoxylin and Eosin stain. Bar = 50 μm.

Fig. 3b. Carotid body tumor cells demonstrating positive expression for Grimelius stain. Bar = 75 μm.

Fig. 3c. Carotid body tumor cells demonstrating positive immunohistochemical expression for neuron specific enolase (NSE). NSE immunohistochemistry. Bar = 75 μm.

Fig. 3d. Carotid body tumor cells demonstrating positive immunohistochemical expression for synaptophysin. Synaptophysin immunohistochemistry. Bar = 75 μm.

(Nisshin Co, Tokyo, Japan). Semi-thin (1 μm thick) sections were stained with 1% toluidine blue to select and locate interesting areas for the electron microscopic examination. Ultra-thin sections stained with uranyl acetate and lead citrate were examined under JEM-100CX electron microscope (Japan Electron Optical Laboratory, Tokyo, Japan).

Histologically, the mass found at the dorsal part of the retropharynx consisted of pleomorphic tumor cells, which were arranged in sheets and clusters separated by fibrovascular connective tissue (Fig. 3a). Tumor cells were irregular in size, showing occasional mononuclear giant cells. The cytoplasm of the tumor cells was eosinophilic, finely granular, and often vacuolated. Some of the tumor cells contained Grimelius' silver-positive granules in the cytoplasm (Fig. 3b). Mitotic figures were uncommon. Foci of coagulative necrosis were scattered among the tumor tissue. Histological findings of the nodular masses observed in the osteolytic lesions of the bone tissues and bone marrow spaces of the 8th thoracic vertebra, ribs, right tibia, liver, ovary, lung and hilar lymph node were similar to the tumor tissue observed at the dorsal part of the retropharynx; hepatocytes around the tumor nodules showed vacuolar degeneration. A number of swollen axons with spheroids formation and moderate spongy changes, which are common findings of the Wallerian degeneration, were observed in the dorsal part of the 8th and 9th thoracic spinal cord segments, where compression by the tumor mass was identified; fibrosis was associated with this lesion. Similar changes with less severe degree were also observed in the 4th lumber spinal cord segment.

Immunohistochemistry showed tumor cells of the masses including the retropharynx mass were positive for NSE (Fig. 3c) and synaptophysin (Fig. 3d), and negative for calcitonin. Electron microscopy demonstrated a number of electron dense membrane-bound granules in the cytoplasm of the tumor cells (Fig. 4).

Medullary thyroid carcinoma is reported to be positive for calcitonin [6]. Tumor cells of the well-differentiated chemodectomas including aortic body and carotid body tumors are positive for NSE, synaptophysin and chromogranin A in immunohistochemistory [1] and cytoplasm of the neoplastic cells are filled with dense and membrane-bound granules [4]. Based on the anatomical location, histological, immunohistochemical and electron microscopic findings, the tumor mass at the retropharnx was considered to be carotid body tumor. Histological features including hemorrhage, necrosis and pleomorphism of the tumor cells with the presence of giant cells are not the distinct findings of the malignant carotid body; these findings are occasionally observed in the small, benign chemodectoma [3]. Multiple osseous metastases with osteolytic lesions indicate that the present tumor is categorized to malignant chemodectoma.

As space occupying lesions, enlarging carotid body tumor masses tend to result in interfering with swallowing, dyspnea, and circulatory disturbances from compression of the large veins in the neck [3]. Present dog showed no such signs related to compression of the cervical area by the tumor mass, resulting in unawareness of the occurrence of the tumor with development of multiple osseous metastases.

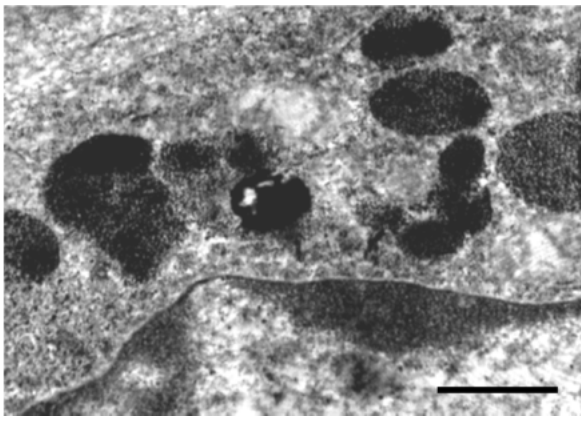

Fig. 4. Electron micrograph of a portion of carotid body tumor cells showing oval membrane-bound dense-core granules in the cytoplasm. Bar = 500 nm.

Metastasis of carotid body tumors in dog occurred in approximately 30 percent of the reported cases, in which lung, bronchial and mediastinal lymph nodes, liver, pancreas, and kidney have been involved [2, 3]. To the best of our knowledge, osseous metastases of the canine carotid body tumors have not been reported in the literature. The present case showed multiple osseous metastases of the carotid body tumor cells, resulting in spinal cord compression with neurological signs.

ACKNOWLEDGEMENT. The authors thank Ms E. Kawahara, Tottori University, for her technical assistance in electron microscopy.

REFERENCES

1. Brown, P. J., Rema, A. and Gartner, F. 2003. *J. Vet. Med. A. Physiol. Pathol. Clin. Med.* **50**: 140–144.
2. Capen, C. C. 1993. pp. 345–347. *In*: Pathology of Domestic Animals, 4th ed., vol. 3 (Jubb, K. V. F., Kennedy, P. C. and Palmer N. eds.), Academic Press, New York.
3. Capen, C. C. 2002. pp. 691–696. *In*: Tumors in Domestic Animals, 4th ed. (Meuten, D. J. ed.), Iowa State Press, Ames.
4. Cheville, N. F. 1972. *Vet. Pathol.* **9**: 166–189.
5. Dellmann, H. D. and Venable, J. H. 1976. pp. 142. *In*: Textbook of Veterinary Histology. (Dellmann, H. D. and Brown, E. M. eds.), Lea & Febiger, Philadelphia.
6. Patnaik, A. K. and Lieberman, P. H. 1991. *Vet. Pathol.* **28**: 223–233.
7. Porth, C. M. and Kunert, M. P. 2002. p. 421. *In*: Pathophysiology Concepts of Altered Health States, 6th ed., Lippincott Williams & Wilkins, New York.