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A Case of Fibrosarcoma on the Perivertebral Surface of a Ferret with Hind Limb Paralysis

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Abstract: A 2.5 year-old female ferret had a stiff palpable mass arising from the dorsal surface of the thoracic (T) to lumbar (L) vertebrae with paralysis of the hind limbs. By myelography the dorsal and ventral lines of contrast were not observed in the area forwarded of L3. Grossly, the tumor encircled the dorsal vertebrae. Microscopically, tumor cells were proliferated intimately and were attached to the vertebrae surface involving surrounding fatty and connective tissues. The tumor consisted of fibroblastic cells with prominent cellular atypia. The bromodeoxyuridine (BrdU) labeling index to examine cellular kinetics was high (11.8%). Based on macro and micropathological features, the present tumor was diagnosed as periosteal fibrosarcoma arising from perivertebral connective tissue.

Key words: ferret, fibrosarcoma, perivertebra

Recently, ferrets have become increasingly popular as exotic pets and for use as laboratory animals [1]. There have been some reports of tumors in pet ferrets [1, 4, 7], of which the most frequent neoplasms were pancreatic islet cell tumors (21.5%), adrenocortical cell tumors (20.1%), and lymphomas (19.1%) [3]. Although a few fibrosarcomas originated from the soft tissue, including vaccine-associated fibrosarcoma, have been reported [6–8], to our knowledge, fibrosarcomas arising from the perivertebral surface has not been reported. This report describes clinical and pathological features of a spontaneous fibrosarcoma arising from the perivertebral surface in a ferret. The proliferative activities of this tumor were analyzed with the bromodeoxyuridine (BrdU) immunolabeling method, which is useful for demonstrating proliferating S-phase

cells [10].

A 2.5-year-old, 1-kg, spayed, male, vaccine-inoculated ferret showed paralysis of the hind limbs. Other physical abnormalities such as anorexia, pain or mass lesion were not observed. No clinical improvement was observed by administration of enrofloxacin and prednisolone. On the 21st day of admission, a stiff palpable mass arising from the dorsal surface of the thoracic (T) to lumbar (L) vertebrae was detected. The animal detested palpation of the abdomen, paralysis of the hind limbs and urination on the lower abdomen. No abnormality was found in a hematological examination. By myelography, the dorsal and ventral lines of contrast were not observed in the area of forward L3 (Fig. 1). On the 22nd day of admission, a dorsal laminectomy was

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Fig. 1. Myelography of the ferret. The dorsal and ventral lines of contrast are not observed in the area forward of L3 (arrowhead).

Fig. 2. Grossly, a white firm mass is seen around the dorsal vertebrae (T15-L2).

performed to decompress the spinal cord and surgical biopsies were obtained. At this time, metastasis was not observed. Histopathologically, the tumor was diagnosed as a fibrosarcoma. After the surgery, the animal had temporary improvement of pain from palpation, although dysuria continued. On the 58th day, the animal experienced severe pain again, and multiple masses were observed diffusely in the lung by X-ray micrograph. On the 67th day, the animal was euthanized by deep anesthesia at the owner's request because of its poor health condition and prognosis. Complete necropsy was done immediately after euthanasia.

Samples were collected from all organs including the spinal cord and fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were cut at 3 μ m thickness and stained with hematoxylin and eosin (HE). Selected sections were also stained with Masson's trichrome stain (MTS).

Immunolabeling of BrdU-incorporating cells was performed as described by Yanai *et al.* [9]. BrdU (5'-bromo-2-deoxyuridine; Sigma-Aldrich Co., St. Louis, MO, USA) was administered intravenously at a dose of 15 mg/kg one hour prior to euthanasia. After dewaxing and rehydrating, paraffin sections were rinsed in PBS and hydrolyzed with 5N HCl at 37°C for 30 min, before being neutralized in Palitisch's boric acid-NaCl-borate buffer (pH 7.6) at 4°C for 15 min. The sections were subjected to a digestive process with 0.04% pepsin (Wako Pure Chemical Industries, Osaka, Japan) in 0.01

N HCl at 37°C for 30 min. After inactivation of endogenous peroxidase and blocking of non-specific binding of the antibody, the primary anti-BrdU mouse monoclonal antibodies (Dako, Glostrup, Denmark; 1 in 50 dilution) were incubated with the sections overnight at 4°C. BrdU-positive cells were counted in a total of 1,000 cells (from 10 high-power [$\times 400$] fields) to give the BrdU labeling index (BrdU LI).

Grossly, white firm mass (4 $\times$ 2 cm) was found throughout T15 to L2 surrounding the dorsal vertebrae (Fig. 2). There was no ulceration on the mass. The dissection between the mass and bone was not clear. There was little periosteal response. The spinal cord surrounded by the mass had moderate liquefactive necrosis and softening. In the lung, white firm masses (0.5–3 cm), similar to the ones arising on the dorsal surface of the vertebrae, were scattered in the whole lobe. No abnormality was observed in the other organs.

Microscopic examination of the mass arising on the dorsal surface of the vertebrae revealed prominent infiltrative growth, which proliferated intimately, attaching to the L1-2 vertebrae surface, and involving surrounding fatty and connective tissues (Fig. 3). The polygonal to spindle-shaped tumor cells were fibroblastic in appearance, arranged interwoven, with scant or moderate amounts of eosinophilic cytoplasm, and showed production of various amounts of collagenous stroma which stained blue with MTS (Figs. 4 and 5). The nuclei of tumor cells were round, oval or polyhedral in shape,

Fig. 3. The polygonal and spinal-shape tumor cells proliferating around the surface and medulla of the vertebra. HE stain.

Fig. 4. The nuclei of tumor cells are oval or polyhedral in shape and contain one or several nucleoli. There is scant or moderate eosinophilic cytoplasm with collagenous stroma. Mitotic figures are frequent (arrowhead). HE stain.

Fig. 5. The cytoplasm of neoplastic cells produces collagenous stroma, which stained blue with MTS.

Fig. 6. BrdU immunohistochemistry. Several nuclei of the neoplastic cells are positive for BrdU. Immunostaining with anti-BrdU mouse monoclonal antibody.

varied in size and contained one or several nucleoli, with higher N/C ratio. Mitotic figures were frequent with 4 to 6 mitoses per high-power field ($\times 400$). In the vertebrae, the tumor invaded into the medullary bone of the vertebrae, then into the meninges and spinal cord, inducing severe necrosis and softening in the spinal cord. There was multi-focal necrosis with secondary bacterial infections in the area of the tumor mass as well as necrotic vertebral bone tissues and spinal cord. These necrotic foci with bacterial colonies were accompanied by a moderate to severe degree of purulent inflammation.

In the lung, there were multi-focal metastatic tumors infiltrating the alveolar septa and space which had similar cytological features to those of the vertebral mass, but with more cellular atypia. Bacterial infections with purulent inflammation were also observed in the lung.

For the cell kinetic study of the present tumor, the bromodeoxyuridine (BrdU) labeling method was performed with the consent of the owner. The original tumor mass in the vertebra showed a BrdU LI of 11.8% on the average (Fig. 6). The pulmonary tumor masses in the lung had a BrdU LI of 13.2% on the average.

Based on topological distribution, cytological and histochemical features, particularly fibroblastic cells attached to the bone surface, the present tumor was diagnosed as periosteal fibrosarcoma originating from periosteal connective tissue of the vertebrae. This tumor also showed aggressive invasion to the medulla of the vertebral meninx and spinal cord.

In general, fibrosarcomas originate mainly from various soft tissues, and rarely from the medullary or periosteal tissues [5]. Canine periosteal fibrosarcoma, occurs most commonly in the bones of head, mandible and maxilla and may also involve the scapula or long bone. These fibrosarcomas are well-differentiated and often accompanied by a large amount of collagenous stroma with only slight cellular atypia and pleomorphism of the tumor cells. The tumor has few mitotic figures and rarely metastasize [5]. It is not always possible to differentiate periosteal fibrosarcoma from a fibrosarcoma of soft tissue origin that secondarily involves bone [5]. In a report on ferrets, fibrosarcoma of the bone originates in either medullary or periosteal tissue and may metastasize rapidly to distant sites [2]. In ferrets, fibrosarcoma of the bone including this case might have severe cellular atypia and aggressive invasion different from typical canine periosteal fibrosarcoma.

Except for the lung and vertebral mass, bacterial infections were not observed in any other organ, and there was no ulceration on the vertebral mass. Accordingly, infection of the mass might have resulted in sepsis infection in the lung.

BrdU LI of the four fibromatous epulides were re-

ported as ranging from 3.7–5.0% (mean 4.2%) [10]. That of a canine fibrosarcoma with pulmonary metastasis was 10.3%, as high as that of the tumor in this case [10]. Regarding BrdU LI, the tumor in this case also appeared to have high malignancy and an aggressive nature.

Based on histological features and BrdU LI, periosteal fibrosarcoma in ferrets might have a tendency to be more malignant and aggressive than those found in other animals including canine cases. Further studies with more reported cases are needed to clarify this point.

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