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Case Report

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Chordoma in the Tail of a Ferret

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A chordoma is an uncommon tumor that originates from the remnants of the notochord and most commonly involves the cranial and caudal regions of the axial skeleton. Chordoma has been described in laboratory animals such as dogs, rats, minks, and ferrets. This report describes a case of a chordoma in the tail of a ferret. Grossly, a grayish-white, expansile, subcutaneous soft-tissue mass was observed in the tail. Histopathologically, the mass was a loosely placed, nodular, unencapsulated neoplasm within the dermis. In the mass, tumor lobules were intermingled with fibrous tissues. Fibrous tissues contained abundant extracellular basophilic material that was consistent with mucin. The tumor was composed of a close pack of adipocyte-like vacuolated cells (physaliferous cells). The cells were centrally or eccentrically located round nuclei and eosinophilic cytoplasm with large vacuoles. Immunohistologically, neoplastic cells were positive for vimentin and S-100 protein. Based on histopathologic findings and special staining characteristics, this case was diagnosed as chordoma.

Keywords: Chordoma, ferret, physaliferous cells, pathology

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Chordomas are uncommon in humans and rare in animals. Up to now, only a few reports have been published about chordomas in dogs (Jabara and Jubb, 1971; Pease *et al,* 2002; Munday *et al,* 2003), mink (Hadlow, 1984), rats (Stefanski *et al,* 1988), cats (Carpenter *et al,* 1990; Carminato *et al,* 2008) and ferrets (Herron *et al,* 1990; Dunn *et al,* 1991; Williams *et al,* 1993). Chordomas originate from remnants of the notochord and may arise anywhere along the vertebral column, but most commonly involve the cranial and caudal limits of the axial skeleton (Carminato *et al,* 2008). In rats, this tumor is most commonly located in lumbosacral vertebrae (Stefanski *et al*, 1988). Only 2 case of feline chordoma developed, in the cervical and coccygeal regions (Carpenter *et al*, 1990, Carminato, 2008). In dogs, the cervical and sacrococcygeal vertebrae, brain, spinal cord, and skin are reported sites for chordomas (Jabara and Jubb, 1971; Pease *et al*, 2002; Munday *et al*, 2003). In ferrets, this tumor mainly occurs in the vertebral column, but also occurs in the sacrococcygeal and sphenooccipital regions (Heffelfinger *et al,* 1973; Koestner *et al,* 1999; Koestner *et al,* 2002).

Ferrets (*Mustela putorius furo*) share many anatomical, metabolic, and physiologic features with humans, which has promoted their use as an animal model. Ferrets are used in biomedical research in a wide variety of studies including cardio-pulmonary, neurological, and gastrointestinal research (Willias and Barrow, 1971). In addition, the ferret is an important experimental animal model for influenza virus (Maher and DeStefano, 2004; van den Brand *et al*., 2010).

This report describes the clinical presentation and histopathological findings of chordoma in a ferret and discusses the differential diagnosis.

Materials and Methods

A 3-year-old, 0.74 kg spayed female ferret presented with a mass in the end of the tail. The ferret was housed in indoor, was fed with a commercial ferret formula and had free access to water. General condition was good with no previous history of any disease. For light microscopy, the entire mass was removed and fixed in 10% phosphate-buffered formalin. Fixed tissue was processed by conventional methods and stained with hematoxylin-eosin (H-E), periodic acid-Schiff (PAS), and toluidine blue. Frozen sections of mass were stained with Sudan III to demonstrate lipids in the cytoplasm.

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Immunohistochemical labeling was performed on formalin-fixed, paraffin wax-embedded sections with the avidin-biotin-complex technique. Sections were deparaffinized and placed in citrate buffer at pH 6.0 for microwave antigen retrieval. Then, slides were incubated with 3% hydrogen peroxide to inactivate endogenous peroxidase activity, followed by several washes in phosphate-buffered saline (PBS). After incubation, the sections were covered with 10% goat normal serum for 30 min and incubated overnight with anti-vimentin (1:200, Dako, Glostrup, Denmark) and S-100 (1:200, Millipore, Billerica, MA, USA). Sections were washed with PBS and incubated with biotinylated anti-mouse IgG (Vector, Burlingame, CA, USA) and peroxidase conjugated strepavidin (Vector) for 30 min, respectively. Diaminobenzidine (DAB) kits (Vector) were used as a substrate for the peroxidase reaction. After developing, the sections were counterstained with hematoxylin and coverslipped.

Results

A physical examination revealed a normal condition. Grossly, an expansile, well-defined solitary mass (1.9×1.6×1.3 cm) was located at the end of the tail. The cut-surface of the mass was lobulated, rubbery-firm, with partly solid regions, and grayish-white in color. The margins were impossible to delineate (Figure 1).

Histopathological examination of the mass revealed a loosely packed, nodular, unencapsulated neoplasm within the dermis. Neoplastic cells were arranged in single or multiple rows between skeletal muscle bundles and fibrous stroma. They were varying from well-circumscribed lesions to tumors having highly infiltrative borders. Cartilage and trabecular bone formation was visible in the center of the mass. The neoplastic cells surrounding the cartilage and trabecular bone caused substantial changes to the bone (Figures 2A, 2B). Most of the subcutaneous tissues and trabecular bone with marrow was destroyed and replaced by tumor cells (Figure 2A). And the tumors grew into the bone marrow cavity with substantial changes of trabecular bone (Figure 2B). Accumulation of extracellular basophilic matrix, interpreted as mucin, was present within most lobules (Figure 2C). The matrix was stained metachromatically with toluidine blue (Figure 2D). The tumors were composed of closely packed adipocyte-like vacuolated polyhedral cells, usually described as physaliferous (having bubbles or vacuoles). The cells showed centrally or eccentrically located, round nuclei and eosinophilic cytoplasm with large vacuoles. Nuclei were small, round to oval, and centrally or eccentrically located (Figure 2E). The cytoplasm of the large tumor cells was eosinophilic and heavily vacuolated.

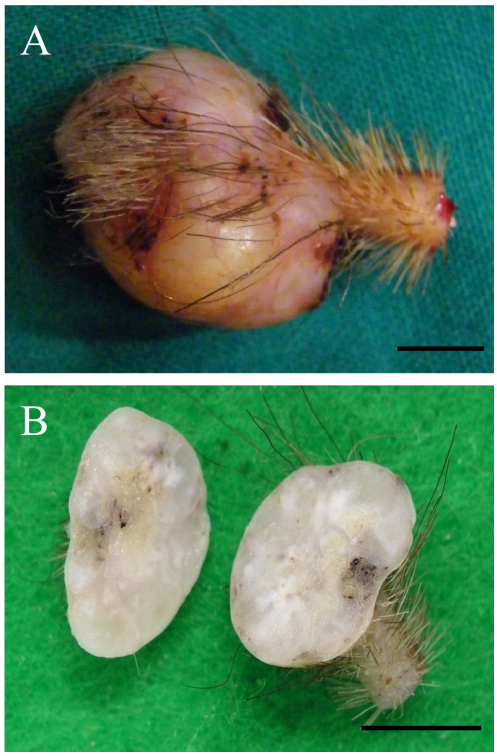

Figure 1. Gross appearance of the mass in the tail of a three-year-old female ferret. Bar=5 mm. (A) Grossly, an expansile, well-defined solitary mass is located at the end of the tail. (B) Cross-section across the middle of the fixed mass showing an infiltrative pattern with an irregular border. Bar=10 cm.

These vacuoles stained weakly with PAS (data not shown), but negative for Sudan III staining (Figure 2F). Immunohistologically, many neoplastic cells were positive for vimentin (Figure 3A) and S-100 protein (Figure 3B).

Discussion

The present report describes a case of chordoma in the tail of a ferret. Chordoma is a rare neoplasm that arises from intraosseous remnants of the notochord and is characterized by dual expression of epithelial and mesenchymal proteins (Dunn *et al,* 1991).

Chordoma has been described in dogs, cats, rats, and mink (Volpe *et al,* 1983). Ferrets appear predisposed to developing tumors of this type. Chordoma commonly arise in the sacrococcygeal region, but they may also occur in the sphenooccipital region and rarely in other locations (Koestner *et al,* 2002). In rats, the tumor is most commonly located in lumbosacral vertebrae (Stefanski *et al,* 1988). Only 2 cases of feline chordoma have developed in the cervical and coccygeal region (Carpenter *et al,* 1990; Carminato, 2008). In dogs, not only the cervical and sacrococcygeal vertebrae,

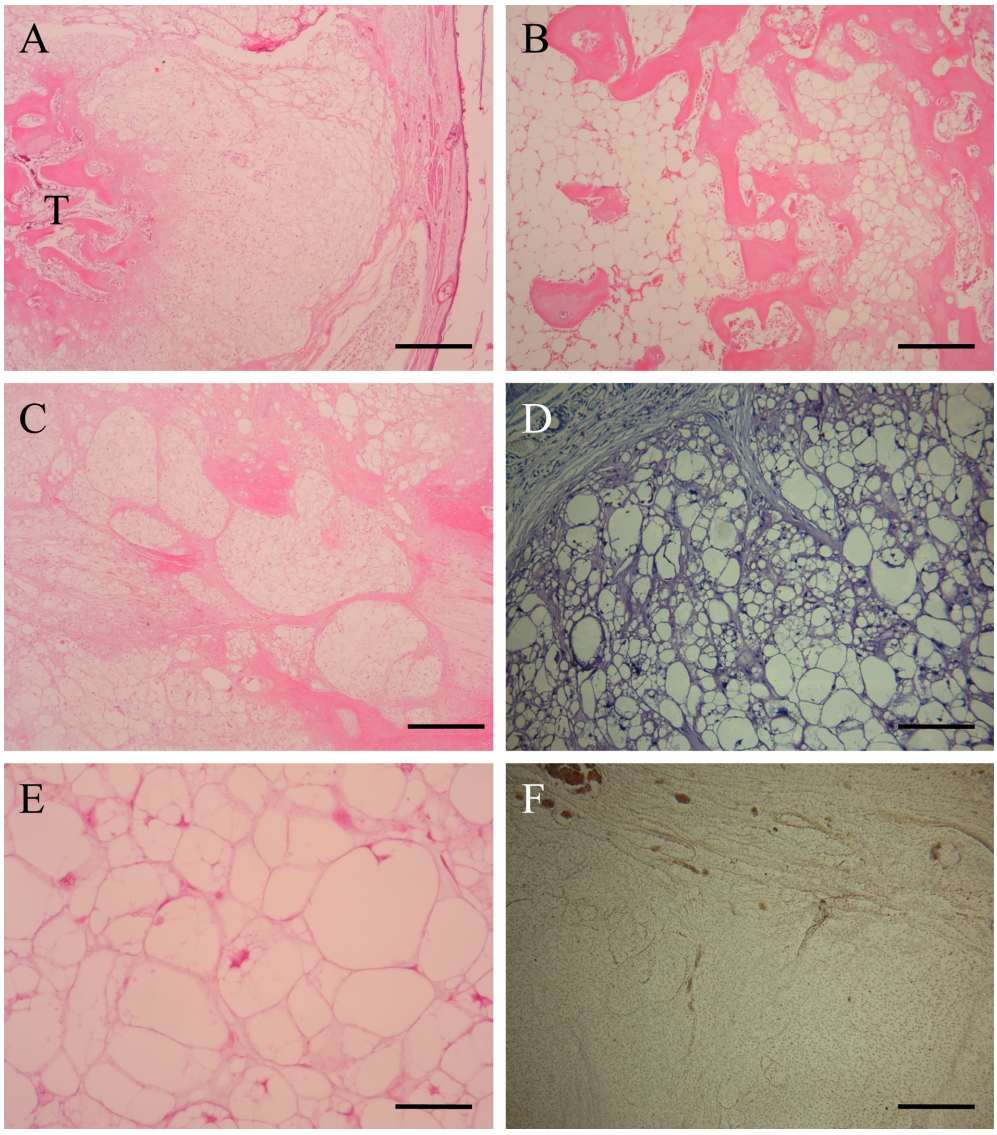

Figure 2. Histopathological lesions of the tumor. (A) Most of the subcutaneous tissues and trabecular bone (T) with marrow is destroyed and replaced by tumor cells. H-E stain, Bar=500 μm. (B) The tumor cells grow into the bone marrow cavity with substantial changes of trabecular bone. H-E stain, Bar=500 μm. (C) Tumor cells are arranged within poorly defined lobules. Within the lobules, pleomorphic cells contain large vacuoles and small dark, centrally located nuclei (physaliferous cells). Also an interlobular amorphous matrix is visible. H-E stain, Bar=500 μm. (D) Between the lobules matrix containing mucin is highlighted with toluidine blue staining. Bar=100 μm. (E) Physaliferous cells contain large clear cytoplasmic vacuoles and small dark nuclei are visible. H-E stain, Bar=50 μm. F) Sudan III staining was negative for lipid. Bar=100 μm.

but also brain, spinal cord, and skin are reported sites for chordoma (Jabara *et al,* 1971; Pease *et al,* 2002; Munday *et al,* 2003). In our case, the tumor arose in the coccygeal vertebrae.

For the differential diagnosis of chordoma, lipo(sarco)ma, myxosarcoma, poorly differentiated chondrosarcoma, infiltrating lipoma, mucinous adenocarcinoma, and sebaceous carcinoma should be considered. Laboratory findings are not helpful in the differential diagnosis of chordoma. A definitive diagnosis of chordoma can only be made based on histopathology. Chordomas have distinct features such as flame figures in histopathologic lesions. The tumors were composed of closely packed adipocyte-like vacuolated cells (physaliferous cells). The nuclei were located centrally or eccentrically and the cytoplasm contained large vacuoles. Fibrous tissues contained abundant extracellular basophilic material that was consistent with mucin (Munday *et al,* 2004).

For accurate diagnosis, immunohistochemical studies are necessary. On immunohistochemistry, physaliferous cells show dual expression of cytokeratin and vimentin intermediate

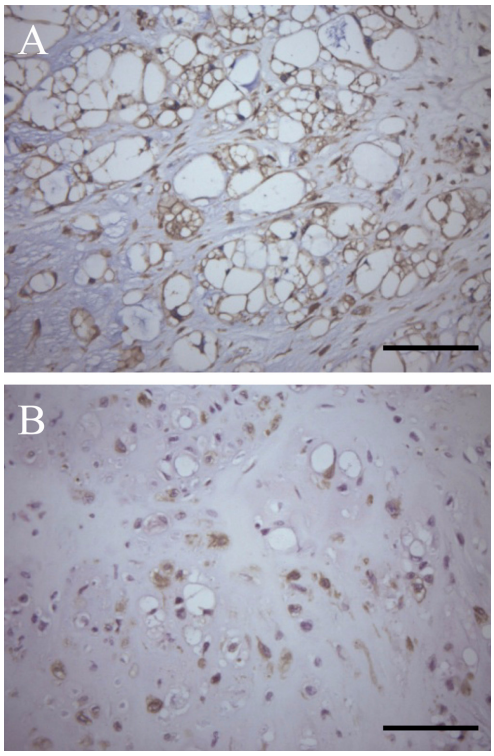

Figure 3. Photographs of the tumor with immunohistochemical stains. (A) Immunohistochemistry for vimentin shows diffuse positive reactions. (B) Immunohistochemistry for S-100 protein shows diffuse positive reactions. ABC method, Bar=100 μm.

filaments and sporadically express S-100 protein (Nakajima *et al,* 1982). This feature offers a clear differentiation from other tumors. In the present study, many neoplastic cells expressed vimentin and S-100 protein (Dunn *et al,* 1991), which can be helpful in distinguishing chordomas from chordoma with chondroid differentiation (Ishida *et al,* 1994).

Limited information is available on the treatment of chordoma. They are best treated by aggressive surgical removal. The rate of recurrence after surgical removal is high in human chordomas (Heffelfinger *et al,* 1973). One canine chordoma, reported in 1933, recurred 12 days after surgery, and the neoplasm returned to presurgical size after 30 days (Ball *et al*, 1933). Complete surgical resection followed by radiation therapy offers the best chance of long-term control in humans. In addition, human chordomas most commonly metastasize to the lung, skeleton, and liver (Volpe *et al,* 1983). Metastases to the lung were reported in 75% of rats (Stefanski *et al,* 1988), and the single feline case metastasized to a local lymph node (Carpenter *et al,* 1990). Adjacent cutaneous metastasis was observed in ferrets (Munday *et al,* 2004).

In conclusion, a grayish-white, expansile, subcutaneous soft-tissue mass was observed in the tail of a ferret. Histopathologically, the tumor was composed of closely packed adipocyte-like vacuolated cells (physaliferous cells) that were positive for vimentin and S-100 immunohistochemically. Based on the histopathologic findings and special staining characteristics, this case was diagnosed as a chordoma.

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