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Sarcomas in Three Free-ranging Northern Sea Otters (*Enhydra lutris kenyoni*) in Alaska

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ABSTRACT: Three sarcomas were diagnosed in wild northern sea otters (*Enhydra lutris kenyoni*) during the mid- to late 1990s. Histologically, the tumors were a chondrosarcoma and two low-grade fibrosarcomas with myofibroblastic cell differentiation. The three sea otters were surviving in the wild and were killed by hunters.

Key words: *Enhydra lutris*, marine mammal, mesenchymal tumor, sarcoma.

Neoplasms that have been reported in the sea otter (*Enhydra lutris*) include leiomyomas, leiomyoma concurrent with a cholangiocellular adenocarcinoma and a pheochromocytoma, lymphosarcoma, malignant seminoma in a retained testicle, carcinoma of probable parathyroid origin, lymphoproliferative disorder, osteosarcoma, oligodendroglioma, and another seminoma (Newman and Smith, 2006). We report three sarcomas in northern sea otters (*Enhydra lutris kenyoni*). All were submitted by subsistence hunters as skinned carcasses to the US Fish and Wildlife Service.

For all cases, formalin-fixed and paraffin-embedded tissue samples were sectioned at 4–6 μm and stained with H&E and Masson's trichrome method for light microscopic evaluation. For cases 2 and 3, immunohistochemical staining was performed on deparaffinized sections with linked streptavidin–biotin (LSAB) complex technique by using the LSAB detection kit (Dako North America, Inc., Carpinteria, California, USA) with diaminobenzidine as the chromogen. Antibodies to the following antigens were used: muscle-specific actin (Enzo, Plymouth Meeting, Pennsylvania, USA; 1:1000), smooth muscle actin (Sigma-Aldrich, St. Louis, Missouri, USA; 1:4000), S-100 protein (Dako North America, Inc.; 1:800), glial fibrillary acidic protein (GFAP) (Dako North America, Inc.; 1:1000), and vimentin (Biogenex, Fremont, California, USA; 1:1600). Microwave epitope retrieval was performed before the immunostaining for S-100 protein. Canine tissue was used for external positive and negative controls. Pre-existing normal sea otter tissue structures were used as internal controls when possible. For ultrastructural studies on cases 2 and 3, tumor tissue was deparaffinized, hydrated, postfixed in 1% osmium tetroxide, dehydrated, cleared, and embedded in epoxy resin. One-micrometer sections were cut and stained with toluidine blue for preliminary light microscopic evaluation. Thin sections (80–90 nm) were cut, stained with uranyl acetate and lead citrate, and examined with an EM10 transmission electron microscope (Carl Zeiss GmbH, Jena, Germany).

Case 1: A mature female sea otter was shot near Sitka, Alaska, USA (57°10′N, 135°10′W) in 1995. The unknown-age animal was in poor body condition, and the skinned carcass weighed 23.6 kg. There was shellfish flesh in the stomach and scant amounts of digesta in the intestines, indicating the animal had continued to forage. There was a 33×30.5×16-cm, 7.7-kg mass within the thoracic cavity just caudal to the proximal aspect of the left scapula attached to the fifth through the ninth intercostal spaces. It infiltrated the seventh rib and caused the eighth rib to bow out laterally. The mass largely filled the thoracic cavity and caused caudal displacement of the diaphragm and dorsal compression of the

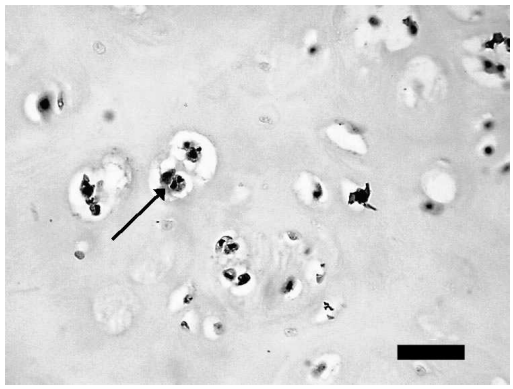

FIGURE 1. Histologic appearance of the chondrosarcoma from case 1. Single to multiple small, condensed cells were present within lacunae (arrow) and these were separated by large amount of basophilic ground substance. Bar=20 μm. 40×.

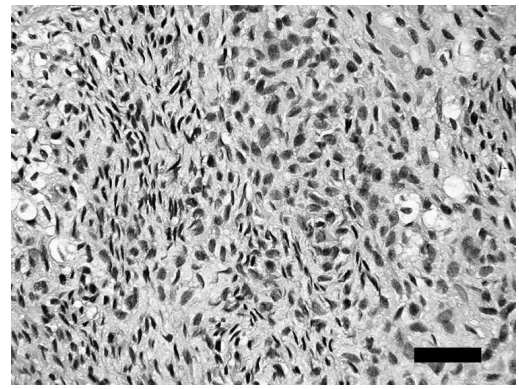

FIGURE 2. Section from the fibrosarcoma from case 2. The majority of cells were spindle-shaped, often oriented in short wavy bundles. Scattered round cells surrounded by clear lacunae also were present (seen on the left and right sides of the photomicrograph). Bar=20 μm. 40×.

lungs and heart. The mass was encased in a glistening white fibrous capsule, and on cut section, there were swirls of translucent white tissue resembling cartilage. Histologically, the majority of the mass was composed of whirls of collagen and wispy, basophilic ground substance. Scattered individual and aggregates of round neoplastic cells surrounded by a clear space and basophilic amorphous material consistent with ground substance (Fig. 1). These cells were plump, had scant cytoplasm with round nuclei, and fine granular chromatin. Mitotic figures were rare. This neoplasm was consistent with a low-grade chondrosarcoma.

Case 2: A 9-year-old (as determined by tooth-sectioning) male sea otter was taken in eastern Prince William Sound, Alaska, USA (65°10′N, 135°40′W) in 1996. The skinned carcass weighed 26.8 kg. On the medial aspect of the left hind leg overlying the distal aspect of the tibia and fibula, there was an ulcerated, dermal and subcutaneous, oval, 13×13×4.5-cm, 455.5-g mass that was not attached to the bone. The surface of the mass was covered with necrotic debris and fibrin. The superficial aspect of the mass was multilobular, soft, white, and of fatty consistency. The deeper aspect of the mass was very firm, infiltrative, and white, with septae extending into the overlying soft mass. The tumor tissue extended up the leg along the tibial nerve bundle. The distal extremity had marked subcutaneous edema. All tissues were moderately autolyzed, obscuring some histologic detail. Histologically, the dense white tissue within the deeper aspects of the mass was composed of thick bands of dense connective tissue with scattered aggregates of lymphocytes and plasma cells. The mass was highly cellular, composed of spindle-shaped to round cells and was divided into lobules by dense collagenous bands. The spindle cells were generally haphazardly arranged with some short and intermediate length bundles with an undulant pattern. The cells had poorly delineated cytoplasmic borders, amphophilic cytoplasm, and oval to round nuclei with fine granular chromatin and rare mitotic figures. In some areas, round cells with sharply delineated borders and clear cytoplasm were embedded in an amorphous to foamy, lightly eosinophilic to lightly basophilic material (Fig. 2). Scattered islands of amorphous eosinophilic material with central, deeply amphophilic to basophilic material were consistent with mineralized osteoid, and entrapped small cells were consistent with osteocytes, interpreted as osseous metaplasia (Fig. 3). Ultrastructurally, the neoplastic cells were elongate, with oval

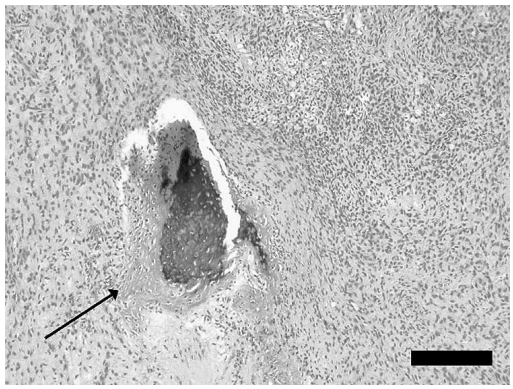

FIGURE 3. In some areas of the fibrosarcoma from case 2, there were islands of woven bone (arrow). Bar=100 μm. 10×.

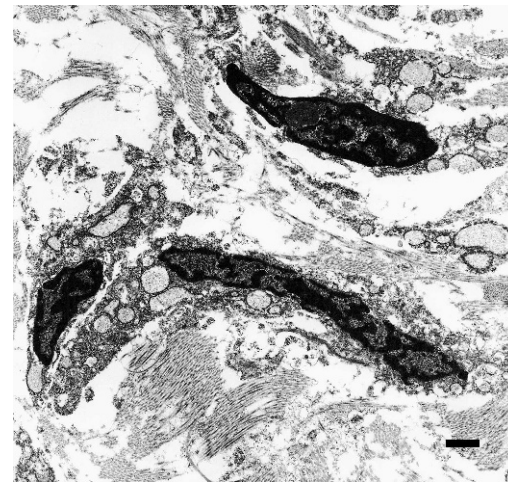

FIGURE 4. Both fibrosarcomas from sea otter cases 2 and 3 had similar ultrastructural features. Neoplastic cells were elongate, with oval to fusiform nuclei containing abundant electron-dense heterochromatin. Cells were separated by collagen bundles. No basement membranes or virus particles were present. Bar=1 μm.

to fusiform nuclei containing abundant electron-dense heterochromatin and were separated by collagen bundles (Fig. 4). No basement membranes were present. No virus particles were detected. Immunohistochemically, the neoplastic cells were multifocally weakly positive for muscle specific actin and smooth muscle actin. Immunostaining for vimentin, S-100 protein, and GFAP were inconclusive. These findings were most consistent with a low-grade fibrosarcoma with myofibroblastic differentiation.

Case 3: Hunters killed an 8-year-old (as determined by tooth-sectioning) male sea otter in Yakutat Bay, Alaska, USA (59°40′N, 139°30′W) in February of 1998. It weighed 30.5 kg after skinning and was in average body condition. In the retroperitoneal space, lateral to the right kidney, there was a 24×20×16-cm, 6-kg mass attached to and blending into the 14th right rib. On section, it was firm, mottled tan, white, and cream. The tan areas were necrotic tissue and made up the majority of the mass. Histologically, in sections taken from the junction between tumor and rib, the tumor infiltrated the cortical bone of the rib. The mass was highly cellular, composed of spindle cells separated by variable amounts of collagen and mucinous ground substance. In general, the cells formed short to long interlacing streams and bundles. The cells were spindle-shaped to round with the spindle cells being serpentine, with oval nuclei with fine granular chromatin, inconspicuous chromatin, and rare mitotic figures (Fig. 5). Similar to case 2, the neoplastic cells were immunoreactive for muscle-specific actin and smooth muscle actin; immunostains for S-100 protein, vimentin, and GFAP were noncontributory. Likewise, the ultrastructural features were comparable to case 2. The diagnosis was low-grade fibrosarcoma with myofibroblastic differentiation.

The chondrosarcoma we report occurred at the costochondral junction, the most frequent site of origin of rib chondrosarcomas in dogs (Pirkey-Ehrhart et al., 1995). Chondrosarcomas are malignant neoplasms consisting of mesenchymal cells that produce a cartilage or fibrillar matrix but not osteoid. They are the second most common primary bone tumor in dogs, accounting for 10% of primary bone tumors in that species. In other domestic species, chondrosarcomas are relatively rare (Thompson, 2007). In all species, they occur most often on flat bones such as ribs and nasal turbinates and are slow to metastasize (Popovitch et al., 1994). There was no evidence of metastasis of the

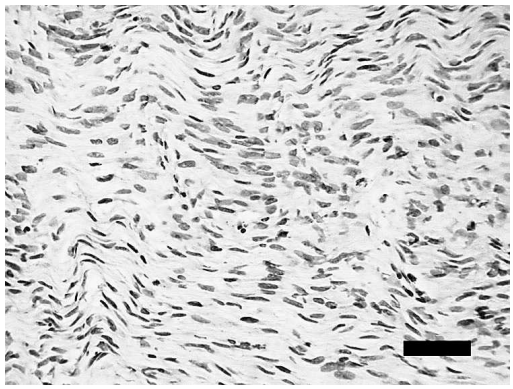

FIGURE 5. The fibrosarcoma from case 3 was composed of long wavy bundles of spindeloid cells. Bar = 20 μm. 40×.

tumor to the lungs, the most frequent site of metastasis of canine costal chondrosarcoma (Thompson, 2007). This tumor was an unusually large mass to be found in a surviving, free-ranging sea otter, comprising approximately one third of the weight of the skinned carcass. Many dogs with chondrosarcoma can survive for months and some for >2 yr, with death resulting from the primary tumor rather than from metastatic disease (Popovitch et al., 1994).

Tumors from two of the sea otters were low-grade fibrosarcomas with no evidence of metastasis. Fibrosarcomas are malignant neoplasms arising from fibroblasts in any anatomic location. In domestic cats and dogs, they are generally low-grade, demonstrating frequent local recurrence and low incidence of metastasis that occurs late in the disease (Popovitch et al. 1994). A high mitotic index is correlated with incidence of recurrence and metastasis (Gross et al., 1992). Immunohistochemistry indicated myofibroblastic differentiation due to the multifocally positive smooth muscle actin reaction. The immunohistochemical staining in these cases was poor, probably due to the postmortem interval and possibly due to the use of nonspecific antibodies. A peripheral nerve sheath tumor was considered initially as a diagnosis for case 2 due to the tendency of the cells to palisade, the presence of mucinous ground substance, and the extension of the mass along the neurovascular bundle. However, GFAP and S-100 staining did not support this diagnosis, and ultrastructurally, a nerve sheath tumor would be expected to display some basement membrane material, which was not present. Osteoid and mineralized bone was present in one case and was interpreted to be osseous metaplasia within the fibrosarcoma because the cells within the bone were consistent with normal osteocytes rather than neoplastic osteoblasts.

Most reports of neoplasms in marine mammals concern those animals, such as California sea lions (*Zalophus californianus*), that are most frequently presented to rehabilitation programs, and those that are held for public display (Newman and Smith, 2006). There are few reports of neoplasia in sea otters. We were not able to determine a cause for these tumors. Viruses have been associated with the occurrence of mesenchymal tumors (Peauroi et al., 1994). The sea otter tumor tissues were evaluated by electron microscopy for virus particles, but none were found. Because these tumors occurred in middle aged to older animals and were in only one site on each animal, they are probably simply spontaneous tumors without a discernible cause.

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