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Case Report

Spontaneous Osteoblastic Osteosarcoma in a Mongolian Gerbil (*Meriones unguiculatus*)

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Spontaneous neoplasms in Mongolian gerbils have an incidence of 20% to 26.8%, but osteosarcomas occur at a much lower rate. Here we report a 1-y-old Mongolian gerbil with a spontaneous osteosarcoma at the level of the proximal tibia, with metastases to the pectoral muscles and lungs. Grossly, the tibial mass obliterated the tibia and adjacent muscles, and an axillary mass with a bloody, cavitary center expanded the pectoral muscles. Microscopically, the tibial mass was an infiltrative, osteoblastic mesenchymal neoplasm, and the axillary mass was an anaplastic mesenchymal neoplasm with hemorrhage. The lung contained multiple metastatic foci. Immunohistochemistry for osteonectin was strongly positive in the tibial, axillary, and pulmonary metastases. Although osteosarcoma is the most common primary malignant bone neoplasm that occurs spontaneously in all laboratory and domestic animal species and humans, it arises less frequently than does other neoplasms. The current case of spontaneous osteoblastic osteosarcoma of the proximal tibia and metastases to the pectoral muscles and lung in a Mongolian gerbil is similar in presentation, histology, and predilection site of both osteoblastic and telangiectatic osteosarcomas in humans. In addition, this case is an unusual manifestation of osteosarcoma in the appendicular skeleton of a Mongolian gerbil.

Mongolian gerbils are used frequently in biologic research,[1,2,4,9,10,12-14] particularly in oncogenic studies and filariasis research studying *Brugia malayi*.[2] There have been several reports[1,6,10,11,13-15] of spontaneous neoplasms, particularly in gerbils 2 y of age and older, typically occurring with the highest incidences in the skin, reproductive tract, and adrenal glands; however, neoplasms have also been reported in the thyroid, thymus, liver, kidney, pancreas, and bone.[1,6,10,11,13-15] The incidence of spontaneous neoplasms occurring in the subfamily *Gerbillinae* ranges from 20% to 26.8%,[1,6,10,11,13-15] depending on the study, age, and sex of the animals.

With a lower incidence than those reported for other neoplasms, osteosarcomas in gerbils have been described in the ramus of the mandible and as an extraskeletal mass throughout the peritoneum.[10,11] The usual age of onset for osteosarcomas in Mongolian gerbils is approximately 3 y (36 to 39 mo); however, no tumor type has been reported at less than 2 y of age in this species.[10,11] Here we report a spontaneous osteosarcoma that occurred at the level of the proximal tibia, with metastases to the pectoral muscles and lung, in a 1-y-old Mongolian gerbil.

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Case Report

A 1-y-old, 95-g, agouti, male Mongolian gerbil (*Meriones unguiculatus*; Charles River Laboratories International, Wilmington, MA) used in filariasis research (*B. malayi*) was examined because of a hard mass (diameter, 3 cm) at the level of the left stifle that prevented normal ambulation. In addition, a firm mass (diameter, 1 cm) at the level of the left axilla and pectoral muscles was noted during routine physical exam. The animal was in good nutritional status otherwise.

The gerbil was used in a *B. malayi* study approved by the University of Georgia's IACUC and housed at a University Research Animal Resources facility at the College of Veterinary Medicine. The University Research Animal Resources unit at the University of Georgia is fully accredited by AAALAC. The gerbil colony is evaluated quarterly for seropositivity to *Clostridium piliforme* as well as ecto- and endoparasites. None were identified nor have been for this colony in at least 3 y. The animal was group-housed, 5 gerbils per rodent cage (17 in. × 8 in. × 8 in., 136 in.^{2} floor space) on irradiated corncob bedding (Purelite Sanitized Corncob Bedding, Harlan Laboratories, Madison, WI). Cages were changed twice weekly, in accordance with standard operating procedures. The gerbil was fed an irradiated commercial rodent diet (PicoLab Rodent Diet 20, Labdiet, PMI Nutritional International, Brentwood, MO) ad libitum, and filtered, bottled drinking water was provided ad libitum. Environmental enrichment consisted of 2 nesting pads (Gusmer Enterprises, Ancare, Waupaca, WI) supplied to each cage.

Necropsy was performed immediately after CO_2 euthanasia due to a presumptive diagnosis of neoplasia. Multiple sections from the masses and other tissues were dissected and fixed in

neutral-buffered 10% formalin fixative solution. The bony mass was demineralized in Kristensen decalcifying solution. Fixed and demineralized tissues were trimmed, routinely processed, embedded in paraffin, sectioned at about 5 mm, and stained with hematoxylin and eosin.

Sections from the tibial and axillary masses as well as lung tissue underwent osteonectin immunohistochemistry. Briefly, paraffin-embedded tissues were sectioned at approximately 4 µm, deparaffinized and rehydrated. The primary antibody used was an antigen affinity-purified polyclonal goat antihuman SPARC–osteonectin antibody (catalog no. AF941, R and D Systems, Minneapolis, MN). Antibody dilutions were tested previously on positive controls to find the greatest dilution of antibody that yielded optimal staining (that is, 1:500 with staining time of 60 min). The SPARC–osteonectin antibody was diluted by using Antibody Diluent (catalog no. S0809, Dako, Carpinteria, CA). Heat-induced epitope retrieval was performed by using citrate buffer (pH 6.0; HK086-9K, Biogenex, San Ramon, CA) at 120 °C for 10 min. Endogenous peroxidase was blocked by using 3% hydrogen peroxide (H312-500, Fisher Scientific, Fair Lawn, NJ). All other blocking was achieved by using Background Punisher (BP974, Biocare Medical, Concord, CA). Positive tissue controls consisted of formalin-fixed, paraffin-embedded gerbil kidney, because murine renal tubular epithelia express osteonectin.[5] Normal goat immunoglobulins diluted in PBS and supplied as a ready-to-use reagent (HK406-5G, Biogenex) were used in place of the primary antibody for a negative antibody control. The secondary antibody used was biotinylated rabbit antigoat IgG (catalog no. BA5000, Vector Labs, Burlingame, CA). Streptavidin conjugated to horseradish peroxidase (catalog no. K1016, LSAB 2, Dako) was used to label the biotinylated antibody.

A procedure similar to that just outlined was used to perform factor VIII immunohistochemistry on sections from the axillary masses. The primary antibody was polyclonal rabbit antihuman von Willebrand factor (catalog no. A0082, Dako), which was provided as a purified IgG fraction of rabbit antiserum. The von Willebrand factor immunogen was isolated from human plasma. The optimal dilution of the polyclonal rabbit antihuman von Willebrand factor antibody was 1:2000 with staining time of 45 min. The primary antibody was diluted by using Antibody Diluent (catalog no. S0809, Dako). Antigen retrieval, endogenous peroxidase, and protein blocking steps were performed as described earlier. Positive tissue controls consisted of formalin fixed, paraffin-embedded canine skin. As a negative control, the primary antibody was eliminated and substituted with a universal negative control, provided as the immunoglobulin fraction of serum from nonimmunized rabbits. The secondary antibody used was biotinylated goat antirabbit IgG (catalog no. BA1000, Vector Labs). The tertiary antibody was streptavidin conjugated to horseradish peroxidase (catalog no. K1016, Dako) in PBS.

For both osteonectin and factor VIII immunohistochemistry, the substrate–chromogen system used was DAB (K3466, Dako). The tissue sections were counterstained with Gills II hematoxylin and bluing; dehydrated in alcohol levels of 70%, 95%, and 100%; cleared in xylene; and mounted by using a xylene-based mounting medium.

Results

Grossly, a light-tan, hard, bony mass (diameter, 3 cm) had obliterated the bony tissues of the proximal left tibia and adjacent skeletal muscles (Figures 1 and 2). On cut surface, the bony mass did not involve the knee joint or the adjacent distal femur. At the level of the left axilla and within the pectoral muscles, a 1-cm dark-red mass was noted (Figure 3). There were no other noteworthy gross findings.

Microscopically, an infiltrative, bone-forming mesenchymal neoplasm with multifocal hemorrhage had destroyed the proximal left tibia, extended into the adjacent skeletal muscles, and spared the knee joint and adjacent femur and patella. The densely cellular neoplasm was composed of pleomorphic spindled cells arranged into interlacing bundles and irregular anastomosed bony trabeculae, with foci of mineralization and osteoid formation, within a fibrovascular stroma (Figure 4). Spindled cells had moderately abundant eosinophilic and vacuolated cytoplasm with indistinct cell borders. The hyperchromatic nuclei varied from round to oval to fusiform, with vesicular and sometimes finely stippled chromatin, and 1 to 3 prominent, magenta nucleoli; 10 mitoses were noted in 10 random fields at a magnification of 400×. Multinucleated cells were present.

The axillary mass consisted of an anaplastic mesenchymal neoplasm, with multifocal hemorrhages, necrosis, and bone formation (Figure 5). The densely cellular neoplasm was composed of pleomorphic spindled cells arranged into interlacing bundles and irregular blood-filled spaces within a fibrillar eosinophilic stromal matrix. We counted 38 mitoses in 10 random fields (magnification, 400×). Hypercellular clusters of neoplastic cells obscured the lung tissues multifocally (Figure 6). Immunohistochemistry for osteonectin was strongly positive in the tibial, axillary, and pulmonary metastases (Figures 4 through 6). Immunohistochemistry for factor VIII was negative in the axillary mass. Additional findings included multiple granulomas in the liver and heart. A single microfilaria, approximately 30 mm in diameter with a curved tail and containing basophilic granular materials, was noted in the liver.

Discussion

The masses found in this Mongolian gerbil were characteristic of an osteonectin-positive osteoblastic osteosarcoma of the tibia with metastasis to pectoral muscles and lung. Histopathologic features of the axillary mass, characterized by interlacing bundles and irregular blood-filled spaces lined by pleomorphic spindled cells that were negative to factor VIII immunohistochemistry, resembled those associated with telangiectatic osteosarcomas in other species.[16]

Osteosarcomas are primary malignant bone neoplasms with high metastatic potential that can be classified by histology into the following categories: poorly differentiated, osteoblastic, chondroblastic, fibroblastic, telangiectatic, and giant-cell.[3,4,8,9,12] Osteosarcoma is the most common primary malignant bone neoplasm, and it occurs spontaneously in all laboratory and domestic animal species as well as humans, but at much lower frequency than do other neoplasms.[3,4,8,9,12]

In a retrospective study, the frequency of occurrence of spontaneous bone neoplasms was less than 1% for most mouse strains, with the exceptions of osteosarcomas in NOD/ShiLtJ (11.5%) and NOD-derived (7.1%) mice derived at the Jackson Laboratory.[3] The age, strain, and sex of mice with spontaneously occurring osteosarcoma varied as well. There are also reports of the induction of osteosarcomas by murine polyomaviruses, bone-seeking radionuclides, and irradiation and in genetically modified mice with

Figure 1. Bone, osteosarcoma. Mongolian gerbil (*M. unguiculatus*). The left tibia has a 3-cm hard mass (arrow). Bar, 1 cm.

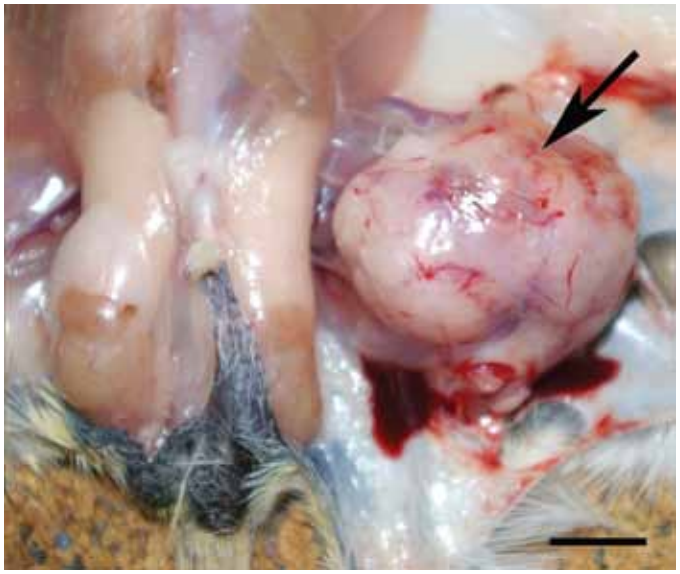

Figure 2. Bone, osteosarcoma. Mongolian gerbil (*M. unguiculatus*). Mass in Figure 1, after deflection of the skin (arrow). The light-tan, hard, bony mass has obliterated the proximal left tibia and adjacent skeletal muscles. Bar, 1 cm.

Trp53 mutations. These features make the mouse a viable model for the study of osteosarcoma and its metastatic properties.[3]

Although rare, spontaneous osteosarcomas and their associated pathologic features have been reported in domestic and laboratory rabbits.[4] Although the predilection sites and metastases for primary osteosarcoma in rabbits are unknown, appendicular skeletal osteosarcomas in the proximal humerus with varying degrees of joint involvement have been reported in that species, as have lesions of the axillary skeleton and left thigh, with metastasis to the lungs (as expected) in other domestic species.[4] Features of osteosarcomas of the bones of the skull and thoracic cavity of

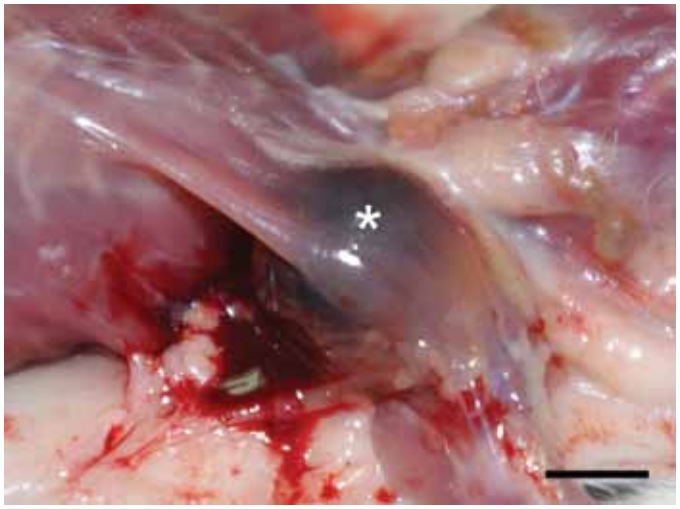

Figure 3. Skeletal muscle, metastatic osteosarcoma. Mongolian gerbil (*M. unguiculatus*). At the left axilla and within the pectoral muscles is a 1-cm diameter dark-red mass (asterisk). Bar, 0.5 cm.

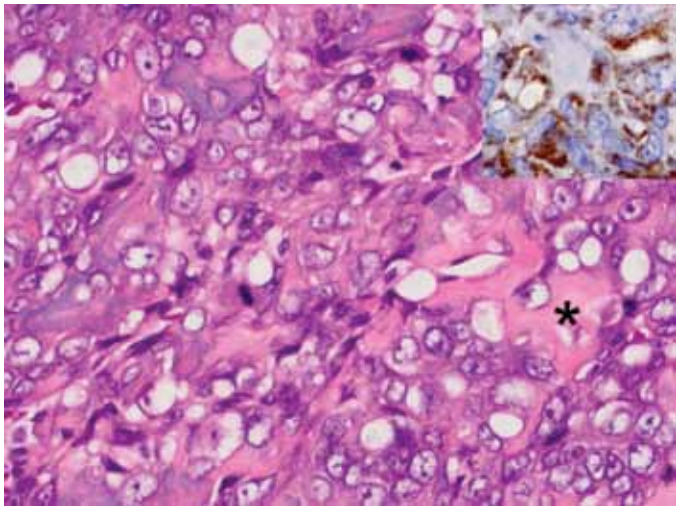

Figure 4. Bone, osteosarcoma. Mongolian gerbil (*M. unguiculatus*). Representative area from the densely cellular neoplasm, with pleomorphic spindled cells in interlaced patterns and osteoid (asterisk). Hematoxylin and eosin stain; original magnification, 400×. Inset. Immunohistochemical staining for osteonectin. Several neoplastic cells stained positively for osteonectin, which is depicted as a granular brown pigment in the cytoplasm. Hematoxylin counterstain; original magnification, 400×.

rabbits are similar to those of previously reported cases of osteosarcomas in Mongolian gerbils.[10,11]

Spontaneous osteogenic sarcomas in rats are rare, but the neoplasm can be experimentally induced in that species by using radiation, chemicals, or the Moloney sarcoma virus and can be transplanted; the induced tumor has been proposed as a model for the study of human osteosarcoma.[7,12] The occurrence of appendicular skeletal spontaneous osteosarcoma in rats has been reported to be similar to that of other domestic species,[4] but unusual presentations, such as in the lumbar, sacral, and coccygeal vertebrae, have been reported.[12] Such examples highlight the possible diverse manifestations of osteosarcoma in domestic species.

The classic osteosarcoma in humans is a rare (0.2% of all malignant tumors), highly malignant tumor, with an estimated inci-

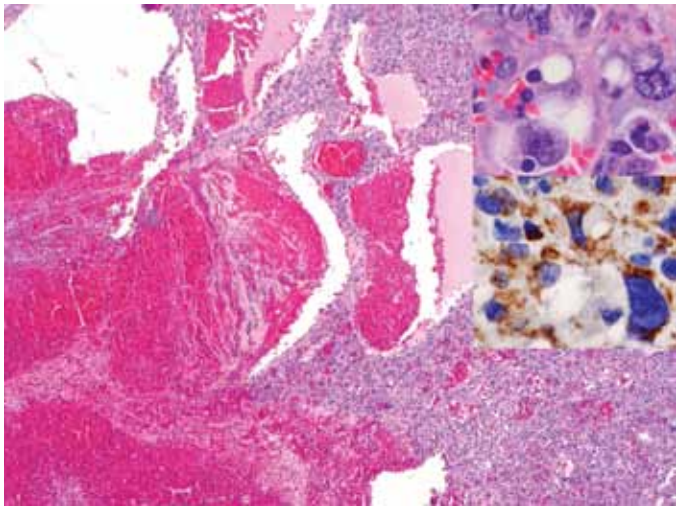

Figure 5. Skeletal muscle, metastatic osteosarcoma. Mongolian gerbil (*M. unguiculatus*). Representative area from the axillary mass, with irregular blood-filled spaces. Hematoxylin and eosin stain; original magnification, 45×. Upper inset. Neoplastic cells are very anaplastic. Hematoxylin and eosin stain; original magnification, 400×. Lower inset. Immunohistochemical staining for osteonectin. Several neoplastic cells stained positively for osteonectin, which is depicted as a granular brown pigment in the cytoplasm. Hematoxylin counterstain; original magnification, 400×.

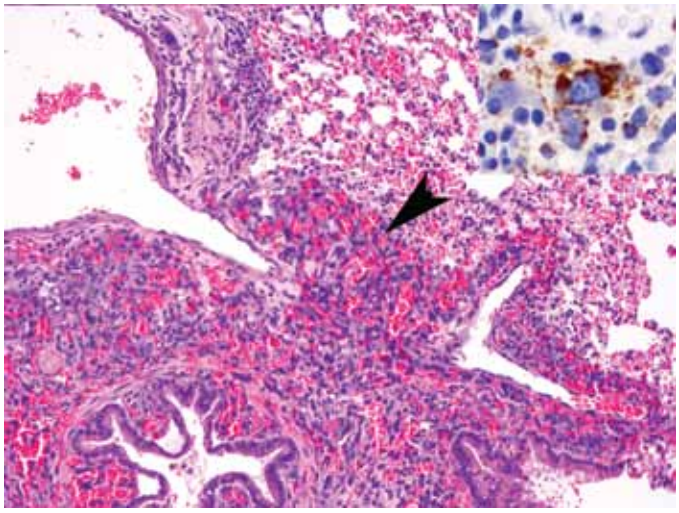

Figure 6. Lung, metastatic osteosarcoma. Mongolian gerbil (*M. unguiculatus*). Arrowhead depicts a hypercellular focus. Hematoxylin and eosin stain; original magnification, 200×. Inset. Immunohistochemical staining for osteonectin. Neoplastic cells stained positively for osteonectin, depicted as a granular brown pigment in the cytoplasm. Hematoxylin counterstain; original magnification, 400×.

dence of 3 cases per million persons annually and predominantly arises (80% to 90% incidence) in the long bones and rarely in the soft tissues.[8] Onset in humans is typically in young adults rather than children; this pattern is similar to the age distribution in other domestic species, including Mongolian gerbils.[1-16] In humans and various domestic species, the femur, tibia, and humerus account for about 85% of extremity tumors, whereas fewer than 1% of osteosarcomas in humans are found in hand and foot bones.[8] The etiology of osteosarcoma in humans is unknown but has been hypothesized to be virally induced, owing to the incidence of virally induced osteosarcomas in laboratory animals as well as the known occurrence of radiation-induced osteosarcomas in humans and laboratory animals.[8]

In humans, telangiectatic osteosarcoma is a rare subtype of osteosarcoma that represents from 2% to 12% of all cases of osteosarcoma.[16] Telangiectatic osteosarcoma is characterized by multiple, aneurysmally dilated, blood-filled cavities with high-grade, sarcomatous cells in the peripheral rim and septae.[16] The case reported herein of spontaneous osteoblastic osteosarcoma of the proximal tibia and telangiectatic osteosarcoma metastases in the pectoral muscles of a Mongolian gerbil is similar in presentation, histology, and predilection site of both osteoblastic osteosarcomas and telangiectatic osteosarcomas in humans, with the exception that both tumor types occurred in the same animal subject.

In conclusion, this case of spontaneous osteosarcoma in a Mongolian gerbil is consistent with the usual manifestation and presentation of spontaneous osteosarcoma in other domestic and laboratory animal species and humans. However, our gerbil was younger than the reported usual age of onset of neoplasms in Mongolian gerbils. Furthermore, this case of spontaneous osteoblastic osteosarcoma with telangiectatic metastases to the pectoral muscles is an unusual manifestation of such a neoplasm in the appendicular skeleton of a Mongolian gerbil. The current report describes the histopathologic and immunohistochemical characterization of an osteoblastic osteosarcoma with telangiectatic metastases in a Mongolian gerbil that likely will further aid in the diagnosis of this very rare neoplasm in this species.

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