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NOTE

Pathology

Chondrosarcoma with undifferentiated neoplastic cell proliferation around the distal tibiotarsus bone in a wild Hooded Crane (*Grus monacha*)

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ABSTRACT. An adult male Hooded Crane was found dead on the Izumi plane. At autopsy, subcutaneous nodules were found around the medial and lateral sides of the left distal tibiotarsus bone. The largest cross-section of the masses revealed a multilobular pattern, with small amounts of viscous mucus. Histopathologically, the nodules were composed of three types of neoplastic cells: chondrocytic cells with abundant lightly basophilic cartilaginous matrices, mesenchymal cells and a small portion of the neoplastic tissue consisted of undifferentiated neoplastic cells exhibiting a high mitotic count and frequent multinucleation. This is the first case of a chondrosarcoma including undifferentiated neoplastic cell proliferation in a wild Hooded Crane.

KEY WORDS: chondrosarcoma, Hooded Crane, tibiotarsus bone, undifferentiated cell

Chondrosarcomas are malignant tumors that produce a cartilaginous matrix and exhibit a low incidence among primary bone tumors [5, 23]. Based on their origins, chondrosarcomas are classified into three categories: the central, periosteal, and extraskeletal types [5, 23]. Central chondrosarcomas arise in bone tissues. Periosteal chondrosarcomas are considered to develop from the periosteum [20], so they form on bone surfaces. Extraskeletal chondrosarcomas arise in non-skeletal soft tissues, e.g., in the subcutis, cardiac and skeletal muscle, retroperitoneum, or mammary gland [2, 16, 17, 23].

Primary periosteal and extraskeletal chondrosarcomas are rare in animals, which is not the case for central chondrosarcomas [23]. In avian species, it is known that avian leukosis/sarcoma viruses not only induce lymphoid leukosis, but can also cause several mesenchymal tumors, including chondrosarcomas in domestic chickens [4]. On the other hand, there have only been four published cases of chondrosarcoma involving wild birds [9, 10, 19, 21]and only a few cases of multiple chondromas and chondrosarcomas involving Sandhill Cranes (*Grus canadensis*) or Whooping Cranes (*Grus americana*) have also been described [6]. Thus, little detailed information is available about cases of chondrosarcoma involving wild birds. Herein, we describe the first case of chondrosarcoma with undifferentiated neoplastic cell proliferation in a wild Hooded Crane (*Grus monacha*).

An adult male Hooded Crane was found dead on the Izumi plane, a major-overwintering site for cranes in Japan. Since highly pathogenic avian influenza viruses were isolated from dead cranes on the Izumi plain in the 2010–2011 winter season [18], all of the crane carcasses found on the Izumi plain are subjected to virological and pathological examinations [14, 15]. At first, swab samples from the conjunctiva, trachea, and cloaca were collected and tested for the influenza A viral gene using reverse transcriptase-PCR, as described previously [14]. The influenza A viral gene was not detected in any of the swab samples.

At autopsy, subcutaneous nodules with ulceration were found around the medial (3.5 cm in diameter) and lateral (7.5 × 5.0 × 3.5 cm in size) sides of the left distal tibiotarsus bone (Fig. 1A). The largest cross-section of the masses showed that the nodules were well demarcated and arranged in a multilobular pattern, because of separation by delicate stromal tissue. Small amounts of viscous mucus were also noted. The overall appearance of the cross-section resembled that of cartilage (Fig. 1B). A hemorrhagic focus was also observed. No adhesion to the bone or invasion into the intertarsal articular space was seen. We also obtained the following minor findings: mild atrophy of both superficial pectoral muscles; small white spots scattered throughout the liver; and diffuse follicular hyperplasia in the spleen. The collected tissues were fixed in 10% buffered formalin, routinely processed, and

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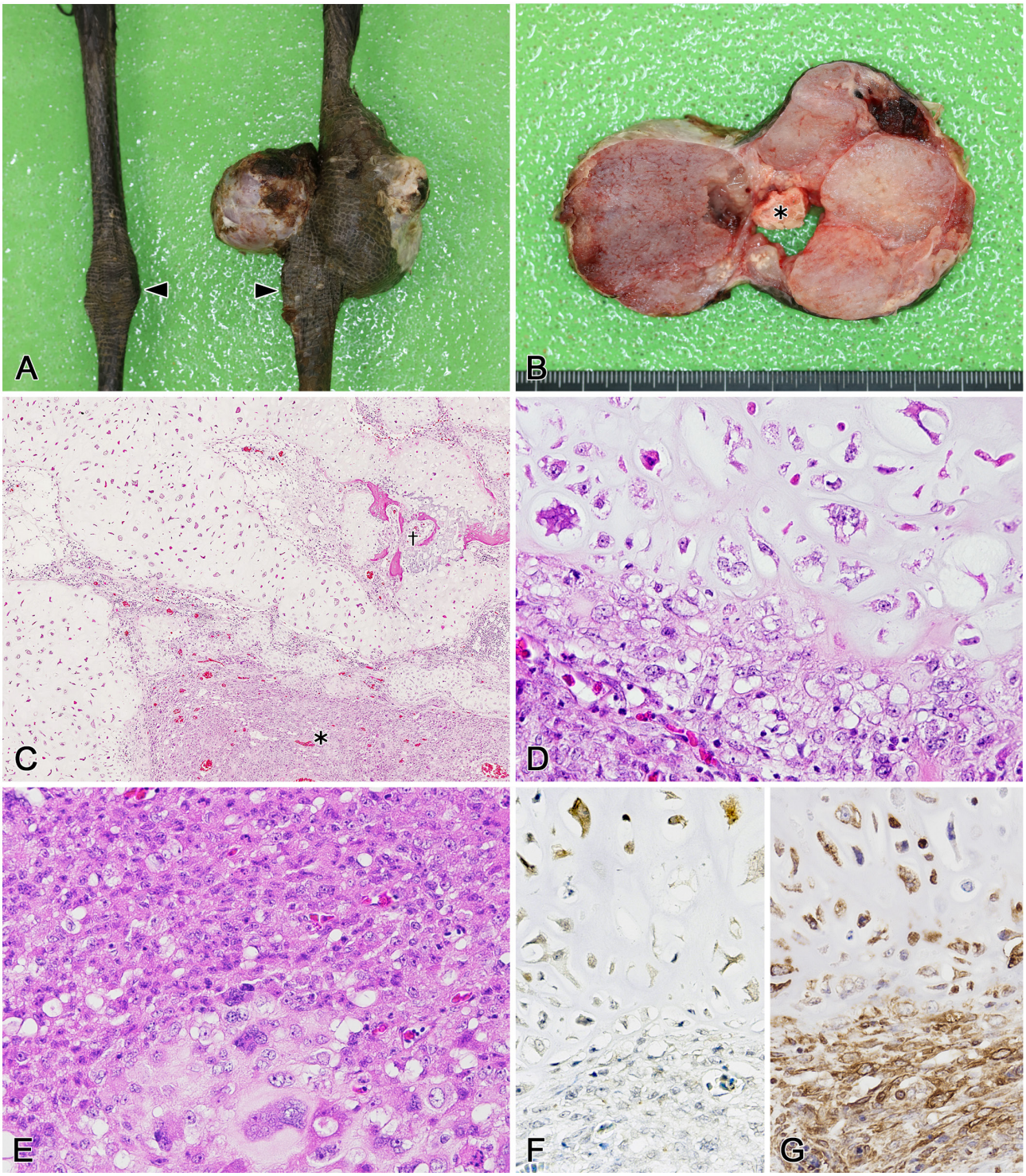

Fig. 1. Chondrosarcoma in a Hooded Crane. (A) The dorsal view of the nodules that form on the medial and lateral sides of the left distal tibiotarsus bone. The arrowheads indicate the intertarsal joints. (B) The distal aspect of the largest cross-section. The masses consist of multiple well-demarcated nodules, which resemble cartilage tissue and are arranged in multiple small lobules. The tibiotarsus bone (*) is intact. (C) The mass consists mainly of cartilaginous tumor with an interstitial portion. Partial proliferation of undifferentiated cells is observed within the mass (*). Scattered endochondral ossification is observed (†). Hematoxylin and Eosin (HE). (D) Chondrocytic neoplastic cells with deposits of abundant basophilic chondroid matrix (upper side). Densely packed mesenchymal neoplastic cells proliferate at the periphery of the chondroid region (lower side). HE. (E) The undifferentiated cells proliferate (upper side) and exhibit weakly eosinophilic deposition and multinucleation (lower side). HE. (F) The chondrocytic neoplastic cells are S-100-positive. Immunohistochemistry (IHC). (G) Both the chondroid and mesenchymal neoplastic cells are vimentin-positive. IHC.

embedded in paraffin. Sections (3-μm-thick) were stained with hematoxylin and eosin or toluidine blue (pH 7.0), or used for the labeled-polymer immunohistochemistry (Histofine Simple Stain MAX PO; Nichirei Biosciences, Tokyo, Japan).

Most of the mass consisted of multiple cartilaginous tumor cell and interstitial part (Fig. 1C); however, a small portion of the neoplastic tissue was composed of undifferentiated neoplastic cells (Fig. 1C*). In the chondral part, two types of proliferating neoplastic cells were seen. The major cells were chondrocytic cells with abundant slightly basophilic cartilaginous matrices (Fig. 1D, upper side), which exhibited metachromasia by toluidine blue staining (Supplementary Fig. 1A). These cells and their matrices formed lobules, which were separated by a loose stroma and displayed varying degrees of hyaline cartilage-like differentiation. The neoplastic cells, which were found in irregular round-to-oval lacunae, included variable size and number of nuclear and cytoplasmic vacuoles, and exhibited occasional binucleation or shrunken nuclei and/or cytoplasm. Endochondral ossification was also scattered throughout this type of neoplastic tissue (Fig. 1C†). The minor cell type was proliferating mesenchymal cells, which were densely packed in the interlobular regions and observed occasionally along the periphery of the cartilaginous regions (Fig. 1D, lower side). These cells had spindle-to-polyhedral, or stellate shapes and possessed pale cytoplasm containing vacuoles. They sometimes formed small foci of cartilaginous matrix. Both types of neoplastic cells exhibited cellular atypia and anisokaryosis with distinct nucleoli, but neither of them displayed obvious invasive growth. The mitotic counts of both types of cells were <1 cell per 10 high power fields (HPFs; 400×, 0.237 mm^2). The undifferentiated cells were round to polyhedral in shape and had eosinophilic to amphophilic cytoplasm (Fig. 1E, upper side). They demonstrated conspicuous cellular and nuclear atypia with frequent multinucleation (Fig. 1E, lower side). In this area, there were few stromal components, but many capillaries and multiple hemorrhages were seen. A small amount of a weakly eosinophilic fine fibrillar substance (Fig. 1E, lower side), which did not display metachromasia by toluidine blue staining, was occasionally deposited around the cells. Moreover, a transition to chondroid structures was observed in a few places. The mitotic count was 10 cells per 10 HPFs. Immunohistochemistry revealed that the chondrocytic neoplastic cells were positive for rabbit anti-human S-100 polyclonal antibody (1 in 300 dilution; Spring Bioscience, Pleasanton, CA, USA) (Fig. 1F) and mouse anti-vimentin monoclonal antibody (clone Vim 3B4; 1 in 200 dilution; DakoCytomation, Glostrup, Denmark) (Fig. 1G), whereas the mesenchymal and undifferentiated cells were only positive for vimentin (Fig. 1G, Supplementary Fig. 1B). The other histological findings were as follows: multiple minimally to mildly lymphocytic and heterophilic infiltration was seen around the hepatic triads and the central veins in the liver, and mild to moderate proliferation of lymphocytes, centered on the white pulp, was noted in the spleen.

The present case was diagnosed as a chondrosarcoma with undifferentiated neoplastic cell proliferation based on the following features: cartilaginous differentiation with abundant matrix, including acidic mucopolysaccharides; apparent structural and cellular atypia of the neoplastic cells with mitotic figures; and small areas composed of undifferentiated neoplastic cells. No metastasis or obvious invasive growth was observed in this case. However, marked cellular and structural atypia were noted. In addition, the presence of mitotic figures in a chondrosarcoma is strongly indicative of malignancy [23].

The foci of undifferentiated cells, which displayed apparent atypia and high mitotic counts, could be discriminated from the cartilaginous part. In humans, two variants exhibiting a characteristic biphasic pattern have been descripted; mesenchymal chondrosarcoma and dedifferentiated chondrosarcoma [5]. Mesenchymal chondrosarcomas exhibit the following features: proliferation of poorly differentiated small round cells with a scant cytoplasm showing an Ewing sarcoma-like appearance; well-differentiated hyaline cartilage islands; occasional osteoclast-like multinuclear giant cells, and osteoid-like matrix formation [5]. Meanwhile, dedifferentiated chondrosarcomas are characterized histologically by an abrupt transition between a well-differentiated (low-grade) hyaline cartilage part and a high-grade undifferentiated sarcoma or another type of sarcoma (e.g., osteosarcoma, angiosarcoma, or giant cell tumor) [5, 8]. In the present case, the undifferentiated cells had a conspicuous eosinophilic-to-amphophilic cytoplasm and did not exhibit any sudden transition to cartilaginous neoplastic components. Furthermore, the chondrocytic neoplastic cells displayed nuclear and cytoplasmic atypia; therefore, they were not "well"-differentiated. Several examples of similarity between the mesenchymal or dedifferentiated parts of the chondrosarcoma were observed, but these findings were not completely consistent.

S-100 protein is expressed in physiological and neoplastic chondrocytes and is utilized as a diagnostic marker of cartilaginous tumors [13, 22]. In the present case, the immunohistochemical examination showed that the mesenchymal and undifferentiated cells were vimentin-positive, but S-100-negative. In several animal cases of chondrosarcoma, the mesenchymal and undifferentiated neoplastic cells were also S-100-negative [7, 12, 17], suggesting that they exhibited undifferentiated profiles, as has been described in human cases [1, 11].

Endochondral ossification, but not *de novo* bone formation by neoplastic cells, can occur in chondrosarcomas [3, 23]. Thus, pathologists have to distinguish chondrosarcomas from chondroblastic osteosarcomas and reactive tissue.

The tumor in the current case formed in the distal peri-tibiotarsal region and was not attached to any bones, so it was considered to have originated in the periosteum or the surrounding soft tissue. However, we could not determine its subtype (periosteal or extraskeletal) since the tibiotarsal region is covered with a very thin layer of soft tissue.

In this case, the primary cause of death was unclear. The overall condition of the crane's body was not too bad: except for the tibiotarsal tumor, other lesions were morphologically recognized, but they were not obviously fatal. The tibiotarsal neoplasm itself might have contributed to the deterioration of the bird's condition and/or affected its motor function, which could have affected its feeding behavior, leading to weakness.

CONFLICTS OF INTEREST. The authors declare that no potential conflicts of interest with respect to the research, authorship, and/or publication of this article exist. The authors received no financial support for the research, authorship, and/or publication of this article.

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