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NOTE

Pathology

Extraskeletal chondrosarcoma in the abdominal cavity of a cow

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ABSTRACT. A 25-month-old female crossbred cow presented with astasia, emaciation, and stunted growth. Macroscopic examination revealed a large mass in the abdominal cavity, approximately 100 × 30 × 30 cm. Microscopic examination revealed that the mass consisted of multilobular mature and immature cartilaginous matrices with chondrocytic cells, surrounded by spindle to pleomorphic mesenchymal tumor cells. The cartilaginous matrices consisted of hyaline and elastic cartilages, as confirmed with Azan stain, and Victoria Blue and Van Gieson stain. Immunohistochemistry revealed that the chondrocytic and mesenchymal cells both expressed S-100. The tumor was diagnosed as an extraskeletal chondrosarcoma in the abdominal cavity of this cow.

KEY WORDS: abdominal cavity, cow, extraskeletal chondrosarcoma

Chondrosarcoma is most commonly observed in cartilaginous tissues; however, it can sometimes develop in extraskeletal soft tissues such as in the abdominal cavity. Extraskeletal chondrosarcoma has been reported in the abdominal cavity of dogs, where the tumor was thought to originate from various tissues including the aorta [6], spleen [8], liver [2], omentum [9] and caudal abdomen [10]. In cattle, extraskeletal chondrosarcoma has been reported in subcutaneous tissues at the base of the neck [11] and in gluteal muscle [14], but not in other tissues/organs including abdominal cavity. We have encountered an extraskeletal chondrosarcoma in the abdominal cavity of a 25-month-old female crossbred cow.

A 25-month-old female crossbred cow presented with astasia, emaciation and stunted growth on arrival at a meat sanitary inspection center, and was euthanized prior to macroscopic examination of the systemic organs. A large mass, approximately 100 × 30 × 30 cm, was found extending from the colorectum to the base of the colon, where the ovaries and uterus were involved by the mass (Fig. 1). The mass consisted of variably-sized multinodular masses ranging from 5 to 10 cm in diameter on cut surface, with smaller masses disseminated throughout the peritoneum and diaphragm. The masses were white to yellow, and occasionally red to brown on cut surface with necrotic and hemorrhagic areas. The mass was separated from the vertebrae, but the connection of the mass to other organs/tissues including gastrointestinal tracts, reproductive organs and ribs could not be examined. Disseminated masses were not observed in the spleen, liver, lung or heart.

The heart, liver, spleen, kidneys, superficial cervical lymph node, popliteal lymph node, internal iliac lymph node and masses at the base of the colon were fixed in 10% neutral-buffered formalin, and processed according to standard protocols prior to being embedded in paraffin. Paraffin sections (5 μm) were stained with hematoxylin and eosin, alongside additional sections stained with toluidine blue (pH 2.0, 4.1 and 7.0) for hyaline cartilage, Azan for hyaline and fibrous cartilages, and Victoria blue and Van Gieson for elastic cartilage, Periodic-acid Schiff-alcian blue (PAS-AB) for cartilage and myxomatous material. For immunohistochemical analyses, paraffin sections of masses were incubated with the following primary antibodies: anti-vimentin (goat polyclonal; 1:200; Santa Cruz Inc., Dallas, TX, U.S.A.), anti-α-smooth muscle actin (α-SMA; mouse monoclonal 1A4; 1:100; Dako, Glostrup, Denmark A/S), anti-S-100 (rabbit polyclonal; 1:400; Dako). For antigen retrieval, deparaffinized sections were heated with: 10 mM citrate buffer (pH 6.0) at 90°C in a microwave for 10 min for vimentin detection, and Dako target retrieval solution (pH 9.0, Dako) at 90°C in a microwave or 121°C in an autoclave for 10 min for a-SMA or S-100 detection, respectively. Signals were detected using a VECTASTAIN® Elite ABC kit (Vector Laboratories, Inc., Burlingame, CA, U.S.A.) with 3,3′-diaminobenzidine/hydrogen

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peroxide as the chromogen. The sections were then counterstained with hematoxylin. For negative controls, the primary antibodies were replaced with non-immunized sera. For positive controls, the positive reactions were confirmed in blood vessels, interstitial cells and peripheral fibers. The peritoneum and diaphragm with disseminated masses were not examined histopathologically.

Microscopic examination revealed that the mass consisted of multilobular cartilage tissues surrounded by mesenchymal tissues (Fig. 2A). Necrosis and hemorrhage were frequently observed. The majority of cartilage tissues were mature, with immature cartilage tissues observed less frequently. Mature cartilage tissues were composed of lobular or irregularly shaped dense basophilic matrices, with relatively large-sized lacunae (Fig. 2A, inset). Immature cartilage tissues were composed of small clusters of eosinophilic to weakly basophilic matrices with small-sized lacunae, and were observed within the periphery of mature cartilage tissues or as individual small cartilage foci. Chondrocytic tumor cells were frequently observed within lacunae in both mature and immature matrices; the cells were characterized by one or a few small to large-sized nuclei with rich to sparse chromatins, with some of them containing one or a few prominent nucleoli (Fig. 2A, inset). The cytoplasm of the tumor cells was abundant and eosinophilic with a well-defined cell border. The cells in mature cartilage tissues were frequently degenerate or necrotic, as shown by their pyknotic nuclei and densely eosinophilic cytoplasm. Proliferation of the spindle to pleomorphic mesenchymal tumor cells was predominantly observed between cartilage tissues to varying degrees, with deposition of mucinous material (Fig. 2A and 2B). The spindle cells had spindle-shaped nuclei and ill-defined eosinophilic cytoplasm. Well-organized spindle cells surrounded each lobule of cartilage tissue, and the cells appeared to transform into intralacunar chondrocytic cells. The pleomorphic tumor cells had small to large, irregularly shaped nuclei with atypia, some of which had a prominent large nucleolus (Fig. 2B). The cytoplasm of these cells was plump and eosinophilic with a relatively well-defined cell border. Multinucleated giant cells were sometimes observed. On average, mitotic figures were 4/10 per high power field (HPF) in areas of dense pleomorphic tumor cell growth, while 0/10 mitotic figures per HPF were observed in areas of sparse pleomorphic or spindle cell growth. The pleomorphic tumor cells diffusely grew, but a hemangiopericytic growth pattern was not observed. Areas of chondrocytic and pleomorphic tumor cell growth were surrounded by interstitial tissues; i.e., smooth muscle layers, arteries, serous connective tissues and mesothelial cells. Neither osteoid nor osseous tissue was observed in the matrix of the tumor mass. No tumor cells were confirmed in other organs.

Blue-colored fine connective fibers were confirmed in the growth areas of spindle cells and pleomorphic cells in Azan-stained sections. Immature cartilage matrices were weakly stained with Azan (blue), while mature cartilage matrices were not stained, except for the peripheral area of the lobules. Weakly stained blue-colored immature cartilage transformed to densely stained blue-colored surrounding connective tissues. Red to purple staining of immature and mature cartilaginous matrices i.e., metachromasia, was identified in toluidine blue-stained sections (Fig. 3A). Blue-colored elastic fibers were observed in mature cartilage matrices in Victoria Blue and Van Gieson stain (Fig. 3B). The periphery of mature cartilage was composed of red-colored connective fibers. Immature cartilage matrices consisted of weakly stained blue-colored elastic fibers and red-colored connective fibers or red-colored connective fibers alone. Blue-colored mature and immature cartilage matrices were observed in PAS-AB stain; weak blue-colored myxomatous materials were observed in the interstitial tissues.

Immunohistochemical analysis demonstrated a positive reaction of vimentin and S-100 in chondrocytic cells, spindle cells and pleomorphic cells (Fig. 4). Densely proliferated pleomorphic cells were negative for S-100.

The present tumor was characterized by the combined proliferation of mesenchymal tumor cells and of lobular cartilage tissues. There were extensive areas of necrosis and hemorrhage in tumor mass, as described in a dog with mesenchymal chondrosarcoma of the ribs [7]. We did not confirm the origin of tumor development; however, the tumor might have been derived from mesenchymal cells in the large intestine or uterus. Unlike conventional chondrosarcoma, the uncommon histological variants of chondrosarcoma include: clear cell, myxoid, mesenchymal and dedifferentiated chondrosarcoma [1]. The present case might mimic mesenchymal or dedifferentiated chondrosarcoma, which occurs in soft tissue and/or bone tissue [1]. Histologically, a mesenchymal-type tumor shows a biphasic pattern, with a highly cellular component of tumor cells resembling chondroblastic cells with low-grade cartilaginous differentiation [1]. "Small cell malignancy" (i.e. relatively small-sized tumor cells) with a hemangiopericytoma-like growth pattern is diagnostic for a mesenchymal-type tumor in human [1]. These characteristics are not necessarily specific in mesenchymal chondrosarcoma in dogs [8, 9]; however, Rhind and Welsh [10] noticed "round-cell morphology" in intraabdominal mesenchymal chondrosarcoma, which appeared to mimic "small cell malignancy" in human [1], and Madarame *et al.* [7] described that proliferated mesenchymal tumor cells showed the typical vascular pattern (stag-horn appearance) in mesenchymal chondrosarcoma of the ribs. Considering immature cartilage tissues observed within the periphery of mature cartilage tissues or individual small immature cartilage foci and the transforming mesenchymal cells into intralacunar chondrocytic cells, the present case might be a mesenchymal-type tumor. A dedifferentiated-type tumor is a high-grade non-cartilaginous sarcoma with a low-grade differentiated cartilaginous tumor: an undifferentiated sarcoma with abrupt transition to cartilaginous component is diagnostic for this type [1]. Similar dedifferentiated-type tumors with a biphasic pattern were elegantly demonstrated in seven dogs and one cat [12]. The sarcomatous components with relatively large-sized pleomorphic cells and a lack of hemangiopericytoma-like growth pattern suggest that the present case also appears to be a dedifferentiated-type tumor. Dedifferentiated cells are known to be either osteoblastic, chondroblastic, or fibroblastic, and less frequently, malignant fibrous histiocytoma-like [4]. The immunopositive reaction for S-100 suggests that the pleomorphic cells could be chondroblastic cells in the present case. However, this type tumor is not fully demonstrated in animals other than dogs and cats [12]. The present case might have a biphasic characteristics of mesenchymal-type and dedifferentiated-type chondrosarcomas.

The mature cartilaginous component was believed to be elastic and hyaline cartilages rather than fibrous cartilage because the tissues stained positively with Victoria Blue and Van Gieson stains, negatively with Azan stain, and displayed metachromasia following staining with toluidine blue. Chondrosarcoma with elastic cartilage matrix has not been reported to our knowledge.

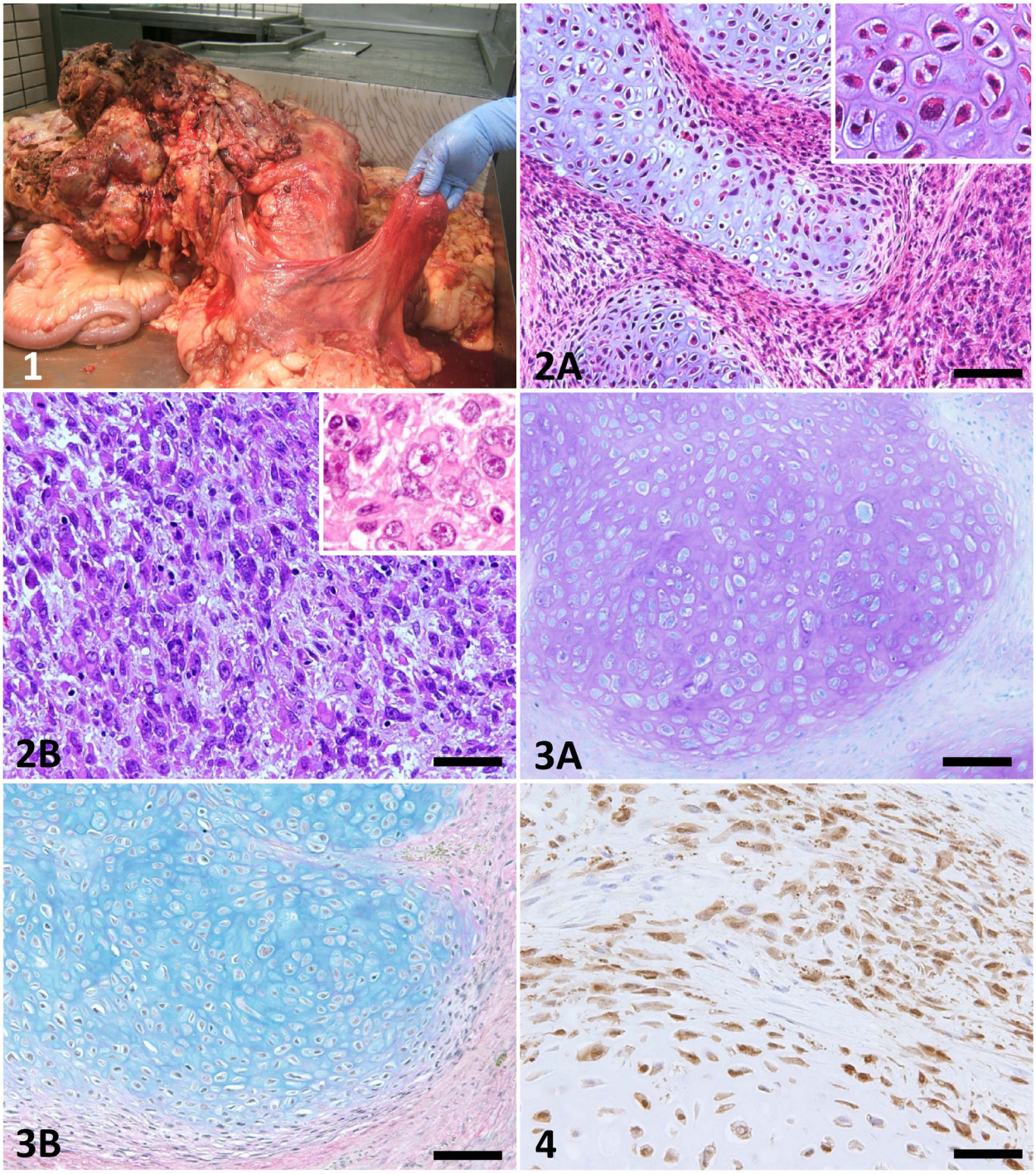

Fig. 1. Macroscopic image of the tumor within the abdominal cavity. Extremely large, yellow to brown masses were observed in the region of the large intestine.

Fig. 2. Histological image of the tumor. (A) The tumor comprised multilobular mature (basophilic) cartilaginous tissues, surrounded by spindle cells and pleomorphic cells. Immature (eosinophilic) cartilaginous tissues were observed in the periphery of mature cartilaginous tissues. Inset: chondrocytic tumor cells with one or a few small to large-sized nuclei were observed within lacunae in mature matrix. (B) The mesenchymal tumor cells were characterized by pleomorphic nuclei and abundant eosinophilic cytoplasm. Inset: a higher magnification of the mesenchymal tumor cells. Bar=100 μm (A) or 50 μm (B).

Fig. 3. Histochemical characteristics of cartilaginous components. Cartilaginous matrix with metachromasia was observed in Toluidine blue (pH4.1)-stained sections (A), and blue-colored cartilaginous matrix was found in Victoria blue and Van Gieson stain with nuclear fast counter-stain (B). Bar=100 μm (A, B).

Fig. 4. Immunohistochemical characteristics of tumor cells. A positive reaction for S-100 was observed in mesenchymal tumor cells (upper right) and chondrocytic cells in cartilaginous components (lower left). The interstitial spindle cells were negative for S-100. Bar=50 μm.

The immature cartilaginous component might be hyaline, elastic and fibrous cartilages, which could be derived from surrounding mesenchymal tumor cells. Interstitial myxomatous materials were detected in PAS-AB stain; however, the myxoid background was a minor component in this tumor mass.

In humans, grade I (low-grade) chondrosarcoma is characterized by poor cellularity and hyperchromatic round nuclei (equal to the size of a mature lymphocyte), with no myxoid background, whereas grade II (intermediate-grade) chondrosarcoma is characterized by increased cellularity and nuclear enlargement [5]. In the majority of dedifferentiated chondrosarcomas, the cartilage portion has been identified as grade I; less frequently, the morphology resembled grade II [4]. Consistent with this, dedifferentiated chondrosarcomas in dogs and cats showed a low-grade (well-differentiated) cartilage matrix formation [12]. Grading of cartilage matrix was not be fully discussed in dogs [2, 6–10] and cow [11, 14] with extraskeletal chondrosarcomas. A marker of chondrocytic differentiation, S-100, was diffusely expressed in the cartilaginous portion in the present case; this was consistent with the findings of grade I and II chondrosarcomas [4]. The present case might be intermediate between grade I and II chondrosarcoma.

The histological appearance of osteosarcoma varies widely, and the matrix may contain bone, osteoid and cartilage. The coexisting of osteoid and a predominant cartilage in sarcomatous tumors indicates chondroblastic osteosarcoma [3, 13]. We did not observe osteoid (i.e. small irregular deposits of hyaline eosinophilic materials) in any sections of the tumor masses by our restricted examination; therefore, we ruled out chondroblastic osteosarcoma as a diagnosis in the present case. Osteoid was found in splenic mesenchymal chondrosarcoma in a dog [8], which should be carefully distinguished from chondroblastic osteosarcoma as described in human [5].

Because of the prominent proliferation of pleomorphic sarcomatous tumor cells within a multilobular cartilaginous matrix, the mass in the abdominal cavity of this cow was diagnosed as an extraskeletal chondrosarcoma. The poorly differentiated sarcomatous cells appeared to be more pleomorphic than those in previously-reported extraskeletal chondrosarcoma of dogs [2, 6, 8–10] and cow [11, 14].

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