NOTE *Pathology*

Carcinosarcoma in a White Rhinoceros (*Ceratotherium simum simum*)

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ABSTRACT. In Rhinocerotidae, there are very few reports of tumors and no reports of a mixed tumor. This paper reports the case of a male 33-year-old southern white rhinoceros. Grossly, there were two masses in the coelomic cavity and solid nodules in the liver. Histologically, all tumors had a biphasic pattern that consisted of malignant epithelial cells (cytokeratin- and E-cadherin-positive) and non-epithelial cells (vimentin-positive) with cartilage. In this case, the prostate could not be identified, and instead, the largest tumor mass was present at that site. Furthermore, since structures regarded as the prostate duct remained in this tumor, we considered that this tumor was very likely to be of prostate gland origin. This case is the first report of carcinosarcoma in Rhinocerotidae.
KEY WORDS: carcinosarcoma, prostate, rhinoceros.

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There are very few reports of tumors in Rhinocerotidae with only 12 case reports to date [16]. In females, vaginal [9] or uterine leiomyomas [13] and adenocarcinoma [17] have been reported as genital system tumors. Although Rhinocerotidae belongs to the Perissodactyla, it has a prostate gland, a seminal vesicle and a bulbourethral gland like a male horse [15]. In male rhinoceroses, there are only two reports of seminomas [10, 12], but no reports of other genital tumors or accessory reproductive gland neoplasms. Carcinosarcomas are very rare tumors in humans and animals, and in veterinary medicine, carcinosarcomas are more often diagnosed in the canine mammary gland [3], but they may also primarily arise from apocrine glands of the skin [1], thyroid gland [6], lung [14] and mandibular salivary gland [11]. There are no reports of carcinosarcoma in Rhinocerotidae. This is the first report of a carcinosarcoma suspected to be of prostate gland origin in a southern white rhinoceros (*Ceratotherium simum simum*).

This case had a history of red mucus occasionally trickling out of the urethral meatus from the summer of 2009. In October 2010, the rhinoceros produced reddish urine and had severe subcutaneous edema around its penis; hence, the penis could not go back inside the foreskin. In November 2010, blood clots and tissues were observed in the urine. Dysuria occurred and gradually progressed to hypouresis. Furthermore, the amount of defecation continued to decline gradually, until eventually the animal could not defecate at all. A rectal examination was performed and revealed a spherical mass located in the ventral side of the rectal wall. As the animal became unable to urinate and defecate by itself, urination by catheter was performed under anesthesia. In December, subcutaneous edema expanded in the lower abdomen and became systemic. The animal died on December 29, 2010, and a complete autopsy was carried out at Gunma Safari Park.

On gross examination, two large masses presented in the coelomic cavity. One was a rugby-ball-sized irregularly shaped mass located between the rectum and the urethra, fixed with the pubic bone slightly to the left side and front edge. The other presented at the right side internal iliac lymph node anatomically (Fig. 1). It was an irregularly shaped mass of about 20 cm in maximum diameter and adhered to the vena cava. Macroscopic findings of the two masses were the same. In other words, they were surrounded by a thick connective capsule, and on cut sections, the tumor tissue was lobulated by abundant connective tissue and formed multiple nodules ranging in size from 1 to 5 cm, including a large amount of milky white necrotic debris. On the surface of the former mass between the rectum and the urethra, both seminal vesicles were observed. The right seminal vesicle adhered to the mass, and the left had been buried in rich connective tissue and become deformed. The prostate gland could not be found. At the cut surface of the whole liver, solid nodules with findings similar to those of the two large tumors were scattered. The nodules were particularly dense in the right lobe of the liver. In addition, no macroscopic or histological abnormalities were found in the testes. The major organs (heart, kidneys, lungs, lymph nodes and liver) and tumor tissues were fixed in 10% neutral buffered formalin and paraffin-embedded, and tissue sections were prepared. These sections were stained with hematoxylin and eosin (HE) and Periodic acid-Schiff (PAS) and subjected to immunohistochemistry by the peroxidase-anti-peroxidase complex method with several primary antibodies as follows: mouse monoclonal antibody (mAb) anti-human cytokeratin (AE1/AE3; Dako Japan Inc., Tokyo, Japan), mouse mAb anti-E-cadherin (C20820; BD Transduction Laboratories,

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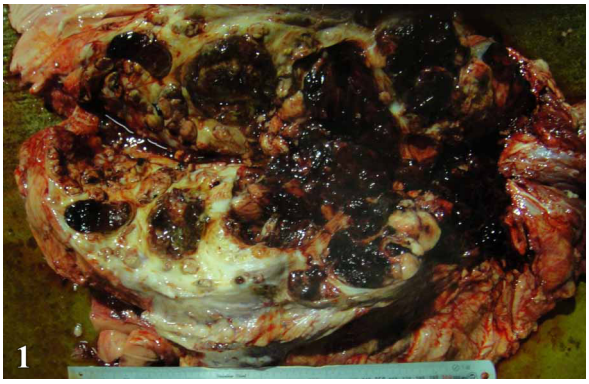

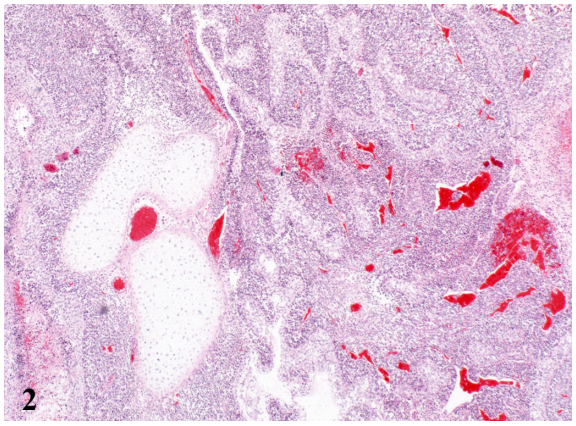

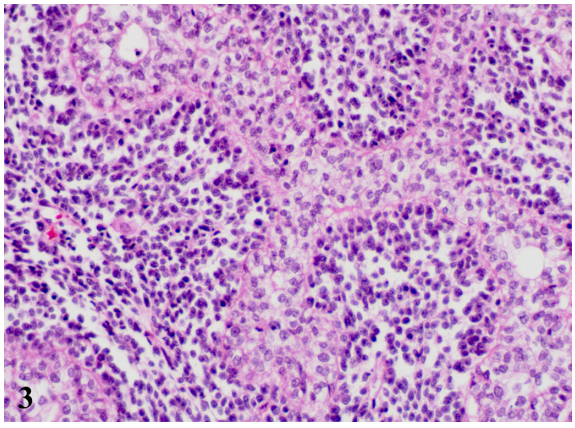

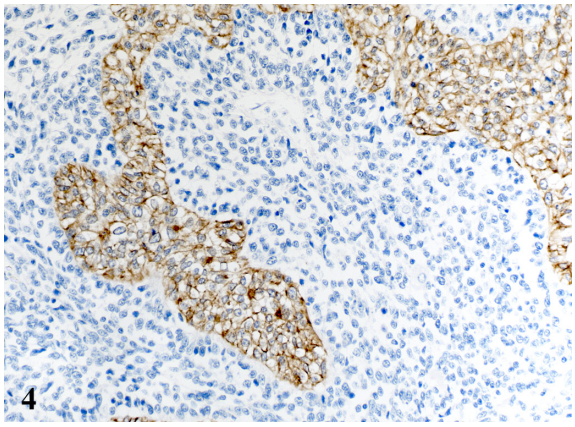

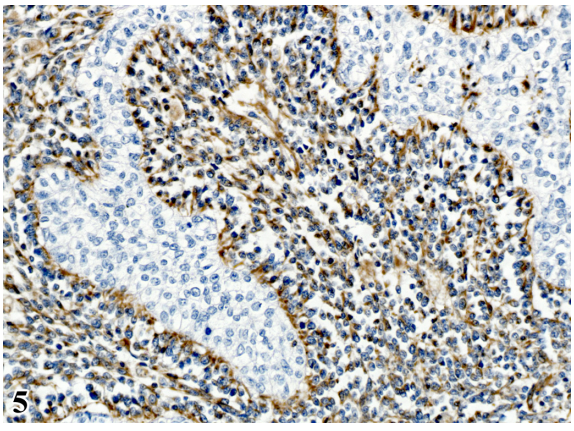

Fig. 1. Cut surface of tumor mass that was considered to be lymph node metastasis. The tumor tissue was lobulated by abundant connective tissue and formed multiple nodules.

Fig. 2. Low magnification of tumor tissue of the liver. The tumor tissue was constructed of epithelial and non-epithelial components. The epithelial component was organized into solid nests and glandular ducts. In contrast, the non-epithelial component developed solidly so as to fill in the spaces between the epithelial components. Cartilage tissues were observed in tumor tissues. HE. Bar=500 μm.

Fig. 3. High magnification of tumor tissue of the liver. Epithelial tumor cells had relatively large and oval or irregularly shaped nuclei with poor chromatin. On the other hand, non-epithelial cells were small and spindle-shaped with weakly eosinophilic cytoplasm. HE. Bar=50 μm.

Fig. 4. Immunostaining for keratin of a tumor nodule section on the liver. The epithelial components were labeled by the anti-human cytokeratin antibody clone: AE1/AE3. Immunostaining technique with a hematoxylin counterstain. Bar=50 μm.

Fig. 5. Immunostaining for vimentin of a tumor nodule section on the liver. The non-epithelial components were labeled by the anti-vimentin antibody clone: V9. Although not shown, cartilage tissues were vimentin-positive. Immunostaining technique with a hematoxylin counterstain. Bar=50 μm.

Lexington, KY, U.S.A.), mouse mAb anti-vimentin (V9; Dako Japan Inc.), mouse mAb anti-α-smooth muscle actin (1A4; Dako Japan Inc.), mouse mAb anti-prostate-specific antigen (PSA) (ER-PR8; Nichirei Inc., Tokyo, Japan), rabbit polyclonal anti-bovine S-100 alpha (Dako Japan Inc.) and mouse mAb anti-human Wilms' tumor 1 (WT1) protein (6F-H2; Dako Japan Inc.) and stained by the double immunofluorescence method with anti-vimentin (V9) and rabbit polyclonal anti-human keratin (Dako Japan Inc.). Histologically, tumor nodules were observed in the two masses of the coelomic cavity and the liver, and they were characterized by similar cell structures. These tumor tissues were composed of two types of cells, namely, epithelial and non-epithelial (Fig. 2). The epithelial components were organized as solid nests and glandular ducts. These tumor cells had relatively large nuclei with poor chromatin, of an oval to irregular shape and with abundant clear cytoplasm and few mitotic figures (Fig. 3). Weakly eosinophilic and PAS stain-positive droplets within the cytoplasm of tumor cells were observed. In addition, around the nests and glandular duct, basement membrane-like structures were observed, but their thickness was irregular, discontinuous in many places and unclear. The non-epithelial component developed solidly to fill in the spaces between the epithelial components, consisting of small and spindle-shaped cells with weak eosinophilic cytoplasm (Fig. 3). Nuclei were irregularly shaped and were highly atypical. Mitotic figures were often seen. In addition, mature cartilage tissues were observed in all tumor tissues (Fig. 2). Some of extra-involucre invasion of tumor tissues was also observed. Moreover, at the peripheral position of the mass located between the rectum and the urethra, a single cuboidal epithelial layer with hyperplasia, similar to the prostate ducts, was observed, but no prostate acinus-like structures were found. The coalesced seminal vesicle and tumor tissue were isolated by thick connective tissue septa. By immunohistochemistry, epithelial tumor cells were found to be positive for cytokeratin AE1/AE3 in the cytoplasm (Fig. 4) and for E-cadherin in the membrane. In contrast, cytoplasm of non-epithelial cells was positive for vimentin (Fig. 5). There were a few cells double-positive for cytokeratin AE1/AE3 and vimentin in the division between epithelial cells and non-epithelial cells (not shown). None of the tumor cells reacted to anti-S100 protein or the anti-αSMA antibody. In addition, the anti-PSA antibody used in this study reacted to the positive control (canine prostate tissue), but did not react to tumor cells. The anti-WT1 antibody did not react to the glomerular podocytes of the present case used as a positive control, so we concluded it did not show a cross-reaction for rhinoceros tissues. The diagnosis of carcinosarcoma in this case was based on the presence of the two malignant components, one epithelial or myoepithelial and the other mesenchymal. These morphological findings were further characterized by immunohistochemistry with sarcomatous and carcinomatous components being immunoreactive for vimentin and cytokeratin, respectively, as previously described [5, 7, 11]. Therefore, it is very important to distinguish between the two components (carcinomatous and sarcomatous). In this case, the tumors were located between the rectum-urethra and internal iliac lymph node, while masses on the liver consisted of biphasic development of epithelial cells (cytokeratin- and E-cadherin-positive) and non-epithelial cells (vimentin-positive). These components had characteristics of malignant tumor cells. Interestingly, we found cartilage on all tumors. From the above, this case was diagnosed as carcinosarcoma. In dog, the tubular structure is dominant as the epithelial component of carcinosarcoma [1, 2, 5, 6, 11, 14]; however, solid epithelial nest formation without a tubular structure was characterized in this case.

This is the first report of a rare case of a mixed tumor in a rhinoceros. Extrarenal nephroblastoma can be mentioned in order to differentiate it from similar diseases. Histologically, nephroblastoma is composed of a mixture of epithelial components and small undifferentiated cells called nephroblast cells, and there is a transition between the two kinds of neoplastic cells. The epithelial components have various degrees of differentiation, such as immature glomerular-like buds and renal tubules [8]. However, in this case, there was no transition into two components, and histological findings were different from nephroblastoma. Moreover, as with carcinosarcoma, pleomorphic carcinoma shows a carcinomatous status, as well as spindle and/or giant cell components, but differentiation into mesenchymal components, such as cartilage and bone, is not seen in pleomorphic carcinoma [4]. In this case, clear biphasic development and cartilage tissue were observed in all tumor tissues examined. Therefore, we concluded that this tumor was not a pleomorphic carcinoma. The prostate is located between the rectum and urethra. However, in this case, the prostate was not observed. In addition, seminal vesicles coalesced with, or were buried in, the surface of the mass and tumor tissue, and they were isolated by connective tissue histologically. Furthermore, since structures regarded as the prostate ducts remained, the origin of the tumor appears to have been the prostate gland. Moreover, even assuming the tumors of the internal iliac lymph node and liver were metastases from a prostate tumor, there is no contradiction. In this case, dysuria and defecation continued for about two months due to the physical pressure of the two large tumors in the body cavity. The deterioration in liver function due to tumor metastasis to the liver in the terminal stage with severe circulatory failure, such as systemic subcutaneous edema, was the cause of death. In conclusion, this is the first report of a carcinosarcoma in a southern white rhinoceros (*Ceratotherium simum simum*).

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