

Case Report

Spontaneous abdominal hemangiosarcoma in a ferret

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Abstract: Herein, we describe the case of a 6-year-old female ferret that died within a few days of the onset of anorexia and reduced spontaneous locomotor activity. Necropsy revealed a dark red abdominal mass of unknown origin between the right lobes of the pancreas and the proximal jejunum, with massive blood retention in the peritoneal cavity. Histopathologically, spindle-shaped or sometimes polygonal tumor cells were proliferating with irregularly shaped vascular spaces containing blood components and surrounding-tissue infiltration. In some areas, tumor cells formed distinctly dilated blood vessel-like structures. Immunohistochemically, most of the tumor cells were strongly positive for CD31, but factor VIII-related antigen immunoreactivity was confined to the area with dilated blood vessel-like structures. Based on these findings, the tumor was diagnosed as an abdominal hemangiosarcoma. Abdominal hemangiosarcoma excluding cases of the liver and spleen are rare in ferrets. (DOI: 10.1293/tox.2018-0060; J Toxicol Pathol 2019; 32: 283–287)

Key words : abdomen, CD31, factor VIII-related antigen, ferret, hemangiosarcoma, tumor

Hemangiosarcoma is a malignant tumor that arises from vascular endothelial cells. In domestic animals, it is most commonly seen in dogs, occurring most frequently in the spleen, liver, and right atrial auricle, especially in German shepherds and golden retrievers¹. In laboratory rodents, spontaneous hemangiosarcoma is seen but not frequently, and the most common primary tumor sites are the liver and spleen^{2–5}. Hemangiosarcoma is more common in mice than in rats, and B6C3F1 mice often have an incidence of over 12%².

The domestic ferret (*Mustela putorius furo*) is a member of the order Carnivora, family Mustelidae. Ferrets are popular pets, but they are also used as experimental animals for various biomedical research^{6, 7}. In ferrets, the incidence of hemangiosarcoma is relatively low, often occurring in the skin or subcutis^{8–11}, and adrenal gland tumors, pancreatic islet cell tumors, and lymphomas are the most common tumors^{8, 9, 12, 13}. The occurrence of hemangiosarcoma is even lower in the abdominal cavity, and most cases are in the liver or spleen^{9, 11, 14}. Here, we describe a case of intra-abdominal hemangiosarcoma in a ferret which occurred in a rare site that was neither the liver nor spleen.

Herein, we describe the case of a 6-year-old spayed female ferret that died within a few days of the onset of

anorexia and reduced spontaneous locomotor activity. The ferret was a domesticated pet which resided in a household and had no noteworthy medical history. At necropsy, the ferret appeared mildly thin, and its body weight was 602 g. The mucosa of the oral cavity and eyelids were severely pale. More than 17 mL of blood was found in the peritoneal cavity. There was a 3.5 × 2.0 × 2.2 cm dark red mass in the mesentery between the right lobes of the pancreas and the proximal jejunum (Fig. 1). Cutting its surface revealed that it was solid and randomly multilocular with abundant blood. The periphery of the mass was overlaid on the proximal jejunum, and on the opposite side, the mass was compressing the pancreas. Abdominal lymph nodes, including pancreatic lymph nodes (Fig. 1), mesenteric lymph nodes, and internal iliac lymph nodes, were red and mildly swollen. The choledoch near the mass was mildly dilated (Fig. 1). Samples of various tissues including the mass, liver, pancreas, stomach, duodenum, proximal jejunum, distal jejunum, ileum, colon, spleen, visceral lymph nodes, heart, lung, thymus, kidney, and brain were fixed in 10% neutral phosphate-buffered formalin and embedded in paraffin wax. Sections were cut at a thickness of 4 μm and stained with hematoxylin and eosin. Additionally, the mass was subjected to Berlin blue staining for detection of hemosiderin deposition. For immunohistochemical examination, sections of the mass were subjected to a labeled polymer staining method using Histofine Simple Stain MAX-PO (MULTI) (Nichirei, Tokyo, Japan). The primary antibodies used for each section were anti-vimentin (prediluted, mouse monoclonal antibody, V9, Dako), anti-human CD31 (1:100, mouse monoclonal antibody, JC70A, Dako), anti-human factor VIII-related antigen (FVIII-RAG; 1:50, mouse monoclonal antibody, F8/86, Dako), and anti-human Ki-67 antibody (1:100, mouse monoclonal antibody, MIB1, Dako). Antigen retrieval for vimentin, CD31, and

Received: 23 October 2018, Accepted: 2 May 2019

Published online in J-STAGE: 6 June 2019

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Ki-67 was performed in 10 mM citrate buffer (pH 6.0) by autoclaving for 15 min at 121°C. For FVIII-RAg, proteinase K (prediluted, Dako) digestion at room temperature for 15 min was used for antigen retrieval. The sections were incubated with each primary antibody overnight at 4°C, visualized with 3,3'-diaminobenzidine (DAB), and then counterstained with Mayer's hematoxylin. The percentage of Ki-67-positive cells was indicated as a Ki-67 labeling index. The Ki-67-positive tumor cells were enumerated in a total of 1,000 tumor cells from 10 high-power fields.

Histopathologically, the mass was not encapsulated and was partially covered with mesothelial cells connected to the mesentrium. The tumor cells were locally invasive to surrounding tissues, including the mesentrium, jejunum serosa, and interlobular connective tissue of the pancreas. In most parts including the central area of the tumor, the tumor cells formed irregularly shaped vascular spaces with blood components and a small amount of eosinophilic interstitial matrix, and they occasionally had a solid proliferative area with some slit-like structures (Fig. 2a–c). The tumor cells were spindle-shaped and had indistinct cell borders with dense proliferation. There was a relatively scant amount of eosinophilic cytoplasm, and there were irregular spindle-shaped to oval hyperchromatic nuclei with one or

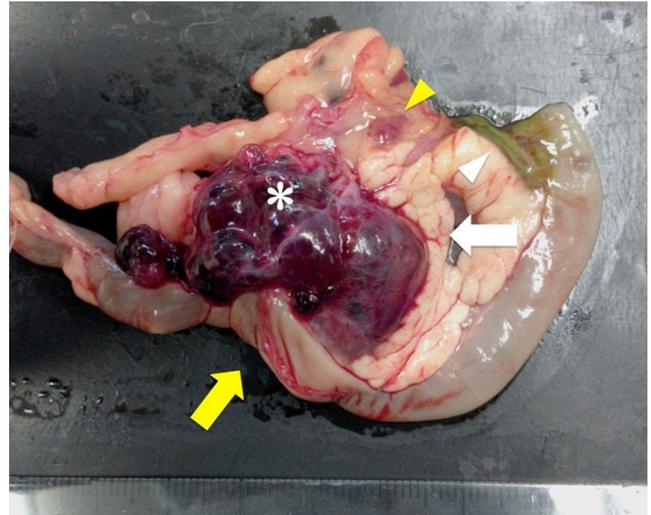


Fig. 1. Gross appearance of a partly multilobular dark red mass (*) formed in the mesentery between the right lobes of the pancreas (white arrow) and the proximal jejunum (yellow arrow). A pancreatic lymph node (yellow arrowhead) showed mild swelling by blood absorption, and the choledoch (white arrowhead) was mildly dilated.

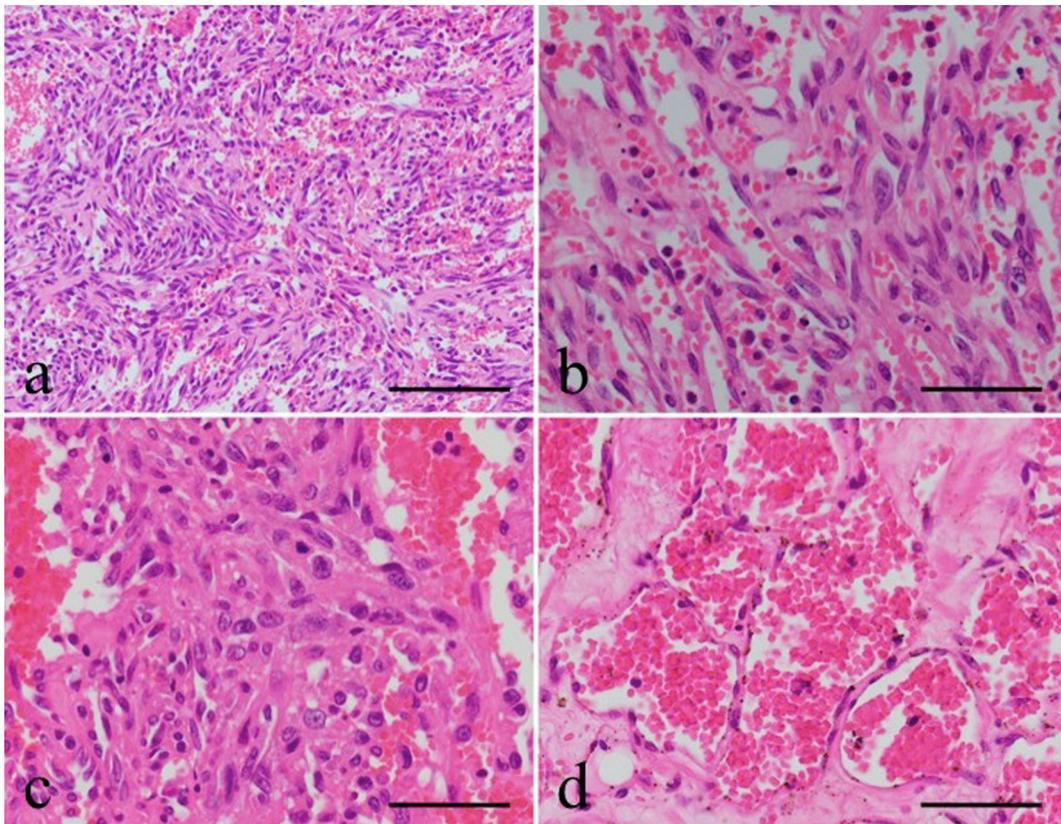


Fig. 2. Histopathology of the tumor after hematoxylin and eosin staining. (a) Tumor cells formed irregularly shaped vascular spaces with blood components. Bar = 100 μ m. (b) Tumor cells were spindle shaped and proliferated densely with a small amount of eosinophilic interstitial matrix. Bar = 50 μ m. (c) Polygonal to spindle-shaped tumor cells formed a solid proliferative area with some slit-like structures. Bar = 50 μ m. (d) Tumor cells formed distinctly dilated blood vessel-like structures. Bar = 50 μ m.

two inconspicuous nucleoli. In the solid proliferative area, the tumor cells showed a mildly pleomorphic polygonal shape. There was moderate anisocytosis and anisokaryosis, and there were one to two mitotic figures counts per high-power field. In some parts of the tumor periphery, the tumor cells formed dilated blood vessel-like structures lined by endothelial cells with enlarged nuclei and no cellular atypia (Fig. 2d). Multiple small and large foci of necrosis, hemorrhagic lesions, and numerous fibrin thrombi were dispersed throughout the tumor. Some of the lesions contained hemosiderin that was identified by Berlin blue staining and yellow pigment presumed to be hematoidin depositions and cholesterol clefts. Immunohistochemically, almost all of the tumor cells were diffusely and strongly positive for vimentin in the cytoplasm (Fig. 3a) and endothelial cell marker CD31 in the cytoplasm and cell membrane (Fig. 3b). Most of the tumor cells were negative for FVIII-RAg in the area where the tumor cells showed dense proliferation with irregularly shaped vascular spaces and a solid proliferative area, and a relatively strong nonspecific reactions was observed throughout the lesion. In contrast, tumor cells in the area with dilated blood vessels in the periphery of the tumor showed positive staining for FVIII-RAg (Fig. 3c). Normal vascular endothelial cells of the jejunum in the same section of the tumor as the internal positive control were strongly

positive for CD31 and FVIII-RAg. A small number of tumor cells were positive for Ki-67 (Fig. 3d) with a labeling index of 10.8%. Other histopathological observations included fibrin thrombus formation in the alveolar capillaries of the lungs; necrosis of centrilobular hepatocytes; mild periportal lymphocyte and plasma cell infiltration and fibrosis in the liver; nodular hyperplasia of pancreatic acinar cells and islet cell adenoma in the pancreas; and moderate blood absorption in the lymphatic sinus of the abdominal lymph nodes, including pancreatic lymph nodes, mesenteric lymph nodes, and internal iliac lymph nodes.

In the present case, histopathological features and the results of immunohistochemical staining suggested that the tumor cells originated from the vascular endothelial cells, and the cells exhibited mild cellular atypia and local invasion. Based on the relatively low mitotic activity and Ki-67 labeling index, this case is considered to be a moderately differentiated and low-grade hemangiosarcoma. Generally, the body weight of spayed female ferrets is 800 g to 1,200 g, and its circulated blood volume is on average 40 mL¹⁵. In this case, it is considered that 42.5% or more of the blood circulation volume was lost into the abdominal cavity from the tumor, which was lethal. Because the patient was thin and had a low body weight, it may have been affected by anorexia due to the intra-abdominal tumor. Although intra-

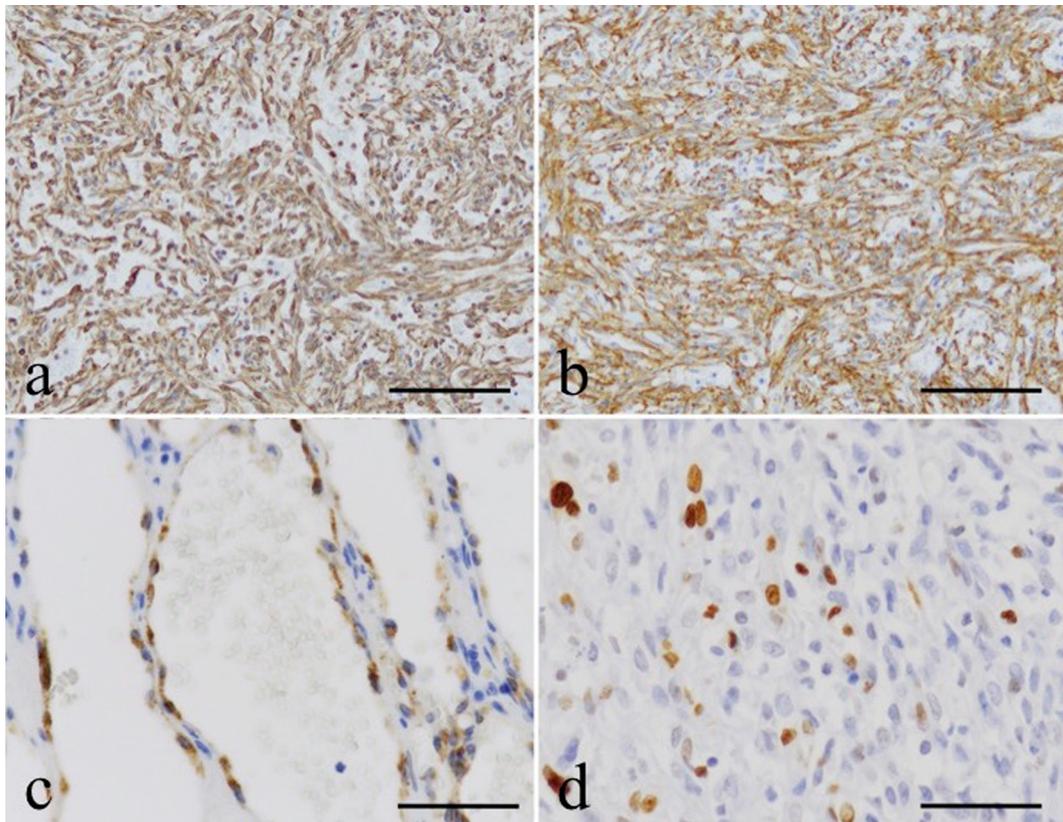


Fig. 3. Immunohistochemical reactivity of tumor cells. Tumor cells were diffusely and strongly positive for vimentin (a) and CD31 (b). Bar = 100 μ m. (c) Tumor cells showed positive staining for factor VIII-related antigen, which was confined to the area with dilated blood vessels. Bar = 50 μ m. (d) A small number of tumor cells were positive for Ki-67. Bar = 50 μ m.

peritoneal hemorrhage from the tumor was the direct cause of death, fibrin thrombi in the capillary beds of the lungs were thought to have occurred following the occurrence of fibrin thrombi within the tumor, and hepatic centrilobular zonal necrosis was thought to have been caused by circulatory disorder or anemia due to blood loss from the tumor, respectively. Because the tumor was situated between the right lobes of the pancreas and the proximal jejunum, the primary site of the tumor was suspected to be the regional lymph nodes of the pancreas, mesenteric lymph nodes, mesenterium, or accessory spleen. It was difficult to definitively determine the primary site of the tumor because it was very large, but at least lymphoid tissue suggestive of lymph nodes and an accessory spleen was not observed in the tumor tissue. Although not definite, since the tumor was continuous from the mesenterium and its surface was partly covered by mesothelial cells, it was suspected to have originated in the mesentery. Based on these findings, the ferret was diagnosed as having an abdominal hemangiosarcoma.

In several large studies, vascular tumors in ferrets including hemangioma and hemangiosarcoma accounted for 7 of 639 (1.1%), 27 of 1,525 (1.8%), 18 of 856 (2.1%), 21 of 945 (2.2%), and 18 of 206 tumor cases (8.7%), among which the incidence of hemangiosarcoma was 6 (0.94%), 16 (1.05%), 5 (0.58%), 15 (1.59%), and 10 cases (4.85%), respectively^{8, 9, 11, 16, 17}. Based on these reports, ferret hemangiosarcoma may not be rare at all. However, among these 52 hemangiosarcoma cases, 41 (78.8%) occurred in the liver, spleen, skin, or subcutaneous tissues. Other cases included a few cases of occurrence in the peritoneum, abdominal cavity, mesenteric lymph nodes, alimentary tract, and sites unspecified^{8, 9, 11, 16, 17}. Pathological features of hemangiosarcoma in ferrets, including the case here, seem to include different degrees of tumor differentiation depending on the case, from cases forming distinct vascular spaces to those growing solid masses^{10, 14, 18}. There was a similar case report of ferret hemangiosarcoma that occurred in the mesentery of the ileum with metastasis in the mesenteric lymph nodes¹⁸. Compared with that case, there were similarities in the gross lesions of the tumors but some differences in histopathological lesions. Here, tumor cells proliferated at a higher density and formed more irregular vascular lumens compared with the previous case report, where they generally seemed to have proliferated with overall clearer cavernous structures. Immunohistochemical staining for FVIII-Rag showed stronger reactivity in tumor cells in the previous report. The above findings suggest that although the degree of differentiation of tumor cells was higher and cellular atypia was milder in the previously reported case, the degree of malignancy seems to have been higher in that case, as metastasis of the tumor cells was not observed in any tissue in our case.

Cellular atypia is reported to be negatively correlated with CD31 and vWF expression in canine hemangiosarcoma¹⁹. Although there have not been detailed CD31 and vWF expression comparisons, Maharani *et al.* mentioned that some tumor cells with high cellular atypia scores were positive for CD31 but negative for vWF¹⁹. Additionally, tumor

cells with mild cellular atypia were more likely to be negative for vWF than for CD31¹⁹.

In the case of human vascular tumors, CD31 has been reported as the most sensitive vascular endothelial marker with well differentiated and poorly differentiated areas^{20, 21}. Conversely, it has been reported that vWF staining is often negative in poorly differentiated hemangiosarcomas²¹ and may even be negative in hemangiomas²⁰. Although there has been no report on the detailed relationship between the degree of differentiation of tumor cells and CD31 and FVIII-Rag immunoreactivity in vascular tumors of ferrets, our result suggests that vascular tumors in ferrets also express CD31 but that expression of FVIII-Rag may often be weak or absent, as observed in human and canine cases^{19–22}. Although the reason why FVIII-Rag staining is sometimes negative in vascular tumors in humans and dogs is still not well understood, many negative or weakly positive cases are reported in poorly differentiated tumors with solid proliferation^{19–22}. In our case, although the tumor cells were relatively well differentiated, FVIII-Rag was not clearly immunostained in the regions where the tumor cells showed dense proliferation. As the immunostaining for FVIII-Rag in our case may have been nonspecific and not accurately determined, the stainability of FVIII-Rag in ferrets needs to be further investigated.

The exact location of the primary tumor site is unknown, but this is the second case report of hemangiosarcoma developing in or near the mesentery. In other animals, development of hemangiosarcoma in the mesentery has been reported in only one case, a South American sea lion, and it occurred between the duodenum and colon²³. In conclusion, the occurrence of hemangiosarcoma in the abdominal cavity excluding the liver and spleen is relatively rare in ferrets. Further investigation is necessary to clarify the characteristics of hemangiosarcoma in this species.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no conflicts of interest to disclose in connection with this report.

Acknowledgments: We thank Mr. Motohiro Nishikawa for his excellent technical assistance.

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