Case Report

Cutaneous Hemangiosarcoma in a Dog

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Abstract: A male golden retriever of unknown age presented with multiple cutaneous and subcutaneous masses from the left elbow to the digits. Histopathologically, multiple tumor foci had formed from the dermis to the subcutaneous tissue. Tumor foci consisted of a vascular structure, alveolar structure and solid proliferative area. The borders among these areas were not clear. Some neoplastic cells resembled a mature endothelium, while others were large pleomorphic cells. Immunohistochemically, the tumor cells were usually strongly positive for CD31 and often positive for PROX-1, the lymphatic endothelial cell marker. Based on these findings, the tumor was diagnosed as a hemangiosarcoma with lymphatic differentiation. (DOI: 10.1293/tox.26.193; J Toxicol Pathol 2013; 26: 193–195)

Key words: canine, hemangiosarcoma, lymphangiosarcoma

Hemangiosarcoma is commonly observed in the spleen of the dog but is less frequently found in the skin1–3. It mostly affects older dogs, both male and female1–3. Histologically, it is a malignant tumor of the endothelial cells, characterized by a vascular channel containing blood. Sometimes it is difficult to correctly differentiate it from lymphangiosarcoma because no blood is found in the vascular space1–3.

A male golden retriever of unknown age presented with multiple 1- to 7-cm diameter cutaneous and subcutaneous masses from the left elbow to the digits. These masses were poorly demarcated from surrounding tissue, and the overlying skin was often ulcerated (Fig. 1). The tumor masses were solid and rubbery. The cut surface of the masses was solid and yellowish white in color. The left forelimb was amputated from the shoulder joint. Two days after surgery, the dog developed neurological symptoms. He was euthanized the next day, but necropsy was not performed, so the cause of the neurological symptoms was not clear. Some of the macroscopic masses obtained at the amputation were fixed in 10% neutral formalin (pH 7.4), dehydrated in a graded series of ethanol and embedded in paraffin, and 4-µm-thick sections were stained with hematoxylin and eosin (H.E.). Immunohistochemical staining was performed by a labeled-polymer method using N-Histofine Simple Stain MAX PO (M or R) (Nichirei, Japan). The primary antibodies used for each section were anti-human CD31 (diluted at 1:20, mouse monoclonal antibody, DAKO, Denmark), anti-human von Willebrand factor (factor VIII-related antigen) (diluted at 1:200, rabbit polyclonal antibody, DAKO), anti-prospero homeobox protein 1 (PROX-1) (diluted at 1:200, rabbit polyclonal antibody, Angio Bio, Del Mar, United States), anti-human smooth muscle actin (α-SMA) (diluted at 1:400, mouse monoclonal antibody, DAKO), anti-human calponin (diluted at 1:200, mouse monoclonal antibody, DAKO), anti-human desmin (diluted at 1:100, mouse monoclonal antibody, D33, DAKO) and anti-cytokeratin (AE1/AE3) (diluted at 1:100, mouse monoclonal antibody AE1/AE3, DAKO).

Histopathologically, multiple tumor foci of varying size had formed from the dermis to subcutaneous tissue. They were divided by dense fibrous tissue continuing from the dermis (Fig. 2). Tumor cells frequently infiltrated into fibrous tissue and formed small foci. Tumor foci consisted of vascular and alveolar structures and had a solid proliferative area. These 3 different areas were admixed in different proportions and transitioned to each other. The vascular structure showed multiple capillaries lined by small endothelial cells lacking cell atypia (Fig. 3a), and the alveolar structure was lined by large pleomorphic cells with a clear lumen (Fig. 3b). The majority of proliferative vascular channels and the alveolar lumen did not contain blood. Moreover, the solid proliferative area consisted of spindle, round or polygonal cells with some slit-like cavities (Fig. 4), but an obvious vascular structure was rarely seen. Mitotic figures increased in the nonvascular area, and immunohistochemically, almost all tumor cells were strongly positive for CD31, a marker for endothelial cells (Figs. 5a, b, c). Differentiated vessels were also strongly positive for factor VIII-related antigen.
Cutaneous Hemangiosarcoma in a Dog

(Fig. 5d), but cells in the solid foci were weakly positive or negative (Fig. 5e). The nuclei of differentiated vessel endothelial cells were negative for PROX-1, which was used as a marker for the lymphatic endothelium, but large atypical nuclei were often weakly positive (Fig. 6). The walls of blood vessels and a part of the fibrous tissue among the tumor foci were positive for α-SMA, but the tumor cells were negative. The neoplastic cells were also negative for calponin, desmin and cytokeratin AE1/AE3. Based on these findings, the diagnosis of cutaneous hemangiosarcoma with lymphatic differentiation was made.

The present case was characterized by three histopathologic findings. First was the similarity of differentiated vessels to normal capillaries. Second was the alveolar structure lined by pleomorphic cells. Third was a solid proliferative area without a clear vascular lumen. The borders among these areas were not clear. Moreover tumor cells formed vascular structures in differentiated areas, suggesting an endothelial cell origin. These features were consistent with high-grade angiosarcoma.

The differential diagnoses included fibrosarcoma, rhabdomyosarcoma and synovial sarcoma. Fibrosarcoma is characterized by a fasciculated growth pattern composed of spindle-shaped cells and no clear vascular differentiation. Rhabdomyosarcoma, especially the alveolar subtype, shows an alveolar growth pattern but does not display clear vascular differentiation. Desmin is also positive, as it is a sensitive marker for rhabdomyosarcoma. Synovial sarcoma, especially the biphasic type, is composed of a glandular structure and fibrosarcoma-like area. The glandular structure is lined by a cuboidal to columnar epithelium and is positive for cytokeratin.

Hemangiosarcomas and lymphangiosarcomas include

![Fig. 1. Multiple nodules on the left toe with ulceration.](image1)

![Fig. 2. Multiple foci are formed and divided by fibrous tissue. H.E. Bar=500 µm.](image2)

![Fig. 3. (a) The vascular structure shows multiple capillaries lined by small endothelial cells. (b) The alveolar structure is lined by large pleomorphic cells with a clear lumen. H.E. Bars=50 µm.](image3)

![Fig. 4. The solid proliferative area consists of round, polygonal (a) and spindle cells (b) with some slit-like cavities (arrows). H.E. Bars=50 µm.](image4)
a broad morphologic spectrum from highly to poorly differentiated tumors. Hemangiosarcomas composed of vascular channels containing blood were easy to differentiate from lymphangiosarcomas. However, highly to poorly differentiated hemangiosarcomas without blood-containing channels could not be distinguished from lymphangiosarcomas. In our case, since the vascular lumen rarely contained blood, lymphatic differentiation was suggested. However, a definite diagnosis was difficult based on the histological features. Thus, immunohistological analysis was used for definitive differentiation. In the present case, immunohistochemical positivity for CD31 and factor VIII-related antigen, in addition to detection of weak and partial positivity for PROX-1, supported the theory that the tumor cells originated from the vascular and lymphatic endothelium. D2-40 (podoplanin) has also been used as a specific marker of the lymphatic endothelium in humans, but we could not stain lymphatic endothelial cells of the dog because of no cross-reactivity. Thus, it is impossible to determine which tumor showed lymphatic versus vascular differentiation. In conclusion, the present case may be diagnosed as an angiosarcoma according to the histologic classification of human soft tissue tumors.1

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