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Immunohistochemical Diagnosis of Primary Renal Hemangiosarcoma in a Dog

Soo-Kyo Jung, Hyoung-Seok Yang* and Jae-Hoon Kim[1]

College of Veterinary Medicine and Veterinary Medical Research Institute, Jeju National University, Jeju 690-756, Korea
**Jeju Self-Governing Provincial Veterinary Research Institute, Jeju 695-963, Korea*

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Abstract : A 7-year-old castrated male Yorkshire Terrier was presented with a palpable intra-abdominal mass. In radiography, a large radioopaque renal mass and small abdominal mass were found on dorsal area of the abdomen. Grossly, red to brown color mass and a cystic structure (hydronephrosis) were embedded in the right kidney. Histopathologically, the mass had many irregular shaped neovascular channels lined by polygonal or oval shaped endothelial cells. These vessels and neoplastic cells had great invasive tendency to adjacent connective or fat tissues. Small abdominal mass had identical morphologic features as in renal mass. According to immunohistochemistry, the neoplastic cells in renal mass demonstrated strong positive signals for vimentin and CD31, and weak positive for von Willbrand factor. However, there were no positive reactions for cytokeratin. Based on the gross, histopathology and immunohistochemistry, this mass was diagnosed as primary renal hemangiosarcoma in a Yorkshire Terrier dog.

Key words **:** CD31, dog, hemangiosarcoma, immunohistochemistry, kidney.

Introduction

Hemangiosarcoma (HSA) is a malignant tumor of vascular endothelium, occurs more commonly in dogs, especially German shepherd breeds, than in other domestic animals. The tumor can arise in any sites of body, however the most common primary sites are spleen, skin (subcutis), right atrium, and liver (7). Renal HSA is a rare anatomic variant in dogs, accounting for 0.01% of all identified canine HSA (6). Depending on where the mass arises, HSA is classified as visceral and nonvisceral forms. In dogs, nonvisceral HSA occur less frequently than visceral HSA (9). Visceral HSA is highly aggressive tumor with poor prognosis, but cutaneous HSA is less aggressive than their visceral counterparts (6).

HSA can be diagnosed through histopathologic examination, but poorly differentiated vascular neoplasm may be indistinguishable from fibrosarcoma or other poorly differentiated sarcoma. Therefore immunohistochemistry is helpful in recognizing the endothelial origin of the tumor cells in such instances (5). Canine vasoendothelial tumors are commonly expressed for CD31 antigen and von Willebrand's factor (vWf; previously named factor VIII-related antigen), which are endothelial cell markers (4,11). Two cases of renal HSA were previously reported in Korea (2,13). These reports described the clinical findings such as radiography and ultrasonography, and final diagnosis were confirmed according to histopathologic or cytotologic features without immunohistochemical examinations. Here we describe the clinical, histopathologic and immunohistochemical features of primary renal HSA in a Yorkshire Terrier dog.

[1]Corresponding author.
E-mail : kimjhoon@jejunu.ac.kr

Case

A 7-year-old, castrated male, Yorkshire Terrier with a palpable intra-abdominal mass was submitted for diagnosis in a local animal hospital. Abdominal radiography showed a large radioopaque mass in dorsal area of the abdomen (Fig 1). The mass was approximately 8 × 5 × 3 cm in size and located in the right kidney. On the cut surface, normal renal parenchyma was almost disappeared and replaced by a large mass and adjacent cystic cavity (Fig 2). Renal mass showed red to brown color with bloody oozing contents and surrounded by dense connective and fat tissues. However cystic cavity contained yellowish turbid urine contents and surrounded by severely atrophied renal parenchyma. During the surgery, another small mass surrounded with fat tissue was also found in lower part of renal mass. Surgically excised renal mass and abdominal mass were immediately fixed in 10% neutral buffered formalin, and then referred to Veterinary Pathology Laboratory in Jeju National University.

The samples were processed routinely for histopathologic examination and sections were stained with hematoxylin and eosin (H&E). To reveal the origin of tumor cells, immunohistochemistry (IHC), streptavidin-biotin peroxidase complex method, was performed using several antibodies such as

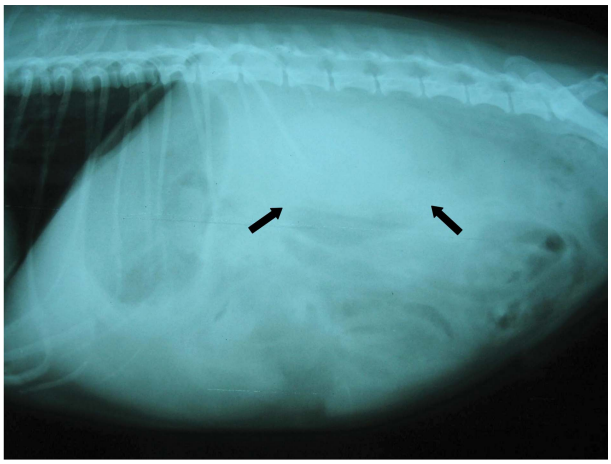

Fig 1. Note a large radioopaque mass (arrows) in dorsal area of the abdomen.

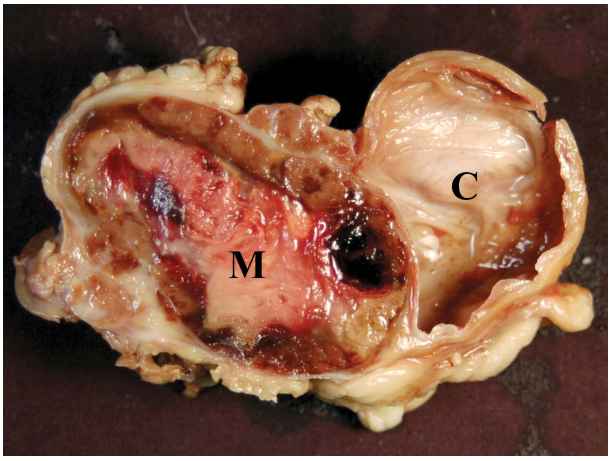

Fig 2. A large red to brown color mass (M) and a cystic structure (C) were embedded in the right kidney.

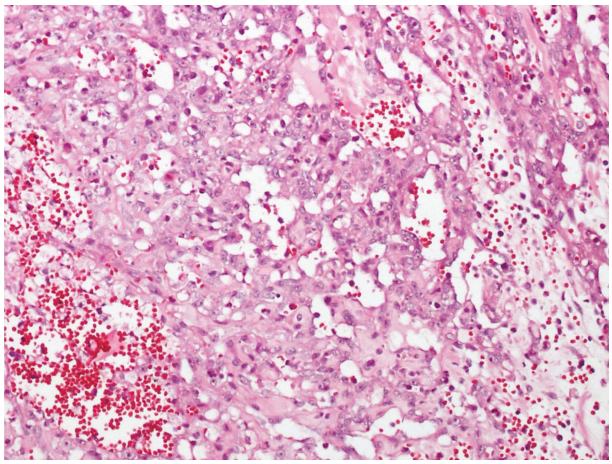

Fig 3. Note numerous new-formed irregular shaped blood vessels lined by polygonal or oval shaped endothelial cells in the mass. H&E, × 200.

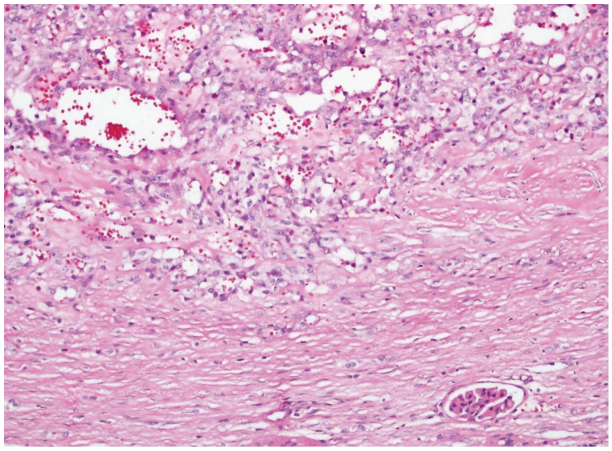

Fig 4. Normal renal parenchyma was severely atrophied. Neoplastic vessels and cells showed marked invasive tendency to adjacent connective tissue. H&E, × 200.

vimentin, CD31, vWf, and cytokeratin (CK). The antibody source, type, and working dilution for each antibody were listed in Table 1.

On histopathologic examination, the renal mass was composed of peripheral, renal capsular or subcapsular, desmoplastic zone and central neoplastic area with multifocal severe hemorrhage and necrosis. There were numerous new-formed or irregular shaped vascular structures that were separated with thin connective tissues in the central area of renal mass. These vascular structures were lined by polygonal or oval shaped endothelial cells with bulging pleomorphic and hyperchromatic nuclei. Large amounts of protein fluids, RBC, fibrin and necrotic neutrophils were occupied in the lumen of vasculatures (Fig 3). Mitotic figures were frequently observed in the high power field (Fig 5A). Spindle like cells also presented around new-formed blood vessels in some areas. These vascular cells had great invasive tendency to adjacent connective or fat tissue (Fig 4), therefore tumor cells emboli were occasionally observed in vasculatures in desmoplastic area. Multifocal severe necro-hemorrhagic foci with fibrin deposition were scattered throughout the renal mass. Cystic

Table 1. Source, type and dilution of antibodies in immunohistochemical examination

Antibody	Type/Clone	Dilution	Source
Vimentin	Monoclonal mouse, V9	1:50	DAKO A/S, Denmark
CD31	Monoclonal mouse, JC70A	1:20	DAKO A/S, Denmark
vWf	Monoclonal mouse, 2F2-A9	1:100	BD biosciences, USA
Cytokeratin	Monoclonal mouse, AE1/AE3	1:100	DAKO A/S, Denmark

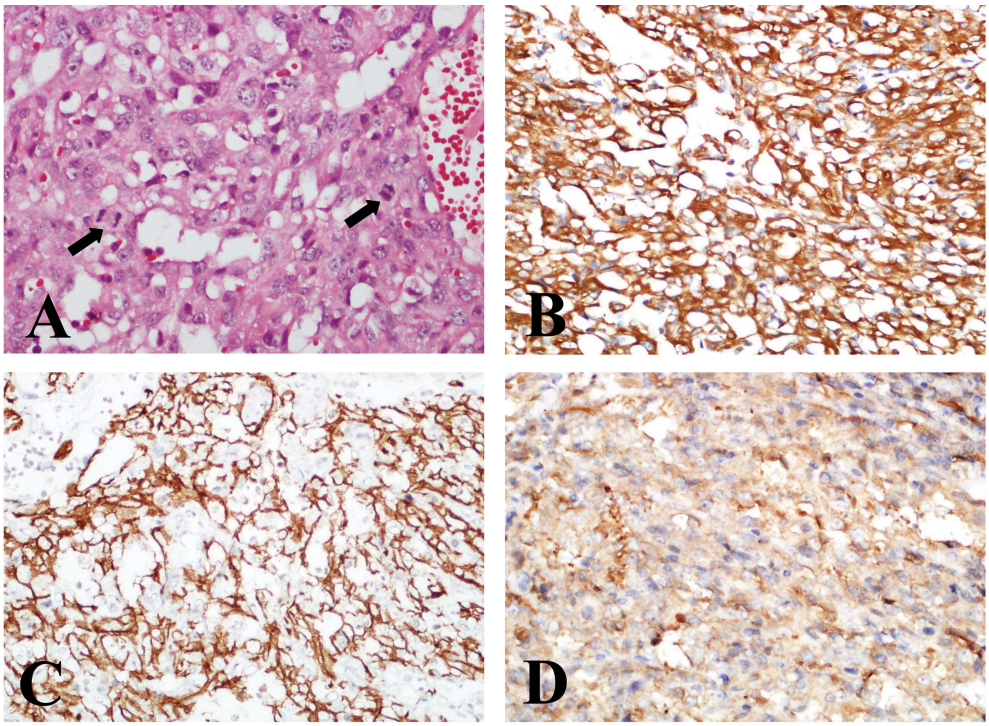

Fig 5. Histopathologic and immunohistochemical findings of renal HSA. Neoplastic cells had polygonal or oval shape with bulging nuclei. Note many mitotic figures (arrows). H&E, × 400 (A). Note strong positive immunoreactivity for vimentin (B) and CD31 (C), and weak positive reaction for von Willebrand's factor (D). Streptavidin-biotin peroxidase stain, × 400.

cavity surrounding tissues were composed of severely atrophied renal tissues including glomeruli and renal tubules without neoplastic foci. Another abdominal mass was composed of central neoplastic foci admixed with hemorrhage and necrosis and peripheral peritoneal fat tissues. Morphologically the tumor cells in abdominal mass was identical that of in renal mass. According to IHC, the tumor cells in renal mass demonstrated strong positive signals for vimentin (Fig 5B), CD31 (Fig 5C), and vWf (Fig 5D). However, there were no positive reactions for CK, the epithelial cell specific marker.

Based on the gross, histopathologic and immunohistochemical features, this case was diagnosed as primary renal hemangiosarcoma.

Discussion

The kidneys are quite common sites for development of metastases, however primary renal tumors are relatively uncommon in both the dog and cat, accounting for only 1.7% and 2.5% of all dog and cat tumors (3). The primary renal tumors are usually grouped according to cellular origin into epithelial, nephroblastic, and mesenchymal tumors (1,8). The most frequent epithelial tumor, accounting for 76% of renal primary tumors, is renal cell carcinoma. About 16% of primary renal neoplasia is mesenchymal tumors such as fibroma and hemangioma, or their malignant counter parts in dogs. Metastatic neoplasms are two times as common as primary ones in dogs. The tumor in this case should be differentiated from these tumors and tumor-like lesions including telangiectasia. Renal cell carcinomas are malignant epithelial tumors without any embryonal differentiation (8). These tumors are subdivided into several types such as papillary, tubular, solid, and clear cell variants based on the predominant cellular type. According to previous literatures renal cell carcinomas demonstrated CK and vimentin positive (8). Differentiation from mesenchymal sarcoma requires IHC staining positive for vimentin and negative for CK. Telangiectasia consists of non-neoplastic proliferations of blood vessels in kidneys. Grossly this tumor-like lesion have very similar morphology to HSA, but the distinction from HSA is that the blood-filled spaces in telangiectasia are lined by simple endothelium, and there is no proliferation of endothelial cells along the blood vessels (8). On the basis of IHC profiles such as vimentin positive and CK negative, the tumor in this case is determined to be mesenchymal origin. In addition, many new-formed and irregular shaped blood vessels lined by polygonal or oval shaped endothelial cells in this case indicate the tumor is vascular origin.

The immunohistochemical detection of vWf and CD31 is regarded as an excellent tool for the identification of endothelial cells and vascular origin tumors (4,11). However overall immunoreactivity of CD31 is more sensitive than vWf (4). According to previous study (5), vWf antigen appears to be present in endothelia of the majority of benign angiomatous neoplasms and tumor-like lesions (98%), however absent in a

large number of angiosarcomas (38%). In addition, the widespread presence of vWf antigen in serum, thrombotic material and body fluids could give rise to false-positive interpretation (12). In this case, strong positive reaction for CD31 was observed on the surface of endothelial cells. However vWf showed weak positive immunoreactivity and a background staining at the site of exudation, hemorrhage and necrotic area. This result indicated that CD31 can be a more useful marker of vascular endothelium than vWf, especially for poorly differentiated HSA (5).

Although distinction between primary and secondary HSA is not always easy, the kidney was probably the primary site because 1) this dog was initially presented with an abdominal mass; 2) the usual primary sites for canine HSA (spleen, liver, and right atrium) were not involved; 3) the kidney was prominently enlarged, whereas another mass in abdomen was very small. Spreading of cells from malignant primary tumors can occur directly into the local tissue or via blood vessels and lymphatics or by invasion of body cavities such as the peritoneum (10). Because there is no evidence of pulmonary metastasis, tumor cells from kidney might be invaded and grown in the peritoneum in this case. One retrospective study demonstrated that distant metastasis at diagnosis appear to occur less frequently in dogs with renal HSA compared with other visceral forms of HSA (6).

Rupture of a primary visceral tumor, as seen in dogs with splenic or hepatic HSA, often leads to hemoperitoneum and subsequent hemorrhagic shock (6,13). Interestingly hydronephrosis was observed in this case. This lesion, occasionally observed in renal pelvis tumors, might be closely related with partial obstruction of urinary tract due to renal HSA (8).

In summary, abdominal mass was detected by radiography in a 7-year-old, castrated male Yorkshire Terrier dog. Histopathologically, we diagnosed this case as primary renal hemangiosarcoma. Especially, through vimentin and CD31 immunostaining, we confirmed that the tumor was vascular endothelium in origin and metastasized into abdominal space.

Acknowledgments

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개에서 발생한 신장원성 혈관육종의 면역조직화학적 진단

정수교 · 양형석* · 김재훈[1]

제주대학교 수의과대학, 수의과학연구소, *제주특별자치도 동물위생시험소

요 약 : 7년령의 중성화된 수컷 Yorkshire Terrier가 복강에 종괴가 촉진되어 이를 주증으로 내원하였다. 방사선 검사상 복강의 등쪽면에 신장 종괴와 작은 복강 종괴가 관찰되었다. 수술적으로 적출된 우측 신장에서는, 육안적으로 붉은 갈색조를 띄는 종괴와 낭상 구조가 함께 관찰되었다. 병리조직검사 상에서 다각형 또는 타원형의 내피세포를 가진 다수의 불규칙한 모양의 신생혈관이 관찰되었으며, 이 혈관들과 종양세포들은 주변조직으로의 강한 침습성을 보였다. 복강의 또 다른 작은 종괴에서는 신장 종괴와 동일한 병리조직학적 소견을 관찰할 수 있었다. 면역조직화학염색을 실시한 결과 종양세포들은 vimentin, CD31 및 von Willbrand factor에 대해 양성 반응을 보였으나, cytokeratin에 대해서는 음성 반응을 보였다. 육안적인 특징, 병리조직학적 소견 및 면역조직화학적 검사를 바탕으로 본 증례는 신장원성 혈관육종으로 진단하였다.

주요어 : CD31, 개, 혈관육종, 면역조직화학염색, 신장