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**Case Report**

# Spontaneous nephroblastoma with oncocytic differentiation in a Japanese White rabbit

Osamu Katsuta[1*], Yumi Kuriki-Yamamoto[1], Toru Shibata[1], Takaharu Mochizuki[1], Miwa Yoshimi[1], Takahisa Noto[1], and Hidetoshi Mano[1]

[1] Toxicology and Pharmacokinetics Group, Non-clinical Research, Research & Development Division, Santen Pharmaceutical Co., Ltd., 8916-16 Takayama-cho, Ikoma-shi, Nara 630-0101, Japan

**Abstract:** Spontaneous nephroblastoma is an uncommon tumor in laboratory rabbits. We recently encountered this tumor, and we describe its histological characteristics in this report. A male 3-year-old Japanese White rabbit (JW/kbs), maintained as a stock animal, suddenly showed poor condition and was found dead a few days later. At necropsy, a large mass was found that extended from one side of the renal pelvis. The cut surface of the mass was dark red in color and velvety to the touch. The kidney on the contralateral side was normal. Microscopically, the tumor mass consisted of biphasic components, which consisted of epithelial (tubular and glomerular) and blastemal (nodular) elements. No sarcomatous proliferation was observed. In addition, some of the tubules were lined by cells with a large amount of eosinophilic cytoplasm. The cells were confirmed as oncocytes by immunohistochemical and electron microscopic examinations. The present case was therefore diagnosed as a nephroblastoma with oncocytic differentiation. (DOI: 10.1293/tox.2016-0067; J Toxicol Pathol 2017; 30: 169–175)

**Key words:** nephroblastoma, laboratory rabbit, oncocytic differentiation, spontaneous

A nephroblastoma is a true embryonal tumor that arises in primitive nephrogenic blastemas[1]. This tumor is commonly observed in pigs and chickens, far less often in calves and dogs, and uncommonly in sheep, horses, and cats[1]. In laboratory rats, it is induced with genotoxic chemicals[2]; however, historical data regarding natural occurrence of the tumor in rats have been reported[3–5]. It is extremely rare in mice[2] and is also rarely observed in laboratory rabbits[6]. Embryonal nephroma, a synonym of nephroblastoma, is commonly found in domestic rabbits and is usually an incidental finding at necropsy[7]. We recently encountered a rabbit bearing a nephroblastoma with oncocytic differentiation; the rabbit had been used in some ophthalmic pharmaceutical studies of eye drops. In this report, we describe the macroscopic and microscopic characteristics of the lesion and compare them with those of tumors in other species.

A male 3-year-old Japanese White rabbit (JW/kbs, Kitayama Labes Co., Ltd., Nagano, Japan), maintained for some months as a stock animal after being used in a previous experiment, suddenly showed symptoms of depression and anorexia. The next day, the animal had a high fever, and it was found dead in the morning three days later. The treatment and handling of animals were approved by the Animal Care and Use Committee of the Nara Research and Development Center, Santen Pharmaceutical Co., Ltd.

At necropsy, a large spherical mass, 5 cm in diameter, was found that extended from the pelvis at the right kidney (Fig. 1). Adipose tissue was observed between the renal hilus and the mass. The capsule continued to the compressed renal tissue. The cut surface of the mass was dark red in color and velvety to the touch (Fig. 1). The left kidney was normal. As for other organs, an ulcer, 1 cm in diameter, was found at the pyloric antrum of the stomach. Blackish contents were seen in the stomach and throughout the entire length of the intestine. No other remarkable findings were detected in the body. Organs/tissues, including the right kidney with the mass, were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE). Additionally, periodic acid-Schiff (PAS) reaction and periodic acid-methenamine-silver (PAM) staining methods were performed on the right kidney. For immunohistochemistry, using a labeled polymer method (Histofine Simple Stain MAX-PO (M), Nichirei Biochemical, Tokyo, Japan), was used to stain selected sections with mouse monoclonal antibodies as follows: anti-cytokeratin (AE1/AE3, 1:100, Dako Japan, Tokyo, Japan), anti-vimentin (1:100, Merck Millipore Corporation, Darmstadt, Germany), anti-desmin (1:100, Dako Japan), anti-α-smooth muscle actin (αSMA) (1:500, Dako Japan),

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*Corresponding author: O Katsuta
(e-mail: osamu.katsuta@santen.co.jp)

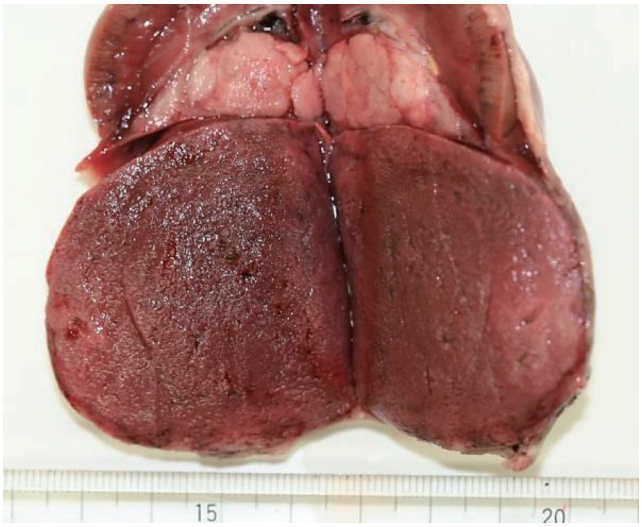


**Fig. 1.** Macroscopic appearance of the tumor mass. The capsule continues to the compressed renal tissue, and adipose tissue at the hilus is included between the kidney and the mass. The cut surface of the mass is dark red in color and feels velvety when touched.

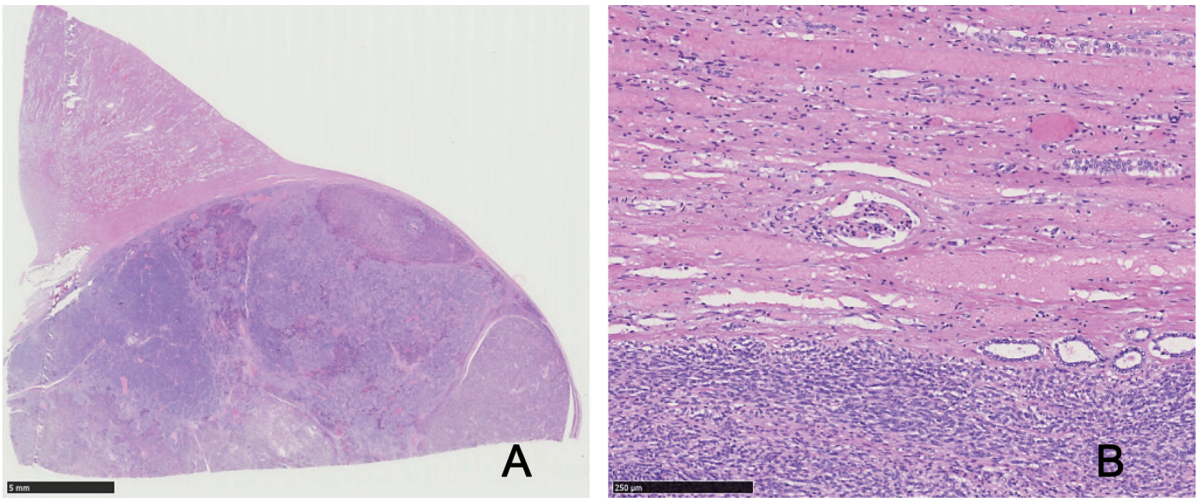


**Fig. 2.** Low magnification of the tumor mass with nonneoplastic renal tissue (A). Higher power view at the border (B). The tumor is bounded clearly from the renal tissue. HE stain. Bars = 5 mm (A) and 250 µm (B).

and anti-prohibitin (1:500, abcam, Tokyo, Japan); staining was visualized with diaminobenzidine tetrahydrochloride as the chromogen. The sections were counterstained with hematoxylin. For electron microscopy, several pieces of the formalin-fixed mass were refixed in 2% phosphate-buffered glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were stained with 2% uranyl acetate and 1% lead nitrate. The sections were then examined with a transmission electron microscope (H-7600, Hitachi Ltd., Tokyo, Japan).

Microscopically, the tumor was encapsulated by thick connective tissue (Figs. 2A and 2B); the renal tissue, however, was markedly compressed and atrophied with increased fibrous components (Fig. 2B). The tumor was composed of tubular structures lined by cuboidal to columnar epithelial cells with hyperchromatic nuclei (Fig. 3A), some of which showed rosette-like patterns (Fig. 3B), primitive glomeruli (Fig. 3C), and ribbon-like arrangements. Also, a whirl or nodular pattern that consisted of basophilic blastemal cells (Fig. 3D), the nuclei of which were round to ovoid in shape and the nucleus to cytoplasm ratios of which were very high, was observed with or without tubules in the periphery. These epithelial components were separated from each other by collagenous connective tissue. This tissue appeared to be a basement membrane when examined in specimens stained with PAS (Figs. 4A–C) and PAM (Fig. 4D). The center of the tumor was necrotic because of insufficient blood supply. The results regarding immunohistochemical reac-

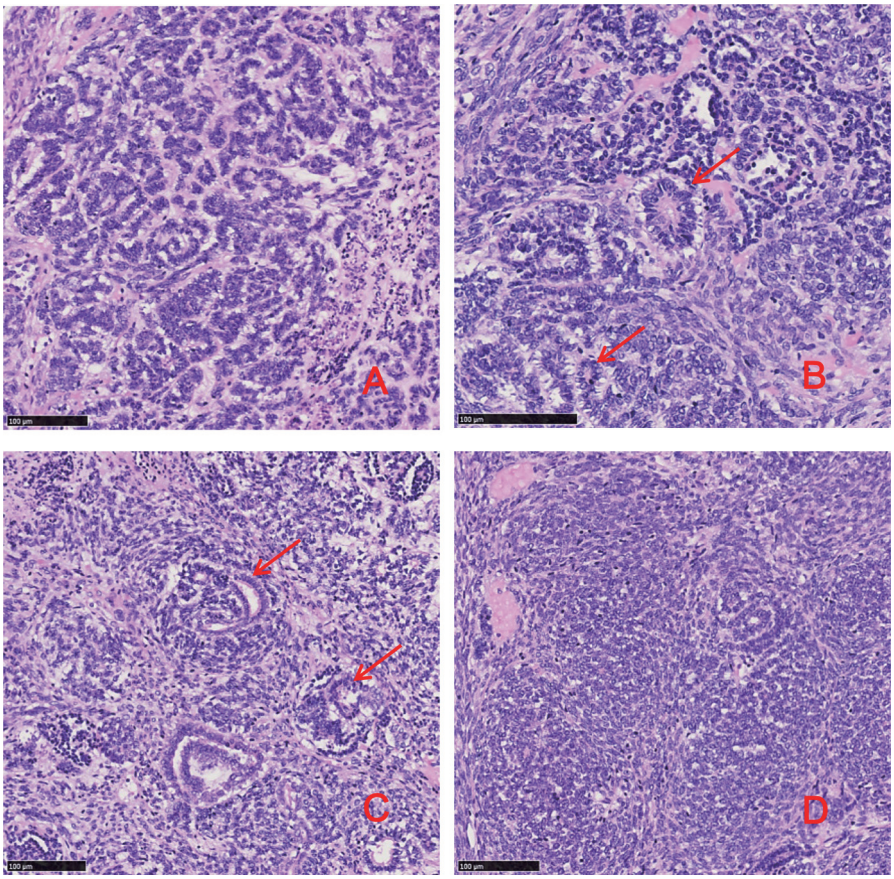


**Fig. 3.** Note the characteristic growth patterns of the tumor: tubular (A), rosette-like (arrows in B), primitive glomeruli (arrows in C), and nodular (D). HE stain. Bars = 100 µm.

**Table 1.** Immunohistochemical Reactivities of the Tumor Components

| Primitive antibodies | Cellular type | | | |
|---|---|---|---|---|
| | Tubular | Primitive glomerular | Blastemal | Oncocytic |
| Cytokeratin | +* or ++ | – | – | ++ |
| Vimentin | – or +/– | ++ | +/– | – |
| Desmin | – | – | – | – |
| αSMA | – | – | – | – |
| Prohibitin | +/– | +/– | – or +/– | ++ |

*Reactivity: –, negative; +/–, slightly positive; +, positive; ++, strongly positive.

tivity in the tumor components are summarized in Table 1. Some of the tubule-composed epithelial cells were positive for cytokeratin (Fig. 5A), and the surrounding epithelial cells of the primitive glomerulus reacted strongly positive for vimentin (Fig. 5B). The basophilic blastemal cells were slightly positive for vimentin (Fig. 5C), and the tumor components did not react to αSMA (Fig. 5D) or desmin at all. In accordance with these findings, the tumor was diagnosed as a nephroblastoma. No metastasis or invasion was noted. Also, mitotic figures of the tumor cells were detected as a rare phenomenon.

It was further noted that a few tubules lined by eosinophilic epithelial cells with abundant fine granules in the cytoplasm and small round nuclei were widely scattered in the tumor mass (Fig. 6A). These findings were shown clearly in PAM-stained specimens (Fig. 6B). The cytoplasm reacted negative for PAS stain, was strongly positive for both cytokeratin (Fig. 6C) and prohibitin (Fig. 6D), and was negative for both vimentin and desmin in immunostaining. In the ultrastructural examination, numerous mitochondria were observed in the cytoplasm of the eosinophilic cells (Figs. 7A and 7B). These findings indicated that the cells were differentiated oncocytes. There were no findings in the kidney of the contralateral side.

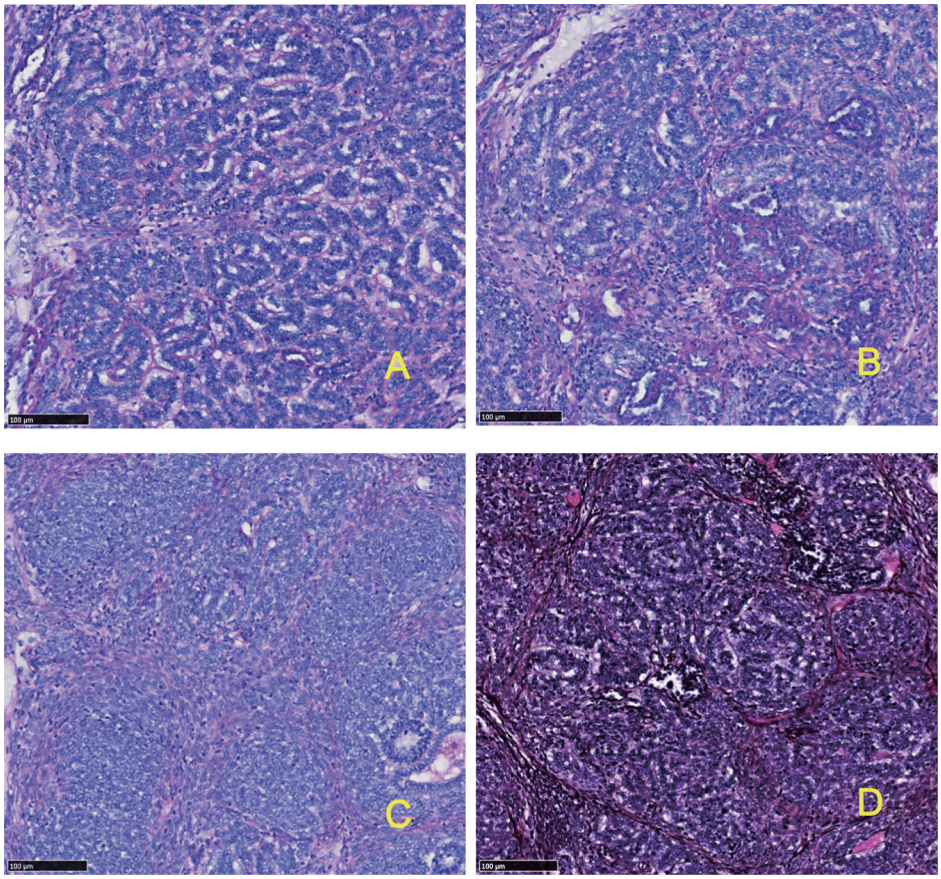


**Fig. 4.**

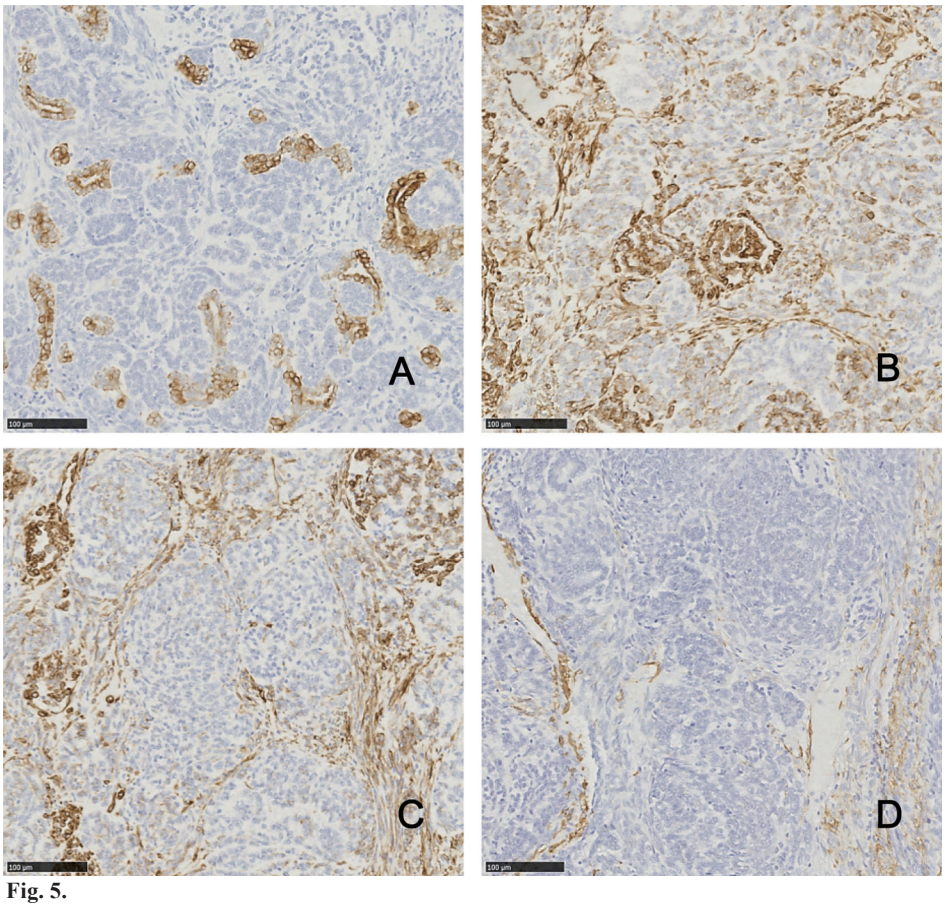


**Fig. 5.**

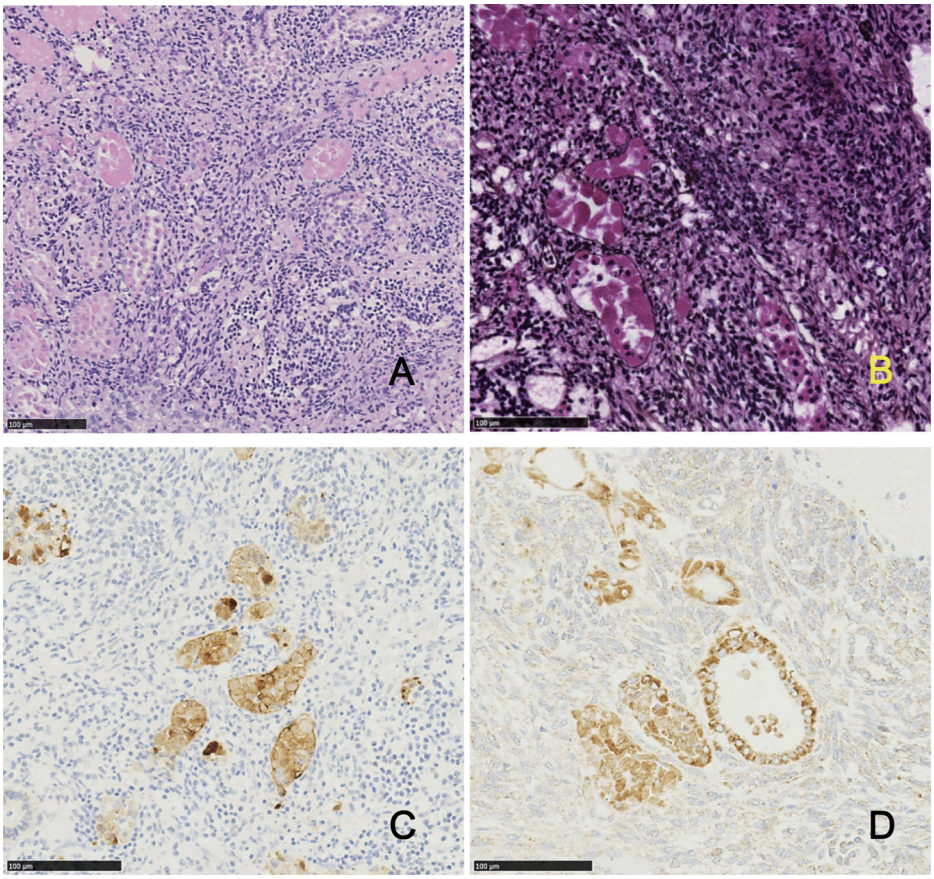


**Fig. 6.** Note the oncocytic differentiations. Eosinophilic epithelial cells can be seen in some tubules (A, HE stain; B, PAM stain). Immunohistochemical staining for cytokeratin (C) and prohibitin (D) shows strong reactions in some tubules. Bars = 100 μm.

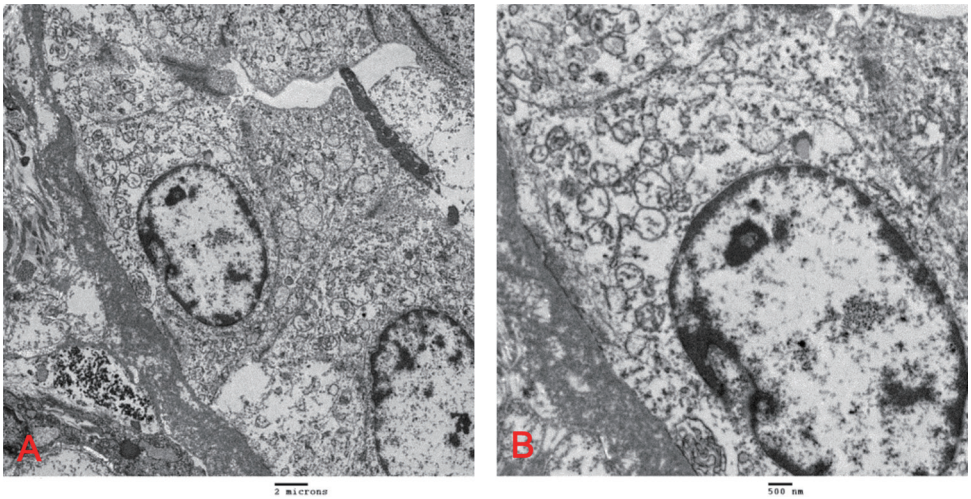


**Fig. 7.** Transmission electron microscopic images of the oncocytic cells (A and B). B shows a higher power view of a portion of A. A lot of mitochondria can be seen in their cytoplasm. Bars = 2 μm (A) and 500 nm (B).

**Fig. 4.** Staining with PAS (A–C) and PAM (D). Wide and/or scanty connective tissues surround the epithelial components of the tumor. Bars = 100 μm.

**Fig. 5.** Immunohistostaining for the tumor components. Cytokeratin shows a positive reaction in some tubular epithelium (A). Vimentin is strongly positive in primitive glomeruli and the connective tissues (B and C) but slightly positive in the blastemal cells (C). αSMA shows a positive reaction in the vascular walls and connective tissue in the interstitium (D). Bars = 100 μm.

Severe ulceration with hemorrhage was seen in the submucosal layer in the stomach, and centrilobular atrophy of hepatocytes with nuclear anisokaryosis was seen in the liver. It was considered that these findings indicated poor systemic conditions of this animal. The mass of the nephroblastoma also might have affected the rabbit's condition because of its huge size. The other organs and tissues were normal.

Hard and Fox have light[6] and electron[8] microscopically demonstrated in rabbits transplacentally exposed to N-ethylnitrosourea (ENU) that nephroblastomas in rabbits contain epithelial structures (tubular and glomerular), and blastemal nodular patterns with no sarcomatous mesenchymal proliferation and that the rabbit nephroblastoma resembles the Wilm's tumor in humans. Also, the triphasic components including sarcomatous proliferation were emphasized in spontaneously occurring nephroblastomas in an Angora rabbit[9]. In the present case, we detected the biphasic components with no proliferation of the sarcomatous element. Concerning the biological behavior of nephroblastomas, nephroblastomas are potentially malignant and are reported with metastatic events in rats[3, 10]. However, nephroblastomas in rabbits are thought to be basically benign[6], because of low metastatic potential and few mitoses even in ENU-induced tumors[7]. Renal cell carcinoma must be considered as a differential diagnosis for nephroblastoma. As for renal cell carcinomas in rabbits[11, 12], the absence of undifferentiated blastemas and primitive glomerular formation in the tumor enables distinction from nephroblastoma.

The present case showed oncocytic differentiation along the neoplastic tubules in the tumor mass. Renal oncocytic hyperplasia/oncocytoma originates from the collecting tubular epithelium in rats[13, 14] and renal oncocytomas[15, 16] and oncocyte-like renal cell carcinoma[17] have been reported in dogs. The diagnostic characteristic of the tumor is the presence of numerous intracytoplasmic mitochondria. Immunohistochemically, the tumor cells react positively for cytokeratin and negatively for vimentin. Furthermore, in the present case the cells reacted strongly positive for prohibitin. Immunostaining with prohibitin, immunized against the inner membrane of mitochondria, is a useful technique for identifying the organelle[18]. The oncocytic appearance in the present tumor indicates the possibility that the epithelial elements of a nephroblastoma differentiate into varied cell types.

Although the present case had been used in some ophthalmic pharmaceutical studies of eye drops, we have no evidence suggesting that the test substances involved induce neoplasms in any organs/tissues, even in combined administrations by instillation (in-house data). Thus, the present case of nephroblastoma is considered to be spontaneous.

We detected a spontaneous nephroblastoma with oncocytic differentiation in a laboratory rabbit. The combination of nephroblastoma with oncocytic differentiation has not been reported in any animal species so far as we know.

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**Disclosure of Potential Conflicts of Interest:** The authors declare that there are no conflicts of interest.

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