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Case Report

Spontaneous nephroblastoma with striated muscle differentiation in an F344 rat

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Abstract: An eleven-month old male F344/DuCrj (F344) rat was found dead and had right kidney mass at necropsy. Histopathologically, the mass was composed of nests of neoplastic stellate cells. At the center of the nests, neoplastic epithelial cells formed a tubular structure. In the fibrous connective tissue surrounding the nests, neoplastic cells with striations demonstrable by phosphotungstic acid hematoxylin were observed. Immunohistochemically, neoplastic stellate cells were partially positive for Wilms Tumor 1 and vimentin, and neoplastic cells with striations were partially positive for desmin. We diagnosed this tumor as a nephroblastoma with striated muscle differentiation. To our knowledge, this is the first case of nephroblastoma with apparent striated muscle differentiation in an F344 rat. (DOI: 10.1293/tox.2017-0004; J Toxicol Pathol 2017; 30: 231–234)

Key words: nephroblastma, striated muscle differentiation, F344 rat, spontaneous

Nephroblastoma is a malignant embryonal tumor derived from metanephric blastemal cells and usually occurs in young animals[1]. In rats, nephroblastoma is induced by direct-acting alkylating agents like *N*-methyl-*N*-nitrosourea[2, 3], but spontaneous nephroblastoma is rare in F344/DuCrj (F344) rats[1]. A nephroblastoma is composed of undifferentiated blastemal cells, epithelial elements, and mesenchymal elements. Mesenchymal elements of a nephroblastoma contain various cells like fibroblasts, bone, cartilage, smooth muscles, or striated muscles[4]. In nephroblastomas of rats, striated muscle differentiation is an extremely rare mesenchymal component. Here, we report a nephroblastoma characterized by striated muscle differentiation in an F344 rat.

An eleven month-old male F344 rat was found dead approximately one day after death. The rat was maintained without treatment under specific pathogen-free conditions in a temperature-controlled room with a 12-hour light-dark cycle at the Animal Facility of Osaka Prefecture University. Food and water had been provided *ad libitum*. The rat was handled according to the Guidelines for Animal Experimentation of Osaka Prefecture University. At necropsy, a 7 × 5.5 × 5-cm mass was observed in the right kidney. The mass was slightly soft, pale red to yellowish white at the cut surface, and wrapped in the renal capsule. In addition, severe hemorrhage was observed in the abdominal cavity. No masses were present in other organs macroscopically.

The mass was fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at a thickness of 4 μm, and stained with hematoxylin and eosin (HE). In addition, phosphotungstic acid hematoxylin (PTAH) staining was performed to visualize striations of the muscles. For immunohistochemical examination, sections from the mass were also subjected to the labeled polymer method using Histofine Simple Stain Rat MAX PO (MULTI) (Nichirei Biosciences Inc., Tokyo, Japan). The primary antibodies used in this study are shown in Table 1. We used the contralateral (left) kidney of the same aminal as a positive control for immunohistochemistry.

Histologically, the mass was composed of neoplastic cells with nest-like growth (Fig. 1A). Adjacent normal renal tissue was compressed by neoplastic cells and became sclerosed and atrophied. The neoplastic cells showing a nest-like growth pattern had scant oval to stellate cytoplasm and round nuclei (Fig. 1B). These neoplastic nests were separated by fibrous connective tissue. At the center of the nests, cuboidal or columnar epithelial cells frequently formed tubular structures (Fig. 1B and C). Neoplastic stellate cells and epithelial cells showed mild to moderate nuclear atypia, and mitotic figures were rarely observed. We judged these epithelial cells to be neoplastic because of nuclear and cellular atypia (Fig. 1B, inset). In the fibrous connective tissue, neoplastic proliferation of the cells with striation was observed (Fig. 1C). These cells had abundant eosinophilic cytoplasm with striations (Fig. 1D). The neoplastic cells with striations showed nuclear atypia and sometimes had multiple nuclei. The striations were clearly demonstrated by PTAH staining (Fig. 1D, inset). A summary of the immunohistochemistry

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Table 1. Primary Antibodies Used in this Study

Antibody	Clone	Supplier	Dilution
Wilms Tumor 1	6F-H2	Thermo Fisher Scientific (Waltham, MA, USA)	1:200
Vimentin	V9	Dako (Glostrup, Denmark)	Prediluted
Cytokeratin	AE1/AE3	Dako	1:1,000
Desmin	D33	Dako	Prediluted
α-smooth muscle actin	1A4	Dako	1:1,000
Ki-67	SP6	Nichirei Bioscience Inc., (Tokyo, Japan)	Prediluted

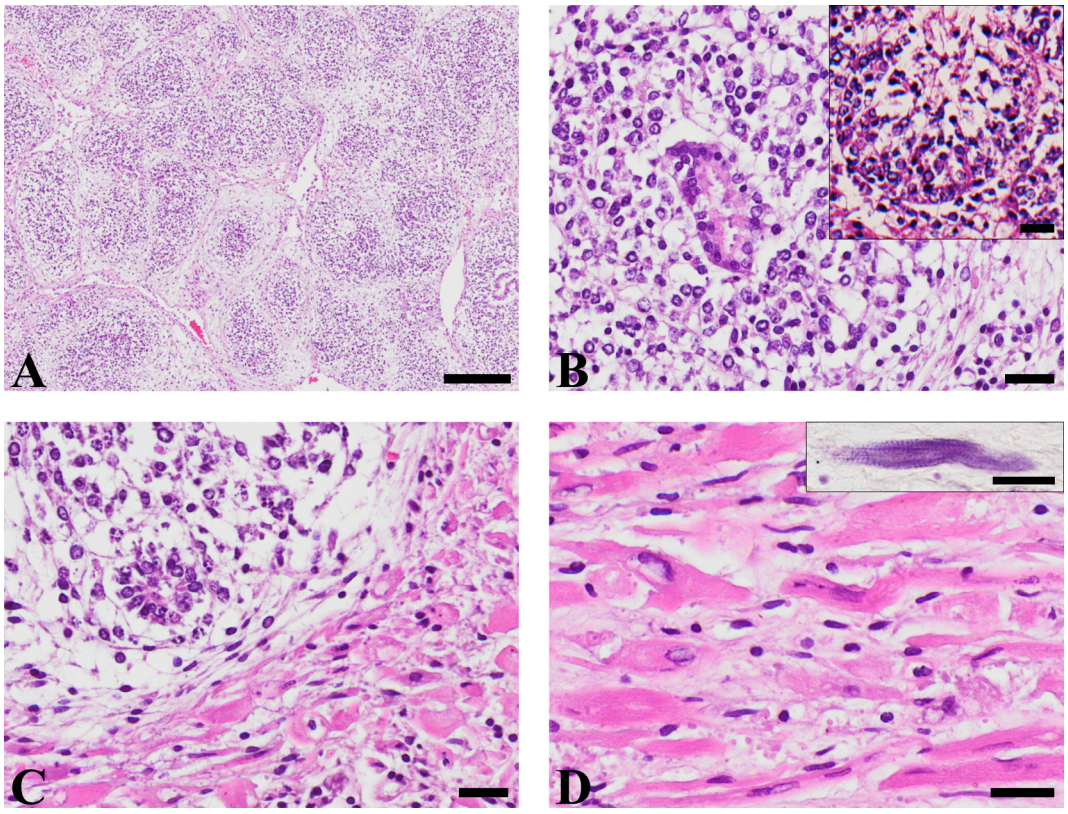

Fig. 1. Histopathological findings of the right kidney mass. Neoplastic cells show a nest-like growth pattern (A). In the center of nests formed by neoplastic stellate cells, neoplastic epithelial cells form a tubular structure (B). Neoplastic epithelial cells show nuclear and cellular atypia (B, inset). Nests of the neoplastic stellated cells are surrounded by tumor cells with abundant eosinophilic cytoplasm (C). The neoplastic cells with abundant eosinophilic cytoplasm have striations in their cytoplasm (D). HE stain. The striations show positive staining for phosphotungstic acid hematoxylin (PTAH) (D, inset). PTAH staining. Bars = 200 μm (A) and 20 μm (B–D).

results is shown in Table 2. Neoplastic stellate cells were partially positive for Wilms Tumor 1 (WT1) and vimentin (Fig. 2A and B). Therefore, these cells were considered to be blastemal cells. Epithelial cells forming tubules were positive for cytokeratin (CK) (Fig. 2C). Neoplastic cells with striations were positive for vimentin and partially positive for desmin but negative for α-SMA (Fig. 2D). All types of tumor cells were infrequently positive for Ki-67, suggesting low proliferative activity. In the present case, WT1-positive blastemal cells and CK-positive epithelial cells were observed. In addition, the tumor was characterized by a mixture of neoplastic proliferation with striated muscles. Taken together, we diagnosed this tumor as a nephroblastoma with striated muscle differentiation.

The differential diagnosis includes renal mesenchymal tumor[5] and rhabdomyosarcoma. In the present case, neoplastic proliferation of epithelial cells was observed, so we excluded renal mesenchymal tumor and rhabdomyosarcoma from the diagnosis. In humans, fetal rhabdomyomatous nephroblastoma (FRN) is known to be one variant of nephroblastoma. Furthermore, the epithelial and blastemal elements of a human FRN are small, and the tumors are predominantly composed of skeletal muscles of the fetal type[6]. In the present case, the neoplastic cells were mainly composed of blastemal cells, so we considered that the present tumor differed from a human FRN in terms of the main type of neoplastic cells.

In conclusion, the tumor in the present case was diag-

Table 2. Summary of the Immunohistochemistry Results

Antibody	Stellate cells	Epithelial cells	Striated muscles	Positive cells in normal rat kidney
WT1 [1)]	+/–	–	–	Podocytes
Vimentin	+/–	–	+	Mesenchymal cells
CK [2)]	–	+	–	Transitional epithelium
Desmin	–	–	+/–	None [4)]
α-SMA [3)]	–	–	–	Vascular smooth muscle cells
Ki-67	+/–	+/–	+/–	Tubular epithelium

[1)] WT1: Wilms Tumor 1. [2)] CK: Cytokeratin. [3)] α-SMA: α-smooth muscle actin. [4)] Desmin-positive cells were not present in the kidney of a normal F344 rat. Intensity of staining was graded as follows: −, negative; +/−, some cells positive; +, positive.

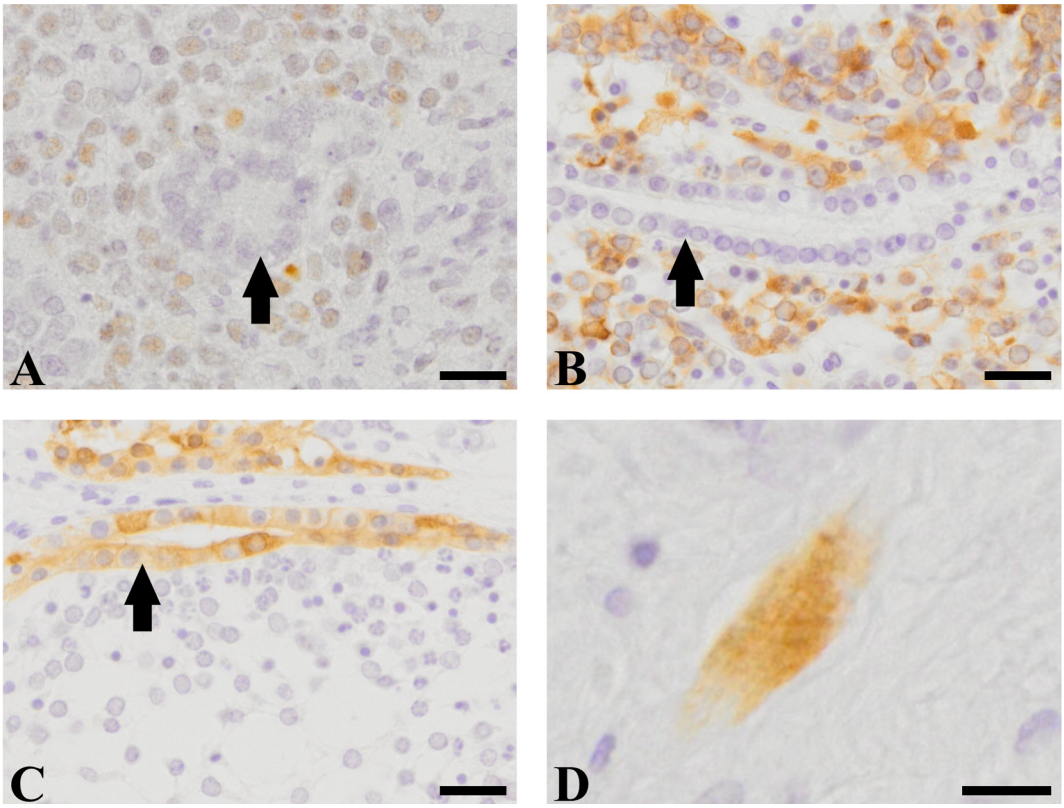

Fig. 2. Immunohistochemical findings of the right kidney mass. Blastemal cells are partially positive for Wilms Tumor 1 (WT1) (A) and vimentin (B). In contrast, epithelial cells surrounded by blastemal cells are negative for WT1 (A, arrow) and vimentin (B, arrow) but positive for cytokeratin (C, arrow). Some striated muscles show a positive reaction for desmin (D). Bars = 20 µm (A–C) and 10 µm (D).

nosed histopathologically as a nephroblastoma with striated muscle differentiation. In human nephroblastomas, striated muscle is the most common stromal cell type[7]. In contrast, striated muscle differentiation has rarely been reported in animals such as swine, cattle, or guanaco[8–10]. In rats, striated muscle differentiation in nephroblastomas has been reported only in two female Sprague-Dawley (SD) rats[11]. Therefore, striated muscle differentiation is considered to be rare in the nephroblastomas of rats. To the best of our knowledge, this is the first case of nephroblastoma showing skeletal muscle differentiation in an F344 rat.

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References

1. Montgomery CA Jr, and Seely JC. Nephroblastoma. In: Pathology of the Fischer Rat. GA Boorman, SL Eustis, MR Elwell, CA Montgomery Jr., and WF MacKenzie (eds). Academic Press, San Diego. 143. 1990.
2. Turusov VS, Alexandrov VA, and Timoshenko IV. Nephroblastoma and renal mesenchymal tumor induced in rats by N-nitrosoethyl- and N-nitrosomethylurea. Neoplasma. **27**: 229–235. 1980. [Medline]
3. Yoshizawa K, Emoto Y, Kinoshita Y, Kimura A, Uehara N, Yuri T, Shikata N, Hamazaki T, and Tsubura A. Arachidonic acid supplementation does not affect N-methyl-N-nitrosourea-induced renal preneoplastic lesions in young Lewis rats. Oncol Lett. **5**: 1112–1116. 2013. [Medline]
4. Meuten DJ. Nephroblastoma (Embryonal Nephroma). In: Tumors in Domestic Animals, 4th ed. DJ Meuten. (eds). Iowa State Press, Ames. 518–519. 2002.
5. Frazier KS, Seely JC, Hard GC, Betton G, Burnett R, Nakatsuji S, Nishikawa A, Durchfeld-Meyer B, and Bube A. Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicol Pathol. **40**(Suppl): 14S–86S. 2012. [Medline] [CrossRef]
6. Saba LM, de Camargo B, and Gabriel-Arana M. Experience with six children with fetal rhabdomyomatous nephroblastoma: review of the clinical, biologic, and pathologic features. Med Pediatr Oncol. **30**: 152–155. 1998. [Medline] [CrossRef]
7. Perlman EJ, Grosfeld JL, Togashi K, and Boccon-Gibod L. Nephroblastoma. In: World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. JN Eble, G Sauter, JI Epstein, and IA Sesterhenn (eds). IARC Press, Lyon. 48–52. 2004.
8. Kashima T, Hara I, Mori N, Iketani O, Abe N, Hirata K, and Nomura Y. A case of fetal swine rhabdomyomatous nephroblastoma. Nippon Juishikai Zasshi. **48**: 809–811. 1995.
9. Yamamoto Y, Yamada M, Nakamura K, Takahashi Y, and Miyamoto T. Nephroblastoma with transcoelomic metastasis in a Japanese black bull. J Vet Med Sci. **68**: 891–893. 2006. [Medline] [CrossRef]
10. Dietrich JG, and Brewer JL. Nephroblastoma with pulmonary metastases in a guanaco. Vet Pathol. **25**: 329–330. 1988. [Medline] [CrossRef]
11. Mesfin GM, and Breech KT. Rhabdomyocytic nephroblastoma (Wilms' tumor) in the Sprague-Dawley rat. Vet Pathol. **29**: 564–566. 1992. [Medline] [CrossRef]