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Case Report

A case of spontaneous nephroblastoma characterized by two distinct morphologies in a Slc:CD(SD)IGS rat

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Abstract: We report a spontaneous case of nephroblastoma in a 26-week-old female Slc:CD(SD) rat. Macroscopically, there was a yellow mass in the left kidney that included another small yellowish-white mass. Histologically, the mass was located mainly in the cortex of the kidney. The tumor showed two distinct morphologies corresponding to the macroscopic findings: a blastemal cell dominant area (blastemal area) with primitive glomeruli and immature tubules and a columnar epithelial tubule dominant area with blastemal cell cuffing on (epithelial area). The epithelial area was located inside the blastemal area and the two morphologies were characterized by the lack of a transition region. Nephroblastoma is known to be biphasic or triphasic and showing transitional features. To our knowledge, there is no report of such nephroblastoma comprising two histologically distinct areas without transition. Therefore, the two distinct morphologies of this case with no transitional characteristic is a rare feature in nephroblastoma. (DOI: 10.1293/tox.2020-0013; J Toxicol Pathol 2020; 33: 291–295)

Key words: nephroblastoma, rat, two distinct morphologies, blastemal type, epithelial type

Nephroblastoma is a neoplasm arising from the metanephrogenic blastema (metanephric blastema), which is of mesodermal origin[1]. Although it is well known that nephroblastoma is induced by chemicals like *N*-methyl-*N*-nitrosourea in rats[2, 3], spontaneous nephroblastoma is rare, with the incidence in SD rats being reported to be around 0.1%[4]. Histologically, nephroblastomas are divided into blastemal, epithelial, and stromal types[5], and commonly show triphasic and biphasic morphologies[3, 5, 6]. Neoplastic cells representing various degrees of differentiation are frequently observed in nephroblastoma[7]. We encountered a rare case of spontaneous nephroblastoma in a rat showing the characteristic of two morphologies that was distinctly divided into two histological types: blastemal and epithelial. The pattern showed two parts; the border between the two areas was distinct and no regions of transition from the blastemal to the epithelial pattern was observed. To the best of our knowledge, there are no previous reports of such two distinct nephroblastomas without transition. Therefore, we report a nephroblastoma with the characteristic of two distinct morphologies and investigate its profile by immunohistochemical and silver staining.

The animal was a 26-week-old female Slc:CD(SD)IGS rat that was maintained under specific-pathogen-free conditions and that had been involved in a pharmacological study without treatment. The animal was obtained from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan) and housed in our animal facility in a suspended stainless-steel cage in an environmentally controlled room that provided a temperature range of 20–26°C, a relative humidity of 35–75%, and a light:dark cycle of 12:12 h. Food was provided daily and tap water was available *ad libitum*. The study was conducted in compliance with the Internal Regulations on Animal Experiments at Nippon Shinyaku Co., Ltd., which are based on the Law for the Humane Treatment and Management of Animals (Law No. 105, October 1, 1973, as revised on June 19, 2019, Ministry of Health, Labor and Welfare).

The animal did not show any clinical abnormalities until being euthanized at 26 weeks. At necropsy, there was a yellow mass (7 mm × 7 mm × 6 mm) in the left kidney. In contrast, no changes were noted in the right kidney. A vertical section of the left kidney revealed the mass included another small yellowish-white mass (Fig. 1). The left kidney, including the mass, was fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin and Watanabe silver stains. Immunohistochemical staining was also performed. Paraffin sections were labeled with primary antibodies against cytokeratin, vimentin, Ki67, aquaporin 1 and calbindin[8] (see Table 1 for details). For immunohistochemical staining, the paraffin sections were dewaxed, pre-treated by heating at 98°C in 10 mM citrate buffer, pH 6.0, for 50 min, and in-

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cubated in 3% hydrogen peroxide for 10 min to quench endogenous peroxidase activity. After treatment with 5% skim milk in phosphate-buffered saline for 50 min, the solution was removed from the slides and the primary antibodies applied. The primary antibodies were incubated overnight at 4°C and processed with the EnVision+ Dual Link system (Abcam, Cambridge, UK), and the end-products were visualized with 3,3'-diaminobenzidine.

Histologically, the lesions were located mainly in the cortex of the kidney and showed two distinct outer and inner lesions (Fig. 2A). In the outer lesion, neoplastic proliferation of the small round cells usually surrounding well-differentiated eosinophilic renal tubules was observed. These cells had scant cytoplasm and resembled blastemal cells. They showed invasive growth into the surrounding tissue and the boundary was not clear (Fig. 2B). Primitive glomeruli in which these blastemal cells were nested and immature tubules were also noted (Fig. 2B). This blastemal cell dominant area is hereinafter referred to as the blastemal area. In contrast, the inner lesion corresponding to the small yellowish-white mass observed macroscopically was well circumscribed and showed expansive growth (Fig. 2C). Thirty serial sections were made from half of the mass including the two regions in order to confirm the end of the inner lesion. Our results indicated that there was no transition between the two histopathological patterns to the end of the inner lesion. In the inner lesion, there were ducts lined with columnar epithelium. Blastemal cells had also proliferated around these columnar epithelial tubules, and the stroma between the neoplastic foci consisted of loose connective tissue (Fig. 2D). This columnar epithelium dominant area is hereinafter referred to as the epithelial area.

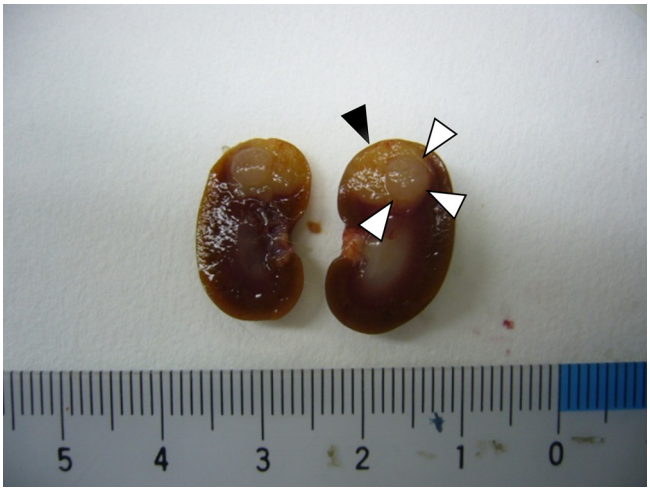

Fig. 1. Vertical section of the left kidney. A yellowish-white mass is located in the cortex (black arrowhead) containing a smaller yellowish-white mass (white arrowhead).

Immunohistochemical staining showed that in the blastemal area, the blastemal cells around the tubules were positive for vimentin (Fig. 3B). The well-differentiated tubules were positive for cytokeratin in the blastemal area (Fig. 3C). While the blastemal cells were often positive for Ki67, there were few positive cells in the epithelium of the well-differentiated tubules (Fig. 3A and D). At the margin of the blastemal area, there were well-differentiated tubules that were positive for aquaporin 1 (data not shown). However, positivity for calbindin was rarely noted (data not shown). In the epithelial area, the blastemal cells were generally positive for Ki67 and vimentin, and columnar epithelial cells were generally positive for cytokeratin and occasionally positive for Ki67 (Fig. 3D–F). No cells were positive for aquaporin 1 or calbindin (data not shown).

Watanabe silver staining showed that the eosinophilic tubules of the blastemal area observed in the hematoxylin and eosin-stained section had clear basement membranes. Immature tubules not showing basement membranes were observed in the blastemal area (Fig. 4A), and there were also tubules showing incomplete basement membranes in the blastemal area (Fig. 4B). In the epithelial area, few columnar epithelial tubules showed clear basement membranes; however, some columnar epithelium had incomplete basement membranes that showed partial absences of the basement membranes (Fig. 4C and D).

Taken together, our findings suggest that blastemal cells that had differentiated into primitive glomerulus or renal epithelium showed two different growth patterns, blastemal and epithelial. Therefore, we diagnosed this tumor as a nephroblastoma showing two distinct morphological types, blastemal and epithelial. Nephroblastomas show three morphological types: blastemal, epithelial, and stromal[5]. In the present case, the pattern showed two parts with the border between the two areas being distinct and the transition region not identified. In addition, Ki67 positivity was higher in the epithelial area than that in the blastemal area, and the epithelial area showed expansive growth. Therefore, it is thought that the epithelial area expansively proliferated to form a nodule after the blastemal area had arisen.

Nephroblastoma is considered to be associated with its precursor lesions, the nephrogenic rest, characterized

Table 1. Primary Antibodies Used in this Study

Antibody	Clone	Dilution	Source	Cells or structures detected
Mouse anti-human cytokeratin	AE1/AE3	Prediluted	Dako (Santa Clara, CA, USA)	Epithelial cells
Mouse anti-human vimentin	V9	Prediluted	Dako	Mesenchymal cells
Rabbit anti-human Ki67	SP6	Prediluted	Nichirei Bioscience Inc. (Tokyo, Japan)	Proliferating cells
Mouse anti-human aquaporin 1	1/22	1:200	Abcam (Cambridge, UK)	Proximal tubules
Rabbit anti-human calbindin	D1I4Q	1:800	CST (Danvers, MA, USA)	Distal tubules

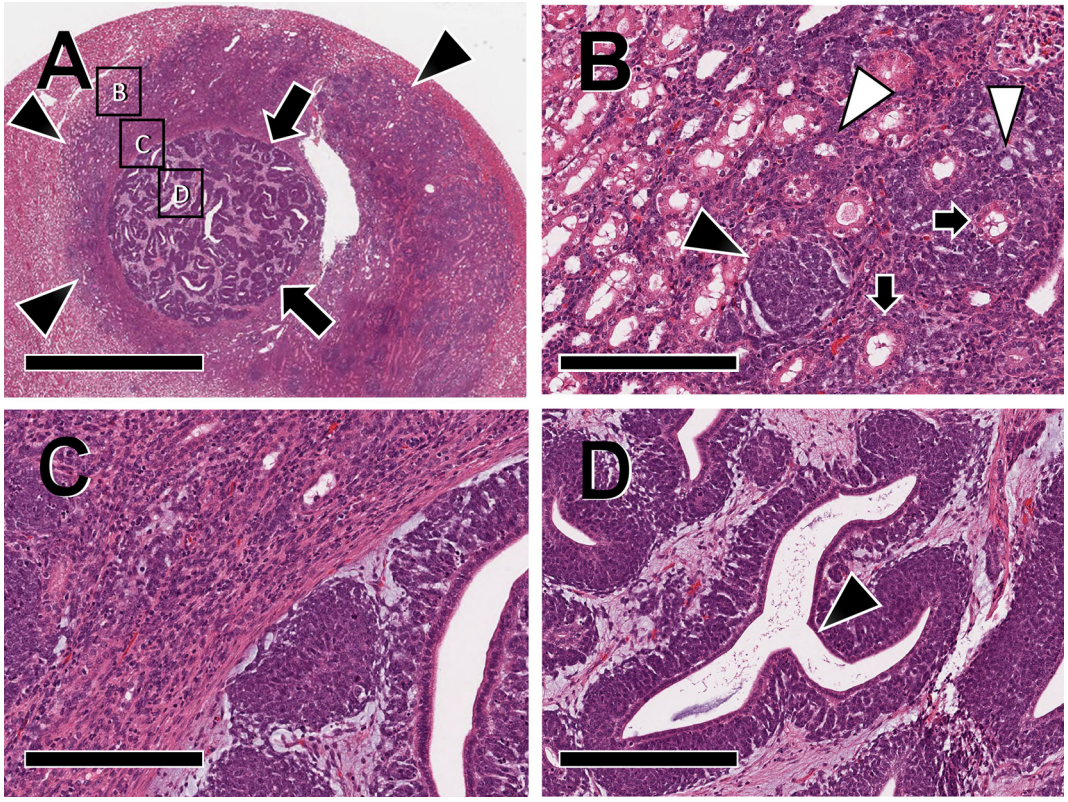

Fig. 2. Micrographs of a section of the mass. (A) The mass consists of an outer blastemal area (black arrowhead) and an inner epithelial area (black arrow). (B) Margin of the blastemal area. A primitive glomerulus (black arrowhead) and immature tubules (white arrowheads) are observed. Well-differentiated eosinophilic tubules located at the center of the blastemal cell focus are also noted (black arrows). (C) Boundary between blastemal area and epithelial area. No transitional region from the blastemal to the epithelial pattern is observed. (D) Epithelial area. Columnar epithelium (black arrowhead) is noted. Blastemal cells have also proliferated around these columnar epithelial tubules. Hematoxylin and eosin stain. Black bar = 4 mm (A), 200 µm (B–D).

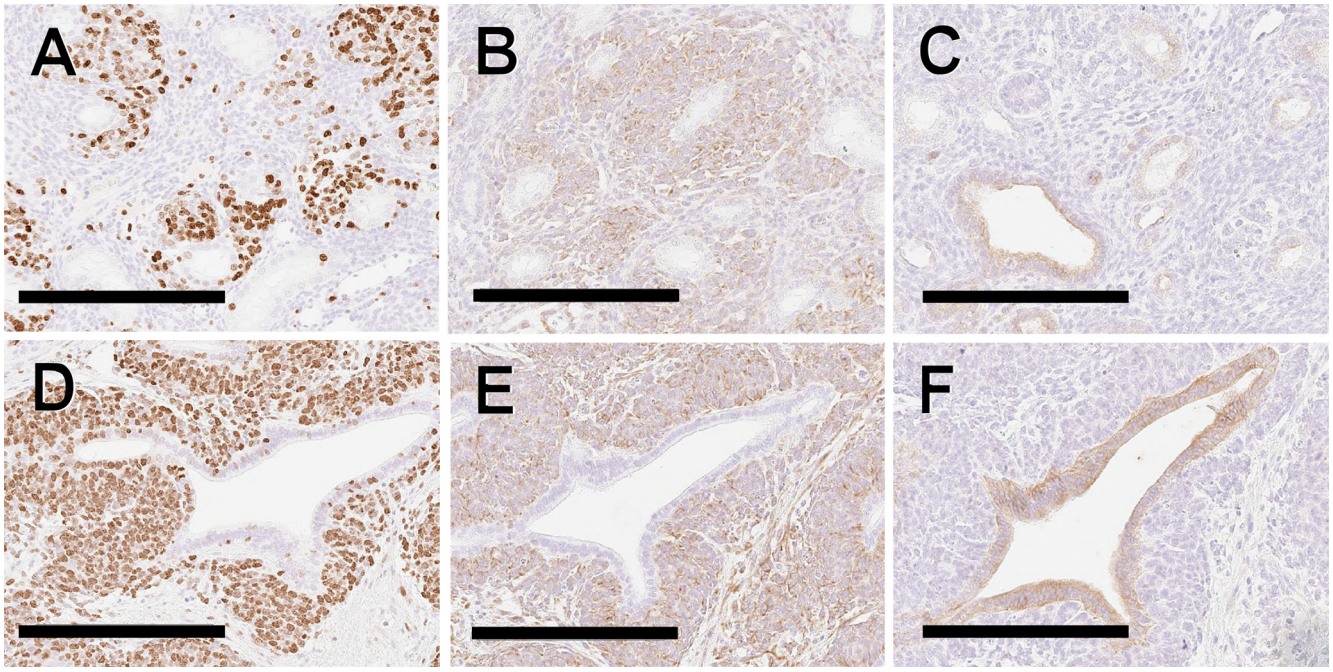

Fig. 3. Immunohistochemical staining of the neoplasm. Blastemal area (A–C) and epithelial area (D–F). The blastemal cells are positive for Ki67, the frequency of positive cells in the epithelial area (D) being higher than that in the blastemal area (A). The columnar epithelium shows some positivity for Ki67 (D). Most blastemal cells are positive for vimentin (B, E). The well differentiated tubules in the blastemal area and in the columnar epithelium are positive for cytokeratin (C, F). Black bar = 200 µm.

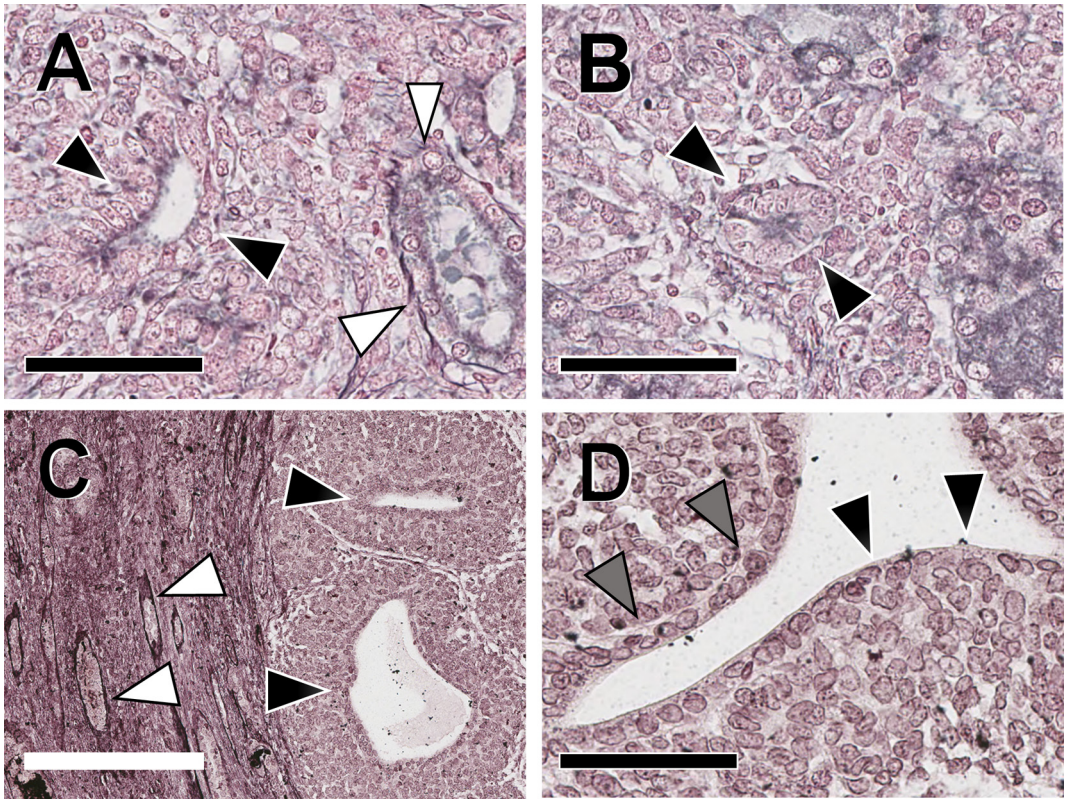

Fig. 4. Micrographs of Watanabe silver staining. (A) Tubules without basement membranes (black arrowheads) and with clear basement membranes (white arrowheads) in the blastemal area. (B) Tubule with incomplete basement membrane in the blastemal area (black arrowhead). (C) Columnar epithelial tubule with incomplete basement membrane in the epithelial area (black arrowhead). Tubules with clear basement membranes in the blastemal area (white arrowheads). (D) A higher magnification of columnar epithelial tubule with incomplete basement membrane (gray arrowhead). A partial absence of basement membrane (black arrow heads). Black bar = 60 μm, white bar = 200 μm.

by proliferation of the nephrogenic blastemal cell[9]. Several genes are reported to be involved in nephroblastoma tumorigenesis. In humans, mutations of *WT1* are considered an "early" event of nephroblastoma tumorigenesis, and its mutations are also observed in nephrogenic rests. *CTNNB1* mutations have been defined as a "late" event in tumorigenesis as they have been observed in nephroblastoma but not in nephrogenic rests[10]. In our case, we inferred the possibility that the blastemal area arose as an early stage of the nephroblastoma where the blastemal cell differentiated to the immature tubules or primitive glomerulus, and then, with subsequent mutations occurring in the blastemal cells in that area, these cells changed their growth pattern and formed the nodular epithelial area as a late stage of the nephroblastoma where the blastemal cells differentiated to the columnar epithelium. These different tumorigeneses may be associated with the development of the nephroblastoma that had the characteristic of two distinct growth patterns macroscopically and histologically.

Nephroblastomas sometimes show well-differentiated neoplastic tubules[6]. In our case, well-differentiated eosinophilic tubules were observed in the blastemal area of the tumor. These tubules showed lower positivity than surrounding normal renal tubules for aquaporin 1 and calbindin which are the renal tubule markers. Although the immunohistochemical detection of renal tubule markers is consistent with the possibility that these tubules were well differentiated neoplastic tubules, the Ki67 positivity was very low and silver staining revealed that these tubules had clear basement membranes. The evidence for proliferating activity and the presence of basement membranes suggests that these eosinophilic tubules were preexisting tubules entrapped in the tumor. Bauchet *et al.*[11] reported the possibility that renal toxicity decreases the immunohistochemical expression of renal tubule markers, aquaporin 1, aquaporin 2, and calbindin. We infer that in our case, the tumor caused degenerations of preexisting tubules, which led to low positivity for the renal tubule markers. In contrast, the silver staining also showed that there were immature tubules with absent or defective basement membranes in the blastemal area. Observation of basement membranes is thought to be useful to distinguish neoplastic tubules from preexisting tubules. In addition, the immunohistochemical detection of vimentin revealed that the blastemal cells around the immature tubules were mesenchymal cells. It is widely known that regeneration of renal tubules occurs where the base-

ment membrane remains intact[12, 13]. Therefore, we can conclude that immature tubules in the foci of small round cells were not regenerated tubules, but had differentiated from mesenchymal cells during the development of the basement membranes. This kind of differentiation of the tubules is thought to represent a mesenchymal-to-epithelial transformation (MET). In MET, which occurs during normal kidney development, metanephrogenic mesenchymal cells aggregate, are converted to epithelia, and lead to the formation of renal tubules[14, 15]. It is thought that MET occurs at the developmental phase; the neoplastic differentiation from the blastemal to immature tubules observed in this case is considered to be MET-like differentiation. The columnar epithelial tubules observed in the epithelial area also had unclear basement membranes and were surrounded by the blastemal cells. Therefore, we consider that these tubules arose from the blastemal cells through MET-like differentiation as well as immature tubules in the blastemal area. Another study reports that the ciliated columnar epithelial duct, originating from the mesonephric tubules, frequently appears in spontaneous nephroblastomas of rats[5]. In our case, the columnar epithelia had no clear cilia but showed morphological similarity to the ciliated columnar epithelia mentioned above. The columnar epithelia in our case are thought to be differentiated from the blastemal cells. These morphological characteristics of the columnar epithelia in our case are suggestive of originating from metanephric blastema rather than mesonephric tubules.

In the present case, the neoplastic cells were not positive for the renal tubule markers aquaporin 1 and calbindin. Because aquaporin 2 is reported not to be expressed in rat nephroblastoma[16], these renal tubule markers can be considered not to be appropriate for the diagnosis of nephroblastoma. The neoplastic components of this tumor were considered to be blastemal cells in the blastemal area, and columnar epithelium and blastemal cells in the epithelial area.

In conclusion, we report a spontaneous nephroblastoma in a rat with the characteristic of two distinct morphologies without transitional features. This morphological pattern is unusual in nephroblastomas.

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

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