Case Report

A Spontaneously Occurring Renal Tubule Carcinoma in a Mouse

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Abstract: Spontaneous occurrence of renal tubule carcinoma in mice is known to be rare. The following features of this tumor are recognized as areas of hemorrhage and massive necrosis and many show atypical cells and numerous mitotic figures. The present renal tubule carcinoma developed in an untreated B6C3F₁ male mouse at the age of 109 weeks. The tumor mass occupied nearly the entire renal cortex and invasion of normal renal tissue was observed. The tumor had solid cell nests, a gland-like structure, definite neovascularization and capsular invasion. The tumor cells had clear eosinophilic cytoplasm, a brush border structure and high proliferative activity. Although tumors derived from the proximal tubule reportedly show basophilic cytoplasm, the tumor cells in this mouse had clear eosinophilic cytoplasm. Consequently, the animal was diagnosed as having an unusual renal tubule carcinoma (classification: solid type). (*J Toxicol Pathol* 2007; **20**: 253–256)

Key words: renal tubule carcinoma, kidney, B6C3F₁ mouse, spontaneously occurring tumor

Spontaneous occurrence of renal tubule carcinoma is known to be rare in mice^{1–6}. However, renal tubule carcinoma can be induced in mice experimentally by carcinogenic chemicals including tris (2,3-dibromopropyl) phosphate or streptozotocin^{1,3}. The present renal tubule carcinoma developed in an untreated B6C3F₁ male mouse used in a carcinogenicity study. From our records, this was the third case at our facility. The detailed histological features of previous cases had solitary, compression of adjacent parenchyma, solid growth pattern, necrosis, mitotic figure, cellular pleomorphism, and invasion (histological classification: solid type), and tumor sizes at tissue trimming were small (4 × 4 mm). The incidence of renal tubule carcinoma in B6C3F₁ mice at our facility is 3 out of 1,650 male animals (incidence rate: 0.18%), and zero in 1,750 females (Table 1). The incidences of renal tubule carcinoma in B6C3F₁ mice complied for this study were similar to those previously reported in other B6C3F₁ mice (Table 1).

The animals were housed individually in a suspended stainless steel wire-mesh cage at a temperature of 23 ± 1°C, a relative humidity of 55 ± 5%, and with lighting for 12 hours a day in a barrier system. All mice had free access to

standard laboratory diet (Modified MIH Open Formula Rat and Mouse Ration, Oriental Yeast Co. Ltd., Tokyo, Japan) and tap water.

The Animal Care and Utilization Committee of the Anpyo Center approved the protocol for this study. On clinical observation, an intra-abdominal mass was palpated in the subject animal at 104 weeks of age. There were no significant changes in body weight, food consumption or hematological parameters.

The subject animal was euthanized under ethyl ether anesthesia at the age of 109 weeks and a complete necropsy was performed. The tissues from this mouse were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 3 µm, and stained with hematoxylin and eosin (H&E), reticulin silver impregnation (Watanabe's method), or the periodic acid-Schiff (PAS) reaction. Proliferating cell nuclear antigen (DAKO, Japan) was stained, immunohistochemically, to confirm the proliferative activity

Table 1. Spontaneous Renal Tubule Carcinoma in B6C3F₁ Mice in the Literature and the Present Case

Histological diagnoses	Male	Female
Adenocarcinoma [6]	1 / 2,543 (0.03%)	2 / 2,522 (0.07%)
Adenocarcinoma [5]	2 / 1,130 (0.18%)	0 / 1,130 (0.00%)
Renal tubule carcinoma*	3 / 1,650 (0.18%)	0 / 1,750 (0.00%)

[]: reference number. *: Our cases, including present animal.

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Fig. 1. Transverse section of the left kidney showing a nodular tumor involving mainly the cortex.

Fig. 2. (a) Capsular border invasion of tumor cells into normal renal tissue (*). H&E, ×20. Bar = 0.5 mm. (b) Small areas of necrosis in the tumor (arrows). H&E, ×330. Bar = 20 μm. (c) In the cell nest, tumor cells show dense proliferation and a gland-like structure. Silver impregnation, ×150. Bar = 10 μm. (d) High magnification of (a). H&E, ×400. Bar = 20 μm.

of the tumor cells. The standard labeled streptavidin biotin technique was performed using a DAKO LSAB Kit. Formalin-fixed kidney tissues from a control 109-week-old male mouse were also prepared for comparison. For electron microscopy, small pieces of formalin-fixed material were prefixed with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer and post-fixed with 1% OsO₄ 0.1 M phosphate buffer. After dehydration through a graded ethanol series, the samples were embedded in Epon 812 resin (Nakarai Tesque Inc., Kyoto, Japan). Ultrathin sections were prepared and stained with 4% uranyl acetate and lead citrate, and examined with a transmission electron microscope (H-7000, Hitachi High-Fielding Corp., Tokyo, Japan).

At necropsy, a white solitary nodule (28 × 18 × 16 mm) occupying most of the renal cortex was found in the left kidney. Other findings included an atrophic spleen and thymus, white spots in the stomach, nodules of the preputial glands and hypertrophic Harderian glands. Tissue trimming of the renal tumor revealed a clearly demarcated white nodule occupying nearly the entire renal cortex (Fig. 1).

Histological findings included a capsular border between the tumor and normal renal tissue, but tumor cells had partially invaded the fibrous capsular and surrounding tissues (Fig. 2a, H&E). Only a small area of necrosis was noted within the tumors (Fig. 2b, H&E). Edema and neovascularization were prominent in the tumoral stroma. The tumor was characterized by numerous solid cell nests showing closely compacted proliferative cells with a thin interstitium. Some of the cell nests showed closely compacted proliferative cells with a gland-like structure (Fig. 2c, silver impregnation). Tumor cells were stained clearly eosinophilic and showed cubic cytoplasm with a round or ovoid nucleus and minimal cellular atypism (Fig. 2d, H&E). There were two types of areas, one of which consisted of uniform sized cells and the other of various sized cells. Mitotic figures were occasionally observed. The PAS reaction revealed a few PAS-positive areas on the luminal side of the glandular lumen. Immunostaining of PCNA demonstrated an increased PCNA labeling index (22%) as compared with normal tissue (2%). These findings suggested that cell proliferative activity was enhanced in the tumor cells. Ultrastructural observation also confirmed the basement membrane, desmosomes, cell surface brush border structures, invaginations of the cell membrane, mitochondria, rough endoplasmic reticulum, glycogen granules and the concentric structure of lysosomes (Fig. 3).

The morphological characteristics of the tumor described above indicated the tumor was possibly derived from the renal epithelium. The brush border structure of the cell surface especially suggested a proximal tubule origin of the tumor. Interstitial edema and small areas of necrosis in the central portion of the tumor were likely to have been caused by a circulatory disturbance. The tumor cells showed little cellular atypism, but the tumor was large and there was capsular invasion of the adjacent normal renal tissue, and structural atypism showing solid proliferation with definite

Fig. 3. Electron micrograph. Brush border structure of the cell surface. Desmosomes (arrows), granular endoplasmic reticulum and glycogen in the cytoplasm can be seen. Bar = 100 nm.

neovascularization and highly proliferative activity. The features of this tumor suggested, in general, a malignancy. Therefore, the tumor was diagnosed as a renal tubule carcinoma (histological classification: solid type).

Spontaneous renal tubule carcinoma is a rare tumor in both rats and mice. Certain diagnostic features of this malignancy are recognized: areas of hemorrhage, necrosis, marked compression of surrounded normal tissue, infiltration of tumor cells into the adjacent parenchyma and prominent neovascular ingrowth. Cell morphology shows a wide range of atypism, from differentiated to pleomorphic and even occasionally anaplastic cell types. Mitotic figures may well be numerous, but are infrequent in some cases^{1-3,7-13}. As for the histological features of this mouse, prominent neovascular ingrowth was recognized, but there was no hemorrhage, only slight cellular atypism, and necrotic foci were small and submicroscopic. Therefore, the morphological characteristics of the tumor differed somewhat from the usual histological features of renal tubule carcinoma. Renal tubule tumors originating from the proximal tubules of both mice and rats have also been known to show basophilic cell staining^{1,14}. However, in the present mouse, the tumor cell cytoplasm showed a clear eosinophilic

staining pattern, just like oncocytomas which are known to be derived from collecting tubules. Oncocytomas are characterized by homogeneous or fine granular cells with abundant eosinophilic cytoplasm. The tumor cells of an oncocytoma have round or oval nuclei with loose chromatin and indistinct cell borders. Ultrastructurally, the cytoplasm contains densely packed atypical mitochondria^{1,11,14}. Therefore, the histological characteristics of the present tumor were different from those of an oncocytoma.

The lack of massive tumor necrosis in this animal was considered to be due to prominent neovascular ingrowth in the central portion. The histological features described above differ from those of ordinary renal tubule carcinomas and are apparently unique, making this atypical tumor quite interesting.

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