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A Novel "Nihon" Rat Model of a Mendelian Dominantly Inherited Renal Cell Carcinoma

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A novel rat model of hereditary renal cell carcinoma (RC) was found in a rat colony of the Sprague-Dawley strain in Japan, and named the "Nihon" rat. In this strain, RCs develop from early preneoplastic lesions, which begin to appear at 4 weeks of age, forming adenomas by the age of 16 weeks. The RCs are predominantly of clear cell type. Southern blot, northern blot and SSCP analyses revealed no change in the *Tsc1*, *Tsc2*, *VHL*, and *c-Met* genes. Thus, the Nihon rat should be a valuable experimental model for understanding renal carcinogenesis, especially clear cell type, which is common among human RCs.

Key words: Nihon rats — *Tsc2* gene mutant (Eker) rats — Renal carcinogenesis — Hereditary cancer

Hereditary cancer was first described in the rat by Eker in 1954.[1)] The Eker rat model of hereditary renal carcinoma (RC) was the first example of a Mendelian dominantly inherited predisposition to a specific cancer in an experimental animal. In 1994, Hino and colleagues identified a germline mutation in the rat homologous to the human tuberous sclerosis gene (*TSC2*) as the predisposing *Eker* gene.[2–4)] Recently, we found a novel hereditary RC model in the Sprague-Dawley rat in Japan. We have named this novel RC model the Nihon rat. In this report, we described the origin, transmission mode, and phenotypic and molecular features of Nihon rats.

All animals were housed individually in stainless steel cages with wood chip bedding in a room controlled at a temperature of 23±2°C, humidity of 55±10%, with a 12 h lighting cycle (from 6 a.m. to 6 p.m.). The rats were fed a standard diet (CR-LPF, Oriental Yeast Co., Ltd., Tokyo) and were allowed tap water *ad libitum*. Animals were killed by exsanguination under pentobarbital sodium anesthesia. After necropsy, tissues were fixed in 10% neutral buffered formalin, processed to paraffin-embedded blocks, and sectioned at 5 μm using standard procedures. Sections were stained with hematoxylin and eosin for microscopical examination. Midsagittal sections of each kidney were also stained with periodic acid-Schiff (PAS), and with alcian blue for acid mucopolysaccharides. Tail and kidney DNAs from the original (parent) female rat were extracted as reported.[4–9)] We used Southern blot, northern blot and SSCP analyses to search for mutations of the *Tsc1*, *Tsc2*, *VHL*, and *c-Met* genes. The gene-specific primers for *Tsc1*, *Tsc2*, *VHL*, and *c-Met* [Accession No. AB012279 (primer for exon 17), AB012280 (primer for exon 18) and AB012281 (primer for exon 19), kindly provided by Dr. Y. Kikuchi] were described previously.[4–9)]

Bilateral, multicentric renal tubule tumors were found in 15 out of 343 rats during 5 toxicity studies during the safety evaluation of 3 unrelated chemicals in our laboratory, although renal tumors were found in both treated and non-treated rats (Table I). The age of the rats ranged from 7 to 16 weeks at termination of the treatment period in each of the studies (Table I). The rats had all obtained from the same supplier (Clea Japan Inc., Shiga). At necropsy, bilateral punctate, clear cystic lesions 1 mm in diameter and yellowish white nodules were macroscopically recognized on the surface of the kidneys. Microscopically, renal tubule tumors were observed, developing through multiple stages from atypical hyperplastic tubules to clear cell and mixed cell (clear/acidophilic cells) adenomas and carcinomas. The clear and mixed clear/acidophilic cell tumors were composed of large clear cells containing nuclei with condensed chromatin and acidophilic, pleomorphic cells with a marked variability in nuclear size. Almost all of the atypical tubules and tumors were of clear cell type or mixed cell type. Some tumors were cystic and papillary projections into the cystic dilatation of the tubules and consisted of acidophilic cells with slightly basophilic cytoplasm. Acidophilic, tubular cell tumors rarely showed brush border-like structures. A few clear cells stained positively for periodic acid-Schiff reaction. Most tumor cells were negative with Alcian blue. Mitotic figures were rare. Carcinomas were large and hemorrhages and necrosis were frequently seen (Fig. 1).

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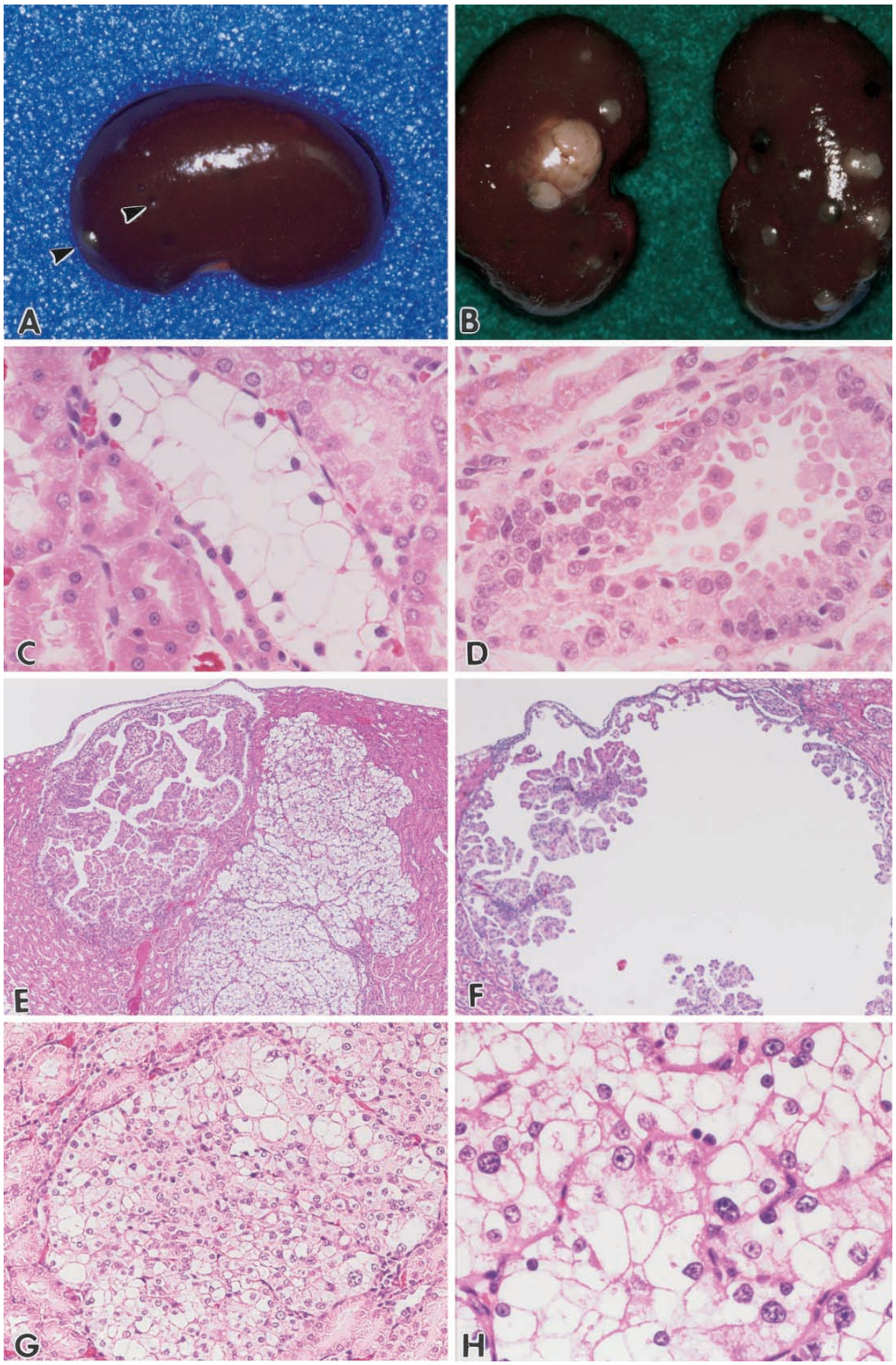

Fig. 1. Gross appearance and histology of the Nihon rat. (A) Gross appearance at 8 weeks of age. Scattered cysts on the surface of the kidneys (arrowheads). (B) Gross appearance at 34 weeks of age. Multiple cysts and yellowish white nodules. (C) Histological appearance of atypical hyperplasia (clear type) and (D) (acidophilic type). HE stain ×160, (E) Histological appearance of papillary acidophilic cell carcinoma and clear cell carcinoma. ×40, (F) cystic acidophilic cell adenoma. ×60, (G) mixed clear/acidophilic cell adenoma. HE stain ×130, (H) Detail of the same tumor at the higher magnification. ×400. All histological panels were stained with hematoxylin and eosin.

Table I. Incidences and Multiplicity of Renal Neoplastic Lesions

Study No.	Test compound	No. of animals examined	Case No.	Sex	Age (week)	Microscopical findings					
						hyperplasia		adenoma		carcinoma	
						clear/mixed cell[a)]	acidophilic cells[b)]	clear/mixed cell[c)]	acidophilic cells[d)]	clear/mixed cell[e)]	acidophilic cells[e)]
1	A	♂21, ♀20	1 (T)[f)]	male	10	6	3	7	10	1	1
2	B	♂22, ♀20	2 (T)	male	10	8	1	7	0	1	0
			3 (T)	male	10	2	0	0	1	0	0
3	B	♂1	4 (T)	male	10	8	2	6	0	0	0
4	C	♂64, ♀64	5 (N)	male	11	8	0	1	1	0	0
			6 (N)	male	15	2	0	2	0	0	0
5	C	♂135	7 (T)	male	7	8	3	0	0	0	0
			8 (T)	male	10	2	1	1	0	1	0
			9 (T)	male	14	1	0	0	0	0	0
			10 (T)	male	15	63	20	6	6	0	0
			11 (T)	male	15	1	2	0	0	0	0
			12 (T)	male	15	1	0	0	0	0	0
			13 (T)	male	16	1	0	0	0	0	0
			14 (T)	male	16	3	0	0	0	0	0
			15 (T)	male	16	2	0	0	0	0	0

a) see Fig. 1C, *b*) see Fig. 1D, *c*) see Fig. 1, G and H, *d*) see Fig. 1F, *e*) see Fig. 1E.
f) (T), treated; (N), non-treated.

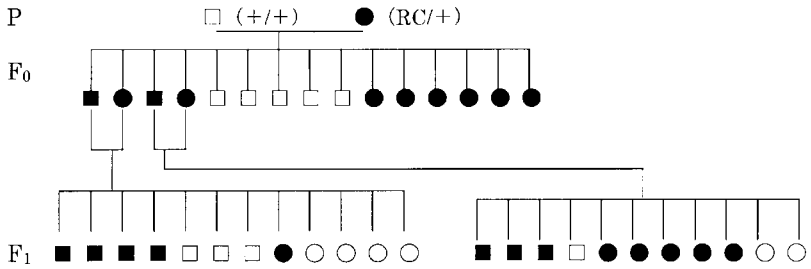

Fig. 2. Pedigree of Nihon rats. P, generation derived from Jcl:SD strain. □, male; ○, female; ■, male with renal tumors; ●, female with renal tumors.

The supplier kindly provided 2 female founder rats. The breeding and pedigree are shown in Fig. 2. A carrier female rat was used in mating with a normal male SD rat to produce F_0 hybrids. Nihon rats were confirmed to be carriers by the detection of macroscopic kidney cysts following unilateral dorsolateral incisions at ten weeks of age. In the F_0 rats, cyst formation was observed in 2 out of 8 (one stillbirth) male rats and in 8 out of 9 (one stillbirth) females. In the subsequent generation, multifocal cyst formation was noted in 7/11 F_1 males and 6/12 F_1 females at 8 weeks of age after abdominal operation. These F_1 rats showing cyst formation were propagated continuously by brother×sister mating. A total of 23 out of 40 rats, namely 10/17 (F_0)+13/23 (F_1), exhibited cyst formation. Thus, the Nihon rat has a heritable disorder with single autosomal dominant inheritance. Surgical intervention at 12–17 days of gestation following matings of two carriers revealed that 26/106 (24.5%) of the fetuses had died. This indicates that homozygous mutant status (expected to be 25%) could be lethal to the fetus. We are now investigating this lethality in detail.

In summary, the Nihon rat macroscopically shows multiple cysts on the surface of the kidney at 8 weeks of age. On the other hand, in the Eker rat, macroscopic evidence of renal neoplasia is generally not seen until eight months of age.[10] Further, although preneoplastic lesions (atypical hyperplasia) have been found microscopically in Nihon rats at 4 weeks of age or less, Eker rats do not usually

show detectable lesions until 2 or 3 months of age.[11] The Nihon rat characteristically shows clear cell type RCs histologically, whereas the Eker rat does not develop clear cell type RCs. The tubular origin in the Nihon rat is compatible with so-called clear and acidophilic cell tumors derived from the proximal tubules or the collecting duct system according to the Bannasch nomenclature.[12] Southern blot, northern blot and SSCP analyses have not revealed any change in the *Tsc1*, *Tsc2*, *VHL*, and *c-Met* genes (data not shown).

In conclusion, the Nihon rat appears to be a novel hereditary renal cell carcinoma model, phenotypically distinct from the Eker rat, and with no mutation in the *Tsc2* gene. Although genetic mapping, isolation and identification of the predisposing *Nihon* gene are necessary, the Nihon rat should be a valuable experimental model for studies on renal carcinogenesis, especially clear cell type, which is common in humans. Further analysis of the Nihon rat might lead to the identification of an additional tumor suppressor gene in man.

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