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Short Communication

Histopathological characteristics of renal changes in human renin-angiotensinogen double transgenic rats

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Abstract: The human renin-angiotensinogen double transgenic rat (dTGR) is a model of hypertension. The aim of this short report was to describe the histopathological characteristics of the renal changes in this rat strain in detail. Seven to nine-week-old male dTGRs were euthanized, and their kidneys were histopathologically examined. At the time of sacrifice, the average systolic blood pressure of the dTGRs was 258 mmHg, while that of age-matched, normal Sprague-Dawley rats was 135 mmHg. In the kidney, histopathological changes were observed mainly in blood vessels, tubules and glomeruli. In blood vessels, changes including medial hypertrophy, intimal thickening, hyaline change and/or fibrinoid necrosis were observed in arteries and arterioles. In tubules, changes including tubular basophilia were observed radially, mainly around interlobular arteries with lesions. In glomeruli, changes including hyaline droplet accumulation in podocytes, which was accompanied by increased expression of desmin, were observed. These changes were similar to those reported in other hypertension models, such as the spontaneously hypertensive rat (SHR). We hope that this short report will be helpful in histopathological examination of renal changes in this or other hypertension models. (DOI: 10.1293/tox.2015-0055; J Toxicol Pathol 2016; 29: 125–129)

Key words: hypertension, kidney, histopathology, human renin-angiotensinogen double transgenic rat

In humans, hypertension causes end-organ damage, especially in the kidney. Similar end-organ damage is also observed in animal models of hypertension, and these models are widely used to investigate the pathogenesis or therapeutics[1].

The human renin-angiotensinogen double transgenic rat (dTGR) is a model of hypertension. This model overexpresses human renin and angiotensinogen, which results in increase of angiotensin II, and develops accelerated hypertension leading to marked damage of the kidney[2].

Though there have been several reports of renal changes in the dTGR[2–4], the histopathological characteristics of the renal changes have not been fully described. The aim of this short report was to describe the histopathological characteristics of the renal changes in this rat strain in more detail.

Animal usage was approved by the Institutional Animal Care and Use Committee of Sumitomo Dainippon Pharma Co., Ltd. Five male dTGRs were prepared by cross-breeding two single-transgenic rat lines supplied by the Max Delbrück Center (Berlin, Germany) that which overexpress the genes for human renin (hREN) and human angiotensinogen (hAOGEN), respectively. The rats were fed commercial chow diet (CE-2; CLEA Japan, Inc.) and tap water *ad libitum*. From 6 weeks old, systolic blood pressure was measured by tail-cuff method, and urinary albumin and creatinine were measured by automated biochemistry analyzer (JCA-BM1650, JEOL, Tokyo, Japan) once a week.

The animals were euthanized by exsanguination under isoflurane anesthesia at 7 or 8 weeks old when they showed poor conditions or otherwise at 9 weeks old. For histopathology, unilateral kidneys were excised and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). In addition, sections of the kidney were stained by the periodic acid-Schiff (PAS) method and periodic acid-methenamine silver (PAM) method. Furthermore, sections of the kidney were subjected to immunohistochemistry (IHC) using a labeled polymer method with Histofine Simple Stain Rat MAX-PO

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Table 1. Antibodies Used in this Study

Antibody	Product number	Antigen species	Dilution	Antigen retrieval	Source
Adipophilin	651102	Human	1:200	Heat	Progen
Albumin	A0001	Human	1:500	Heat	Dako
Aquaporin 2	AB3274	Rat / Mouse	1:750	Heat	Millipore
Desmin	N1526	Human	Prediluted	None	Dako
LAMP-2	L0668	Mouse	1:200	Heat	Sigma-Aldrich

Heat: pressure cooker/Serotec target unmasking fluid mark2 (acidic) (AbD Serotec, Oxford, UK), 10 minutes. Sources: Progen, Heidelberg, Germany; Dako, Glostrup, Denmark; Millipore, Temecula, CA, USA; Dako, Carpinteria, CA, USA; Sigma-Aldrich, St. Louis, MO, USA.

(MULTI) (Nichirei Biosciences Inc., Tokyo, Japan). Table 1 shows the primary antibodies that were used in this study.

For comparison with the dTGRs, five 7-week-old normal male Sprague-Dawley (SD) rats were treated and examined in the same manner as the dTGRs and sacrificed at 9 weeks old.

Systolic blood pressure was high in the dTGRs from 6 weeks old, and at the time of sacrifice, it was 254 ± 12 mmHg in the dTGRs and 135 ± 14 mmHg in the normal SD rats (the numbers represent the group mean and standard deviation, respectively). Also, the urinary albumin/creatinine ratio was high in the dTGRs from 6 weeks old, and at the time of sacrifice, it was 8.9 ± 5.6 mg/mg in the dTGRs and 0.0 ± 0.0 mg/mg in the normal SD rats. In the kidney, histopathological changes were observed mainly in blood vessels, tubules and glomeruli. The changes in each unit are described as follows.

In blood vessels, medial hypertrophy was observed diffusely from interlobar arteries to efferent/afferent arterioles (Figs. 1A, B). From interlobar to arcuate arteries, minimal infiltration of inflammatory cells in the intima and focal degeneration of media were occasionally observed (Fig. 1A). In addition, from arcuate arteries to efferent/afferent arterioles, a minimal increase in fibroblasts was observed in the adventitia or surrounding interstitium in a few vessels (Fig. 1A). From the interlobular artery to efferent/afferent arterioles, focal intimal thickening, which was accompanied by rupture and/or duplication of the internal elastic lamina (Figs. 1B, C), and hyaline change and/or fibrinoid necrosis of the media were observed in many vessels (Figs. 1D, G). The blood vessels with hyaline changes and/or fibrinoid necrosis were positive for PAS and albumin (Figs. 1E, F). In the vessel wall of the affected efferent/afferent arterioles, PAM-positive granules were observed (Fig. 1H), as seen in normal afferent arterioles.

In tubules, basophilia was observed radially from the outer layer of the outer medulla to the superficial cortex across the kidney (Figs. 2A, B). These basophilic tubules were finely vacuolated and were accompanied by minimal single-cell necrosis (Fig. 2B). PAS-positive, brush border-like structures were observed in the apical surface of these basophilic tubules (Fig. 2B). These changes were observed mainly around interlobular arteries with the above-mentioned lesions. The vacuoles were positive for adipophilin (Fig. 2C), and the basophilic tubules contained many LAMP-2-positive granules (Fig. 2D) (in the normal SD rats, positive reaction for adipophilin was observed only slightly in proximal tubules, and many LAMP-2 positive granules were observed in proximal tubules). Collecting ducts, which were positive for aquaporin 2, were dilated from the cortex to outer layer of the outer medulla along nephrons across the kidney (Fig 2E). The aquaporin 2 staining pattern or intensity was similar to that seen in normal collecting ducts. Some collecting ducts also showed basophilia in the inner medulla. Hyaline casts were observed multifocally from the cortex to inner medulla along nephrons. Some of these hyaline casts contained red blood cells, and red blood cell casts were also observed. Small, focal hemorrhages were scattered in the interstitium in the cortex.

In some glomeruli, accumulation of hyaline droplets was observed in the cytoplasm of some or many podocytes (Fig. 3A). These droplets were positive for PAS (Fig. 3B) and albumin (Fig. 3C). Desmin expression was increased in the podocytes (Fig. 3D). In addition, focal, segmental increase in mesangial cells and mesangial matrix were observed (Fig. 3E). Changes in the glomerular basement membrane were not obvious, while the basement membrane of Bowman's capsule was thickened in a few renal corpuscles. These glomerular changes tended to be observed more often and to be severer in the juxtamedullary area than in the superficial area.

As previously reported[2–4], the dTGRs used in this study showed an increase in blood pressure from as early as 6 weeks old, which was accompanied by an increase in urinary albumin and histopathological changes in the kidney.

In blood vessels, changes including medial hypertrophy, intimal thickening, hyaline change and/or fibrinoid necrosis were observed in arteries and arterioles. The blood vessels with hyaline changes and/or fibrinoid necrosis were positive for PAS and albumin, suggesting accumulation of plasma proteins in the vessel wall. In the vessel wall of the affected efferent/afferent arterioles, PAM-positive granules were observed. These granules were thought to be renin granules[5], and it was suggested that the affected vessels were afferent arterioles. The vascular changes may have been caused by mechanical stress from hypertension or caused directly by increased angiotensin II via proliferation of vascular smooth muscle cells or increase of vascular permeability[3, 6].

In tubules, basophilia was observed radially. These tu-

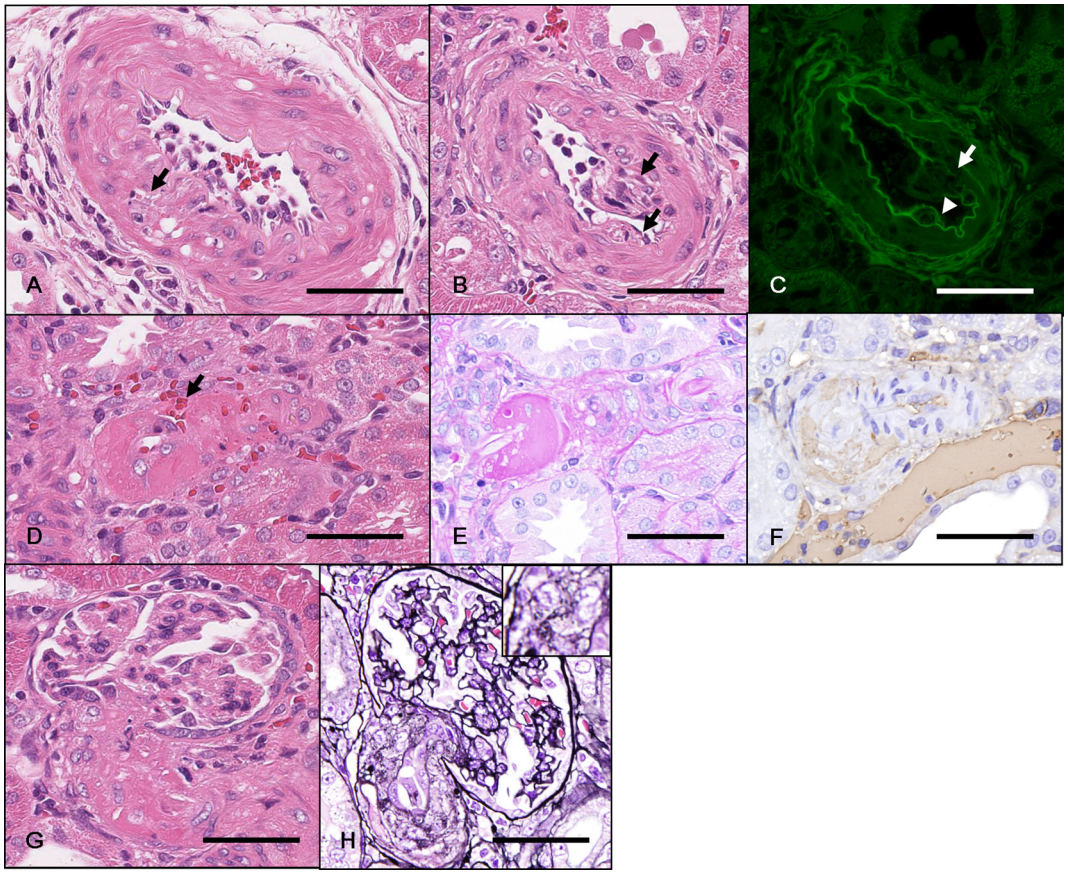

Fig. 1. Histopathological changes in blood vessels. (A) An arcuate artery. In the intima, minimal infiltration of inflammatory cells can be seen. In the media, hypertrophy and focal degeneration (arrow) can be seen. In the adventitia or surrounding interstitium, minimal increase of fibroblasts can be seen. HE. (B) An interlobular artery. Focal intimal thickening can be seen (arrows), along with medial hypertrophy. HE. (C) The same field as in (B) observed under fluorescent microscope. Rupture (arrow) and duplication (arrowhead) of the internal elastic lamina can be seen with intimal thickening. HE. (D, E, F) An interlobular artery. Hyaline change and/or fibrinoid necrosis of the media can be seen with some extravasated red blood cells (arrow) (D). HE. The hyaline substance is positive for PAS (E) and albumin (F). Immunohistochemistry. (G, H) An efferent/afferent arteriole. Hyaline change and/or fibrinoid necrosis of the media can be seen (G). HE. In the affected vessel wall, PAM-positive granules can be seen (H). Inset: A higher magnification of the vessel. Bars = 50 µm.

bules were suspected to be mainly proximal tubules, since they had brush border-like structures and contained many LAMP-2 positive lysosomes, as seen in proximal tubules. In addition, the vacuoles observed in these basophilic tubules were positive for adipophilin and were revealed to be lipid droplets[7]. Based on its distribution around interlobular arteries with injuries, the tubular basophilia was thought to be a degenerative change secondary to the vascular damage, probably through ischemia and/or ischemia/reperfusion.

In glomeruli, accumulation of hyaline droplets positive for PAS and albumin was observed in the podocyte cytoplasm. In addition, increased expression of desmin, a known marker of podocyte injury[8], was observed in podocytes. Thus, it was suspected that podocyte injury caused functional loss of the glomerular filtration barrier and hyperfiltration of plasma proteins, which resulted in accumulation of hyaline droplets in podocytes, hyaline cast formation, and increase of urinary albumin. The appearance of red blood cells in tubules was also thought to reflect glomerular injury. The glomerular changes may have been caused by mechanical stress from hypertension or caused directly by increased angiotensin II[3]. Though it is believed that plasma protein leaking from injured glomeruli can cause tubular injury[9], it was thought to be unlikely that the tubular changes in the present study were caused by this mechanism, considering that there was no correlation between the distribution of the glomerular changes and that of tubular changes.

Similar histopathological changes in blood vessels, tubules and glomeruli have also been reported in kidneys of other hypertension model rats, such as the spontaneously hypertensive rat (SHR; Table 2)[10–12]. Thus, at least qualitatively, we could not find a clear histopathological difference in the present dTGRs compared with other hypertension model rats.

In this short report, we described detailed histopathological characteristics of the renal changes in the human renin-angiotensinogen double transgenic rat. We hope that this short report will be helpful in histopathological examination of renal changes in this or other hypertension models.

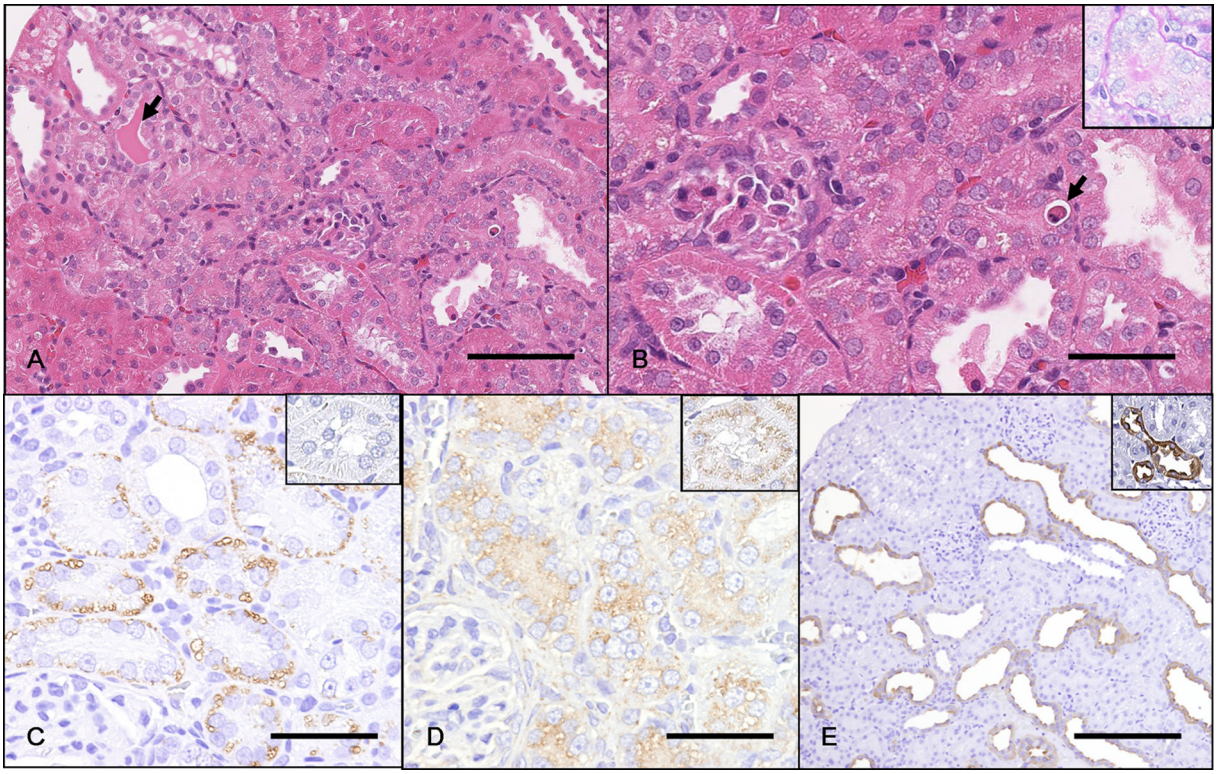

Fig. 2.

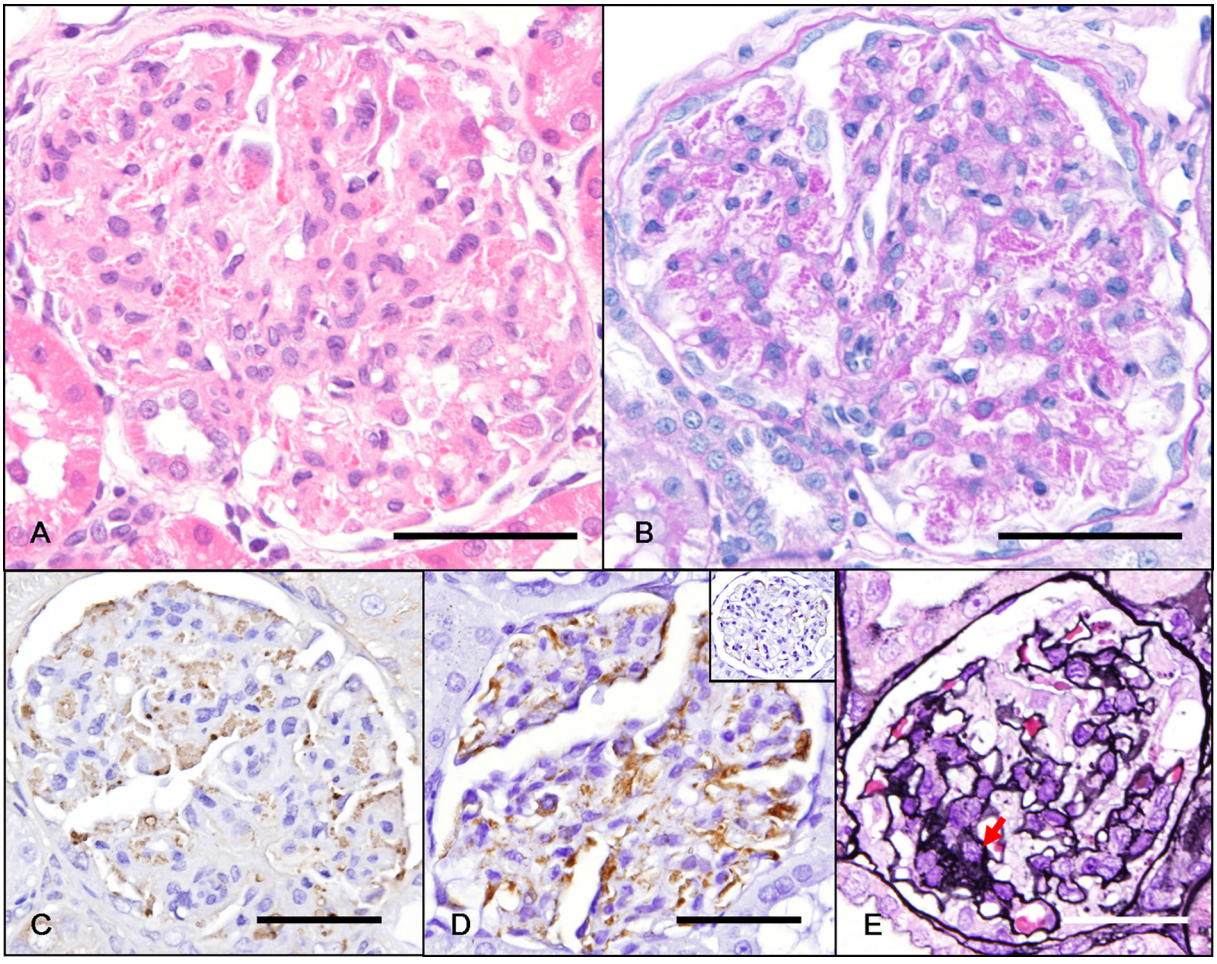

Fig. 3.

Table 2. Comparison of Major Renal Histopathological Changes in the dTGRs with those Reported in the SHRs[10–12]

Units/strains	dTGR	SHR
Blood vessel	• Medial hypertrophy • Intimal thickening • Hyaline change and/or fibrinoid necrosis	• Medial hypertrophy • Intimal thickening • Hyaline change and/or fibrinoid necrosis
Tubule	• Basophilia • Dilatation of collecting ducts • Hyaline cast	• Degeneration • Dilatation • Hyaline (protein) cast
Glomerulus	• Accumulation of hyaline droplets in podocytes • Increase of mesangial cells and mesangial matrix	• Hyaline material within the capillary tuft • Increase of mesangial cells • Sclerosis

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Fig. 2. Histopathological changes in tubules. (A) A focus of basophilic tubules can be seen in the cortex. A hyaline cast can also be seen (arrow). HE. Bar = 100 µm. (B) Higher magnification of (A). The basophilic tubules are finely vacuolated and are accompanied by minimal single-cell necrosis (arrow). HE. Bar = 50 µm. Inset: A basophilic tubule with a PAS-positive, brush border-like structure in the apical surface. (C) The vacuoles in the basophilic tubules are positive for adipophilin. Immunohistochemistry. Bar = 50 µm. Inset: A normal proximal tubule of a normal SD rat. (D) The basophilic tubules contain many LAMP-2-positive granules. Immunohistochemistry. Bar = 50 µm. Inset: A normal proximal tubule of a normal SD rat. (E) Collecting ducts, which are positive for aquaporin 2, are dilated. Immunohistochemistry. Bar = 200 µm. Inset: A normal collecting duct of a normal SD rat.

Fig. 3. Histopathological changes in glomeruli. (A) In the glomerulus, global accumulation of hyaline droplets can be seen in podocyte cytoplasm. Slight increase of mesangial cells can also be seen. HE. Bar = 50 µm. (B, C) The droplets are positive for PAS (B) and albumin (C). Bars = 50 µm. (D) Increased expression of desmin can be seen in the podocytes. Bar = 50 µm. Inset: A normal glomerulus of a normal SD rat. (E) Segmental increase of mesangial cells and mesangial matrix can be seen (arrow). PAM. Bar = 75 µm.