J. Comp. Path. 2013, Vol. 149, 280–290

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SPONTANEOUSLY ARISING DISEASE

Development of Podocyte Injuries in Osborne–Mendel Rats is Accompanied by Reduced Expression of Podocyte Proteins

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Summary

Osborne–Mendel (OM) rats spontaneously develop glomerulopathy with progressive podocyte injury. Changes in protein expression levels in the foot processes of podocytes have been suggested to play an important role in the development of renal disease. The aim of this study was to investigate the temporal relationship between the expression of five podocyte proteins (nephrin, podocin, synaptopodin, α-actinin-4 and α_3-integrin) and the development of podocyte injuries, proteinuria and glomerulosclerosis in OM rats. Male OM rats 5–20 weeks of age and age-matched Fischer 344 rats were used. Semiquantitative analysis of expression of the five podocyte proteins was performed by immunofluorescence labelling. Nephrin mRNA expression was determined by quantitative real-time reverse transcriptase polymerase chain reaction and nephrin protein expression was determined by mass spectrometry. Progressive reduction in expression of the podocyte proteins correlated with the progression of podocyte injuries, the development of proteinuria and the subsequent development of glomerulosclerosis. Nephrin mRNA expression and nephrin concentration also showed temporal decreases in OM rats. Altered expression of podocyte proteins preceded the development of proteinuria and glomerulosclerosis, suggesting that this event contributes to podocyte dysfunction and progression to glomerulosclerosis.

Keywords: glomerulopathy; Osborne–Mendel rat; podocyte; proteinuria

Introduction

Osborne–Mendel (OM) rats develop obesity when fed a high-fat diet (Pittman *et al*., 2008). In parallel, they also develop cardiac and renal hypertrophy, hyperglycaemia and hyperinsulinaemia (Madiehe *et al*., 2000; Fitzgerald *et al*., 2001). Progressive glomerular injuries are present in the kidneys of young male OM rats that have not been fed a high-fat diet (Yasuno *et al*., 2010). This glomerulopathy is characterized by early development of proteinuria associated with focal and segmental glomerulosclerosis. The glomerular injuries are followed by severe tubulointerstitial inflammation and fibrosis, which occurs by 20 weeks of age. Podocyte injuries, including deposition of hyaline droplets, vacuolation, foot process (FP) effacement and a reduction in the number of glomerular podocytes (podocytopenia), precedes the development of glomerulosclerosis. These findings suggest that podocyte damage may be an important factor in the pathogenesis of glomerulopathy in OM rats.

Podocytes are highly specialized epithelial cells that cover the outer layer of the glomerular basement membrane (GBM). They form a tight network of interdigitating cellular extensions (known as FPs) that are bridged by slit diaphragms (SDs). SDs act as a size-selective filter and are composed of a number of proteins including nephrin, P-cadherin, CD2-associated protein (CD2AP), zonula occludens (ZO)-1, FAT,

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2013.01.012

podocin and Neph1 (Mundel and Shankland, 2002; Tryggvason *et al.*, 2006; Patrakka and Tryggvason, 2010). FPs are anchored to the GBM through $\alpha_3\beta_1$-integrin, dystroglycans (Regele *et al.*, 2000; Patrakka and Tryggvason, 2010) and a number of actin-binding proteins. Synaptopodin and α-actinin-4 are important molecules that maintain the structure and functions of podocytes (Asanuma *et al.*, 2006; Dandapani *et al.*, 2007; Faul *et al.*, 2007).

In-vivo mutation analyses of podocyte proteins including nephrin (Kestila *et al.*, 1998; Lenkkeri *et al.*, 1999), podocin (Boute *et al.*, 2000) and α-actin-4 (Kaplan *et al.*, 2000; Kos *et al.*, 2003) have shown that the podocyte and SDs have critical roles in maintaining the function of the glomerular filtration barrier. Mutation of these proteins results in progressive glomerular injury and is associated with severe proteinuria. Expression of synaptopodin (Barisoni *et al.*, 1999; Srivastava *et al.*, 2001; Garovic *et al.*, 2007) and integrin (Regele *et al.*, 2000; Chen *et al.*, 2006; Pozzi *et al.*, 2008) is altered in several glomerular diseases.

The aim of the present study was to investigate alterations in expression levels of five podocyte proteins in the early stage of glomerulopathy in male OM rats. Morphological, immunohistochemical, molecular and biochemical features of the kidneys of OM rats were compared with those of Fischer 344 (F344) rats, which are used in long-term toxicological studies and often develop chronic glomerulopathy in old age (Solleveld and Boorman, 1986).

Materials and Methods

Study Design

The study design is shown in Fig. 1. Male F344/NSlc rats ($n = 28$; Japan SLC, Shizuoka, Japan) and male OM (OM/NSlc) rats ($n = 44$; Japan SLC) were divided into four groups for each strain. At the end of the observation period, systolic blood pressure

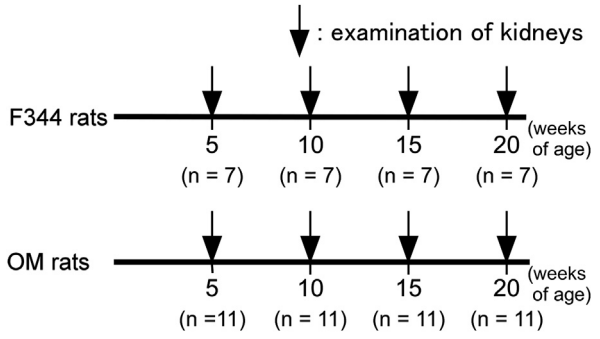

Fig. 1. Study design. The kidneys from F344 and OM rats were examined at 5, 10, 15, and 20 weeks of age.

was measured by tail plethysmography (BP-98A; Softron, Tokyo, Japan) in conscious animals and urinary excretion was determined by 24 h urine collection. All rats were housed in a barrier facility with a 12 h light/dark cycle, temperature of 21 ± 1°C and relative humidity of 50–60%. Rats were fed CLEA rodent diet CE-2 (CLEA Japan Inc., Tokyo, Japan) with a sodium content of 0.36% and crude protein content of 25.1% and were given free access to tap water. All procedures in this study were in accordance with the guidelines approved by the institutional Animal Research Committee. At the end of the study, the rats were killed and a blood sample and the kidneys were collected.

Isolation of Glomeruli

For mass spectrometry (MS) analysis, glomeruli were isolated from whole kidneys of three rats in each group using a sieving method (Kawakami *et al.*, 2011). Renal cortices were pooled into cold phosphate buffered saline (PBS; pH 7.2, 0.01 M). The cortices were minced and sieved through 250 μm and 150 μm stainless steel meshes and then washed with cold PBS over a 75 μm stainless steel mesh. Glomeruli that were trapped on the 75 μm mesh were collected and placed into a low protein binding tube (SUMILON Proteosave; Sumitomo Bakelite, Tokyo, Japan). The samples were stored at −80°C until western blotting or MS analysis.

Morphometric Analysis of Glomerulosclerosis

Kidney tissues were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (3 μm) were stained with periodic acid–Schiff (PAS) and examined under a light microscope. Glomerular volume (V_G) was determined (Yasuno *et al.*, 2010) using the equation: $V_G = (\beta/k)\ (A_G)^{3/2}$, where A_G is the mean glomerular tuft cross-sectional area, $k = 1.1$ (the size-distribution coefficient) and $\beta = 1.38$ (the shape coefficient for spheres as described by Weibel, 1979). The renal sections were also graded for the degree of glomerulosclerosis as 0, 0% of glomeruli affected; 1, 1–25% of glomeruli affected; 2, 26–50% of glomeruli affected; 3, 51–75% of glomeruli affected; and 4, 76–100% of glomeruli affected. The glomerulosclerosis score was calculated as (1 × % grade 1) + (2 × % grade 2) + (3 × % grade 3) + (4 × % grade 4). A total of 50 glomeruli were examined in each kidney section.

Transmission Electron Microscopy

Cortical tissues were cut into 1 mm^3 cubes, fixed in 2.5% glutaraldehyde and post-fixed in 1% OsO_4 for