Peroxisome proliferator-activated receptor-δ activation ameliorates albuminuria by preventing nephrin loss and restoring podocyte integrity in Type 2 diabetes

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Abstract

Background. Peroxisome proliferator-activated receptor (PPAR)-δ is a ligand-activated transcription factor in regulating gene expression and is believed to play an important role in various kidney diseases including diabetic nephropathy. This study investigated the efficacy of GW610742, a highly specific agonist for PPAR-δ, for the treatment of diabetic nephropathy.

Methods. Type 2 diabetic Otsuka Long-Evans Tokushima Fatty rats were randomized into an untreated diabetic group (n = 9) and a GW610742-treated diabetic group (n = 9). The GW610742 was administered (10 mg/kg/day) orally for 11 weeks. Long-Evans Tokushima Otsuka rats (n = 9) were used as a non-diabetic control.

Results. Albuminuria was markedly increased and renal PPAR-δ expression was decreased in diabetes. Diabetic albuminuria and renal injury markers, such as glomerular basement membrane thickening, decreased number of slit pores between podocyte foot processes, decreased nephrin expression, increased desmin expression and increased CCL2 expression, were significantly reversed through the treatment with GW610742. PPAR-δ agonist GW610742 markedly increased nephrin expression in cultured podocytes. Nephrin mRNA expression was markedly decreased in response to high glucose in cultured podocytes and effectively prevented by GW610742.

Conclusions. PPAR-δ activation by GW610742 ameliorates albuminuria by preventing diabetes-induced nephrin loss and restoring podocyte integrity, implying that GW610742 may be a potential therapeutic agent for diabetic nephropathy.

Keywords: albuminuria; diabetic nephropathy; nephrin; podocyte; PPAR-δ

Introduction

Peroxisome proliferator-activated receptor (PPAR)-δ is a nuclear receptor protein that acts as a ligand-activated transcription factor in regulating gene expression and is implicated in cellular metabolism, differentiation, proliferation, survival and inflammation [1]. PPAR-δ is also believed to play an important role in many kidney diseases [2]. PPAR-δ+/− and PPAR-δ−/− mutant mice exhibit much greater kidney injury and dysfunction than wild-type mice after renal ischemia/reperfusion injury. Genetic deletion of PPAR-δ-developed proteinuria suggests that PPAR-δ activity could influence renal damage and dysfunction [3]. Conversely, PPAR-δ activation has been reported to decrease renal injury and the markers of glomerular dysfunction in the models of renal ischemia/reperfusion [4, 5], and the overexpression of PPAR-δ provides the protection against hypertonicity-induced cell death in cultured medullary interstitial cells, which suggests that PPAR-δ is an important survival factor in the kidney [6].

With regard to diabetic nephropathy, however, the role of PPAR-δ in the pathogenesis of kidney injury has barely been studied. Renal PPAR-δ mRNA expression is known to be markedly decreased in proteinuric Type 1 diabetic mice [7], and PPAR-δ protein expression is markedly decreased in response to insulin-like growth factor-1 in cultured mesangial cells [8]. These findings suggest that reduced renal PPAR-δ expression possibly represents an underlying mechanism involved in diabetic kidney injury, and that PPAR-δ agonists may be considered a novel means of conferring renal protection in diabetic nephropathy. Newly developed synthetic PPAR-δ ligands have been under recent evaluation for their efficacy in animal models of insulin resistance and in Type 2 diabetes.
mellitus patients; however, the potential therapeutic role of PPAR-δ in diabetic kidney disease has not yet been evaluated [9, 10].

In this study, we examined whether diabetes influences renal PPAR-δ expression, and investigated the effects of a highly specific synthetic ligand for PPAR-δ, GW610742, on functional and structural renal changes in Type 2 diabetic Otsuka Long-Evans Tokushima fatty (OLETF) rats. We also attempted to elucidate the mechanism of renoprotective effects of PPAR-δ activation in cultured podocytes.

Materials and methods

Experimental animals

All protocols using rats were approved by the Institutional Animal Care and Use Committee. All rats were kindly provided at 5 weeks of age by Otsuka Pharmaceutical (Tokushima, Japan), and animals were provided with food and water ad libitum. At 25 weeks of age when all OLETF rats were hyperglycemic, diabetic rats were randomized into an untreated diabetic group (n = 9) and a GW610742-treated diabetic group (n = 9). The GW610742 (GlaxoSmithKline Pharmaceuticals, Stevenage, UK) [11], a specific and high-affinity agonist for PPAR-δ, was administered (10 mg/kg/day) via oral gavage once a day for 11 weeks. Male Long-Evans Tokushima Otsuka rats (n = 9) were used as a non-diabetic control. Body weights and plasma glucose levels were checked every other week, the latter using glucose oxidase methods (Surestep; Lifescan, Korea). Urine albumin concentrations were determined using ELISA (Exocell, Philadelphia, PA). The rats were anesthetized with Zoletil® (Virbac, France, 70 mg/kg IP). Blood was collected from the left ventricle during final anesthesia, centrifuged and the plasma was stored at −70°C for subsequent analyses. The rats were killed by cervical dislocation, followed by systemic perfusion with PBS, following which both kidneys were extracted. One kidney was preserved using a quick freeze method with liquid nitrogen, and we determined renal CCL2 (R&D System, Minneapolis, MN, USA) using ELISA. The contralateral kidney was fixed in 4% paraformaldehyde for 24 h, and then embedded in paraffin for histological examination and immunohistochemical staining.

Renal pathology

The paraffin embedded tissues were cut into 3 μm thick sections. To examine the effect of PPAR-δ agonist on glomerular area and mesangial matrix area, an analysis was performed on periodic acid Schiff staining to the method of Weibel and Gomez, as reported previously [12]. The glomerular matrix index was scored semi-quantitatively by a blinded observer, under ×200 magnification in a masked field. A minimum of 70 glomeruli per kidney were randomly selected to be included in the study. The glomerular area up to 25%; Grade 2, 25–50%; Grade 3, 50–75% and Grade 4, 75–100%.

For the ultrastructural evaluation, the kidney tissue was fixed in 3% glutaraldehyde, postfixed in 1% osmium tetroxide, immersed in uranyl acetate and embedded in epoxy resin (epon). The specimen was then sectioned and examined under a transmission electron microscope (JEOL 1200EX II, JEOL, Tokyo, Japan).

Electron micrographs of 5–10 glomeruli per kidney were randomly taken at magnification ×1000 and ×20000 for each rat. The mean glomerular basement membrane (GBM) thickness was calculated using the measurements from three different sites in the cross section, with the aid of Image J. Tangentially sectioned GBM was also excluded from the analysis. Photomicrographs of the GBM were also analyzed for the density of slit pores between the podocyte foot processes using published methods [13, 14]. The number of slit pores was counted and divided by the GBM length (mm) to arrive at the linear density.

Measurement of renal lipid contents

Renal lipids were extracted by the method of Bligh and Dyer with slight modifications [15], as follows: a portion (50 mg) of the kidney was homogenized in 1 mL of water and the homogenate was added to 1.25 mL of water and 5 mL aliquots of methanol-chloroform (1:1, v/v). The mixture was extracted by shaking the tube horizontally for 10 min in a shaker and then centrifuged at 2000g for 10 min. The lower chloroform phase was withdrawn, and cholesterol and triglyceride contents were measured using assay kits (Cayman Chemical Company, Ann Arbor, MI, USA). Renal free fatty acid levels were also measured using commercial kits (Abcam, Cambridge, UK).

Immunohistochemistry staining

Immunoperoxidase staining for PPAR-δ, nephrin, desmin and ED-1 was performed. Small blocks of kidney tissue were immediately fixed in 4% buffered paraformaldehyde for 24 h before being embedded in paraffin. Following dewaxing, 3 μm thick sections were microwaved for 5 min twice in a 0.1 M citrate buffer. Endogenous peroxidase was quenched and supplemented with protease inhibitors and 3% hydrogen peroxide in methanol. Non-specific binding was blocked with 10% normal goat serum in PBS. Kidney sections were stained for PPAR-δ (1:100, Abcam, Abcam), in antibody diluent solution (Golden Bridge International, Mukilteo, WA), nephrin (1:100, Abcam), desmin (1:100, Abcam) and ED-1 antibodies (1:100, Millipore, Billerica, MA) in a humidified chamber at 4°C. Polymer HRP-linked anti-mouse or anti-rabbit IgG (Golden Bridge International) were used as secondary antibodies. Sections were covered with a 3,3-diaminobenzidine substrate solution (Sigma-Aldrich, St. Louis, MO), then dehydrated in ethanol, cleared in xylene and mounted without counterstaining. All of these sections were examined in a masked manner using light microscopy (Olympus BX-50; Olympus Optical) equipped with a digital camera (Olympus DP2; Olympus Optical). Glomerular desmin staining was graded as follows: signal area in the glomerular capillary tuft of 0% = 1; 1+ = 1–25% 2+ = 26–50%; 3+ = 51–75% and 4+ = 76–100% [16]. ED-1 positive macrophage numbers were assessed on paraffin-embedded sections. A total of 50 glomeruli were analyzed per animal by a blinded observer.

A human kidney specimen for PPAR-δ immunostaining was obtained from a diagnostic renal biopsy performed at the Soon Chun Hyang University Cheonan Hospital (Cheonan, Korea). As a normal control, non-tumor kidney tissue from a patient who had renal cell carcinoma and underwent a nephrectomy was used. Studies using human tissues were approved by the institutional review board.

Western immunoblotting

The kidney cortex was homogenized in PRO-PREP™ Protein Extraction Solution (Intron Biotechnology, Korea). The supernatant protein concentrations were measured using the Lowry assay (Bio-Rad, Hercules, CA) and equalized with the addition of Laemml buffer prior to sodium dodecyl sulfate-based electrophoresis through a sodium dodecyl sulfate–PAGE gel. Following electrical wet transfer of the proteins to a nitrocellulose membrane, the membrane was incubated with anti-PPAR-δ (Progen Biotechnik, Heidelberg, Germany) or anti-β-actin (Sigma). After the incubation with horsedardish peroxidase-conjugated secondary antibody (anti-guinea pig IgG (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-mouse IgG (Sigma)), the chemiluminescent reaction was developed using SuperSignal West Pico (Pierce, Rockford, IL). Computer-assisted densitometry (Image J) was used to quantify the bands captured on radiographic film, and after normalization for the quantity of β-actin, the mean results for each protein in the three groups of rats were graphed as a percentage of the control, which was assigned a value of 100%.

Cell culture

Conditionally, immortalized mouse podocytes (gift of Dr Mundel, University of Miami, Miami, FL) were cultured as previously described [17, 18]. The cells were allowed to differentiate at 37.5°C without interferon in DMEM containing 5.5 mM glucose. Differentiated podocytes were synchronized into quiescence by growing cells in a serum-free medium for 24 h prior to the experiment. To examine the effects of PPAR-δ activation on PPAR-δ target gene transcription, podocytes were treated with PPAR-δ agonist GW610742 and/or PPAR-δ antagonist GW9662.
Higher than that of the control rats. Treatment with a PPAR-δ agonist ameliorates diabetic albuminuria

The effects of PPAR-δ agonist on the clinical characteristics of the diabetic rats

As expected, the diabetic OLETF rats were more obese and hyperglycemic than the non-diabetic control rats. Body weight and fasting glucose concentrations were not affected by GW610742 (Table 1). Renal function assessed by blood urea nitrogen and serum creatinine was not affected by GW610742 (Table 1). Blood levels of total cholesterol and triglycerides were increased in the OLETF diabetic rats compared with those of the non-diabetic rats; however, renal contents of total cholesterol, triglycerides and free fatty acids were not different between the diabetic and non-diabetic groups. GW610742 did not affect blood and renal lipid concentrations, either (Table 1).

Table 1. Effects of GW610742 on body and kidney weights, fasting blood glucose levels, renal lipid concentrations and renal functional parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 9)</th>
<th>DM (n = 9)</th>
<th>DM + GW610742 (n = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>518 ± 12</td>
<td>623 ± 19*</td>
<td>627 ± 9*</td>
<td>0.000</td>
</tr>
<tr>
<td>Kidney weight (g/100 g bw)</td>
<td>0.64 ± 0.01</td>
<td>0.70 ± 0.02*</td>
<td>0.71 ± 0.02*</td>
<td>0.028</td>
</tr>
<tr>
<td>Fasting blood glucose (mM)</td>
<td>5.4 ± 0.1</td>
<td>8.0 ± 0.4*</td>
<td>7.1 ± 0.2*</td>
<td>0.000</td>
</tr>
<tr>
<td>Blood urea nitrogen (mM)</td>
<td>3.31 ± 0.35</td>
<td>3.42 ± 0.25</td>
<td>2.73 ± 0.25</td>
<td>0.257</td>
</tr>
<tr>
<td>Serum creatinine (mM)</td>
<td>19.6 ± 1.3</td>
<td>16.7 ± 1.0</td>
<td>13.3 ± 1.9</td>
<td>0.066</td>
</tr>
<tr>
<td>Blood total cholesterol (mM)</td>
<td>4.6 ± 0.1</td>
<td>5.7 ± 0.4*</td>
<td>6.0 ± 0.4*</td>
<td>0.012</td>
</tr>
<tr>
<td>Blood triglycerides (mM)</td>
<td>0.34 ± 0.04</td>
<td>2.31 ± 0.36*</td>
<td>3.12 ± 0.56*</td>
<td>0.000</td>
</tr>
<tr>
<td>Renal total cholesterol (μM)</td>
<td>145 ± 8</td>
<td>163 ± 9</td>
<td>149 ± 6</td>
<td>0.240</td>
</tr>
<tr>
<td>Renal triglycerides (mM)</td>
<td>1.12 ± 0.09</td>
<td>1.37 ± 0.14</td>
<td>1.20 ± 0.03</td>
<td>0.216</td>
</tr>
<tr>
<td>Renal free fatty acid (μM)</td>
<td>154 ± 8</td>
<td>148 ± 13</td>
<td>147 ± 7</td>
<td>0.852</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SEM. DM, diabetes mellitus, bw, body weight. P: overall P-value for the ANOVA. *P < 0.05 from post-hoc analysis versus non-diabetic control rats.
and GW610742 treatment effectively improved podocyte injury (Figure 3C and D). However, the calculated glomerular volume and mesangial expansion assessed by the glomerular matrix index, which were markedly increased in the untreated diabetic rats compared with the control rats, were not changed by treatment with GW610742 (data not shown).

**PPAR-δ agonist recovers nephrin expression in diabetic kidney tissue**

Nephrin mRNA expression was markedly decreased in the OLETF diabetic rats as compared with the non-diabetic control rats. In the diabetic rats that were treated with GW610742, nephrin expression was effectively restored (Figure 4B). The intensity of nephrin was decreased in the diabetic rats as compared with the non-diabetic control rats, as assessed by immunohistochemistry. The administration of GW610742 also significantly restored glomerular nephrin levels in the diabetic rats (Figure 4C).

**CCL2 and macrophage accumulation in diabetic kidney**

CCL2 mRNA expression by real-time RT-PCR was markedly increased in the kidneys in the OLETF diabetic rats as compared with the non-diabetic control rats (Figure 5A). Renal CCL2 protein expression measured with ELISA was also markedly increased in the OLETF diabetic rats as compared with the non-diabetic control rats (Figure 5B). GW610742 treatment effectively inhibited renal CCL2 mRNA expression, but renal CCL2 protein expression is only partially decreased in the diabetic kidneys (Figure 5A and B). The number of ED-1 positive macrophages was negligible in the kidneys of non-diabetic normal control rats. Although the number of renal macrophages looks slightly increased, there was no statistical difference among three groups by ANOVA test (Figure 5C and D).

**Renal expression of PPAR-δ in diabetes**

In the non-diabetic normal control kidney tissue, PPAR-δ was constitutively expressed in both humans (Figure 6A) and rats (Figure 6B). PPAR-δ was also expressed in the podocytes (Figure 6B). Glomerular PPAR-δ expression was markedly decreased in the proteinuric diabetic human and rats as compared with the non-diabetic human and rat tissue, as assessed by immunohistochemistry (Figure 6A and C). However, PPAR-δ expression was not modified by GW610742 in the diabetic rats (Figure 6C and D).

**PPAR-δ ligand increases nephrin expression**

To determine whether PPAR-δ activation directly regulates nephrin gene expression in the kidneys, differentiated mouse podocytes were treated with incremental doses of PPAR-δ agonist GW610742. Podocytes constitutively express moderate levels of nephrin mRNA, and this level increased following the activation of PPAR-δ. Compared with the control, GW610742 markedly increased nephrin mRNA expression in a concentration-dependent manner, as measured using quantitative real-time RT-PCR (Figure 7A). To examine the role of PPAR-δ in nephrin gene regulation in diabetic nephropathy, we investigated the effect of high glucose on nephrin expression in vitro. Nephrin mRNA expression was significantly decreased in response to high glucose in cultured podocytes. PPAR-δ agonist GW610742 markedly prevented high glucose-induced decrease in nephrin expression. GW9662, which is an irreversible inhibitor of PPAR-δ, significantly abrogated GW610742-induced changes of nephrin expression (Figure 7B). These data indicate clearly that nephrin
expression is regulated in the diabetic milieu in a PPAR-δ-dependent manner.

**Discussion**

We have identified PPAR-δ as a newly recognized participant in the pathogenesis of diabetic kidney disease. Renal PPAR-δ expression appears to be decreased in Type 2 diabetes, and the diabetic rats treated with GW610742, a highly specific agonist for PPAR-δ, were functionally and morphologically protected against diabetic renal injury. This is the first report showing the therapeutic benefit of PPAR-δ agonist GW610742 in albuminuric Type 2 diabetic kidney disease.

We determined the role of PPAR-δ in the development of diabetic renal injury. We firstly confirmed PPAR-δ expression in normal kidney tissue and cultured podocytes and then found that renal PPAR-δ expression was markedly decreased in diabetic rat and human tissues. Although a decrease in the expression of PPAR-δ has been described in the diabetic heart [19], reports of the same condition occurring in kidney tissues are lacking. Until now, only two groups have shown the renal PPAR-δ expression in Type 1 diabetes; however, the results were discordant [7, 20]. Ours is the first to show decreased renal PPAR-δ expression in proteinuric Type 2 diabetic rats and human kidney tissue, which raises the possibility of an important role of PPAR-δ in the protection of kidney injury in Type 2 diabetes. And the fact that the altered PPAR-δ expression in diabetes is associated with albuminuria and well-known diabetes-induced changes, including GBM thickening, podocytopathy and loss of slit diaphragm integrity, support the essential role of PPAR-δ in maintaining urinary filtration barrier integrity.

We also evaluated the therapeutic benefit of PPAR-δ activation on functional and structural diabetic renal injury. GW610742 treatment effectively inhibited albuminuria and diabetic renal injury markers. It also effectively restored diabetes-induced renal morphological changes, including foot process effacement, GBM thickening and distorted slit diaphragms. This is the first study to investigate the effect of this PPAR-δ selective agent on the ultrastructural changes of the diabetic kidney.

Finally, we examined the mechanism underlying the anti-albuminuric effect of the PPAR-δ agonist in diabetes. Nephrin, a major component of the slit diaphragm and in glomerular urinary filtration barrier integrity, was evaluated. Untreated diabetic OLETF rats showed lower nephrin protein expression than non-diabetic controls, as we expected [21, 22]. Interestingly, we found that PPAR-δ activation restores nephrin expression in diabetic kidneys. Recently, Miglio et al. [5] showed that a PPAR-δ agonist increases nephrin expression in podocytes after oxygen/glucose deprivation-reoxygenation in vitro. The
Fig. 3. Podocyte injury. Representative electron photomicrographs show where podocyte slit pore (arrowhead) density is decreased in diabetes, and its prevention in the GW610742-treated diabetic rats is evident by visual inspection (A). As assessed by electron microscopy, the number of slit pores between the podocyte foot processes is expressed per millimeter length of GBM to arrive at the mean numerical density for each of the three groups of rats. The diabetic rats had decreased slit pore density, but these changes were significantly prevented by treatment with GW610742. The data are displayed as the slit pore density mean ± SEM as evaluated in at least 10 glomeruli from each rat (B). The intensity of desmin, a marker of glomerular podocyte injury, was markedly increased in the diabetic rats as compared with the non-diabetic control rats, and GW610742 effectively improved podocyte injury (C and D). The overall P value from ANOVA is shown in the graph. *P < 0.001 versus non-diabetic control rats, †P < 0.005 versus untreated diabetic rats.
The relationship between PPAR-δ and nephrin has never been previously evaluated in vivo, although it has been published in other isoforms of PPAR, such as PPARα or PPARγ [23, 24]. One important and interesting finding in this study is that PPAR-δ regulates podocyte function and morphology. The data presented here, that PPAR-δ is expressed constitutively in the podocytes and PPAR-δ agonist increases nephrin as PPAR-δ target gene expression in cultured podocytes, suggests that PPAR-δ is functionally active in podocytes and regulates podocyte...
function and morphology. And the production of the nephrin gene was decreased in response to high glucose in cultured podocytes. Treatment with PPAR-δ agonist was markedly prevented high glucose-induced decrease in nephrin expression in vitro. Also PPAR-δ antagonist abolished the effect of PPAR-δ activation on nephrin. These findings suggest that PPAR-δ is an endogenous regulator of nephrin gene expression.

After ligand binding, PPAR-δ acts by modifying their shape and binding with the specific DNA consensus sequence known as peroxisome proliferator-responsive element. As peroxisome proliferator-responsive element occurs in the promoter region of a nephrin gene [23], in the present study, PPAR-δ agonist GW610742 increased nephrin gene transcription via the peroxisome proliferator-responsive element in the promoter region of the nephrin gene, which discloses a novel mechanism of renoprotection for the PPAR-δ agonist.

PPAR-δ might also exert direct and indirect anti-inflammatory activities. This study shows that the renal
Fig. 6. Glomerular expression of PPAR-δ. (A) In non-diabetic normal human kidney tissue, PPAR-δ is expressed in glomerular cells, including podocytes. Renal PPAR-δ expression was markedly decreased in diabetic human kidney tissue as compared with non-diabetic human kidney tissue, as assessed by immunohistochemistry. (B) PPAR-δ was constitutionally expressed in the normal rat kidney tissues and cultured mouse podocytes. (C) PPAR-δ expression by immunohistochemistry was markedly decreased in the diabetic renal glomeruli as compared with the non-diabetic rats, and PPAR-δ expression was not modified by GW610742 treatment. (D) PPAR-δ expression was markedly decreased in the diabetic renal cortex as compared with the non-diabetic rats, as assessed by immunoblotting, and PPAR-δ expression was not modified by GW610742 treatment. The overall P value from ANOVA is shown in the graph. *P < 0.05 versus control.
CCL2 mRNA and protein expression is markedly increased in diabetic rats as compared with the non-diabetic control rats. And GW610742 treatment effectively inhibited renal CCL2 mRNA expression in diabetes, although GW610742 did not affect ED1 immunostaining. This finding is also consistent with our in vitro podocyte culture study [18], which showed that CCL2 causes podocytes to increase permeability to albumin, migrate and change cytoskeleton. We did not measure blood pressure during the experiment, although blood pressure is important in the development of diabetic kidney injury. However, we anticipate the effect of PPAR-δ on BP might be negligible as Takata et al. [25] showed that the administration of the PPAR agonist GW0742 (1 or 10 mg/kg) did not alter blood pressure.

In conclusion, the deficiency of renal PPAR-δ in a model of Type 2 diabetic nephropathy likely contributes to the aggravation of albuminuria and the pathologic changes observed in this model. PPAR-δ activation by GW610742 ameliorates diabetic albuminuria by preventing diabetes-induced nephrin loss and restoring podocyte integrity, implying that GW610742 may be a potential therapeutic agent for diabetic nephropathy.

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**Conflict of interest statement.** None declared.

**References**


Urine podocin:nephrin mRNA ratio (PNR) as a podocyte stress biomarker

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Abstract

Background. Proteinuria and/or albuminuria are widely used for noninvasive assessment of kidney diseases. However, proteinuria is a nonspecific marker of diverse forms of kidney injury, physiologic processes and filtration of small proteins of monoclonal and other pathologic processes. The opportunity to develop new glomerular disease biomarkers follows the realization that the degree of podocyte depletion determines the degree of glomerulosclerosis, and if persistent, determines the progression to end-stage kidney disease (ESKD). Podocyte cell lineage-specific mRNAs can be recovered in urine pellets of model systems and in humans. In model systems, progressive glomerular disease is associated with decreased nephrin mRNA steady-state levels compared with podocin mRNA. Thus, the urine podocin:nephrin mRNA ratio (PNR) could serve as a useful progression biomarker. The use of podocyte-specific transcript ratios also circumvents many problems inherent to urine assays.

Methods. To test this hypothesis, the human diphtheria toxin receptor (hDTR) rat model of progression was used to evaluate potentially useful urine mRNA biomarkers. We compared histologic progression parameters (glomerulosclerosis score, interstitial fibrosis score and percent of podocyte depletion) with clinical biomarkers [serum creatinine, systolic blood pressure (BP), 24-h urine volume, 24-h urine protein excretion and the urine protein:creatinine ratio (PCR)] and with the novel urine mRNA biomarkers. Results. The PNR correlated with histologic outcome as well or better than routine clinical biomarkers and other urine mRNA biomarkers in the model system with high specificity and sensitivity, and a low coefficient of assay variation.

Conclusions. We concluded that the PNR, used in combination with proteinuria, will be worth testing for its clinical diagnostic and decision-making utility.

Keywords: glomerular disease; podocyte; proteinuria; urine biomarker; urine podocin:nephrin mRNA ratio

Introduction

An ideal biomarker for prevention of progression of glomerular diseases will predict who will progress to end-stage kidney disease (ESKD) (unless effective intervention is implemented) and who will not progress (and therefore does not need expensive monitoring or exposure to