Overexpression of thioredoxin1 in transgenic mice suppresses development of diabetic nephropathy

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Abstract

Background. Oxidative stress has been suggested to play an important role in the pathogenesis of diabetic nephropathy. In the present study, the effects of thioredoxin1 (TRX1) overexpression, a small protein with antioxidant property, on the development of diabetic nephropathy in streptozotocin-induced diabetic animals were investigated using TRX1 transgenic mice (TRX1-Tg).

Methods. Eight-week-old male TRX1-Tg and wild-type mice littersmates (WT) mice were treated either with streptozotocin (200 mg/kg) or vehicle alone. After 24 weeks of treatment, diabetic nephropathy and oxidative stress were assessed in these four groups of mice, by biochemical analyses of blood and urine, as well as by histological analyses of the kidneys.

Results. Haemoglobin A1c (HbA1c) levels of diabetic TRX1-Tg were not significantly different from those of the diabetic WT. Nevertheless, an augmented urinary albumin excretion observed in diabetic WT was significantly diminished in diabetic TRX1-Tg. Histological study revealed that pathological changes such as mesangial matrix expansion and tubular injury were significantly prevented in diabetic TRX1-Tg accompanied by a reduced tendency of expression of transforming growth factor-β (TGF-β) as compared with diabetic WT. In parallel, urinary excretion of 8-hydroxy-2'-deoxyguanosine and acrolein adduct and the immunostaining intensities of these markers in the kidney were significantly higher in diabetic WT compared with non-diabetic mice. The markers were significantly suppressed in diabetic TRX1-Tg, an indication of systemic and renal oxidative stress attenuation by TRX1 overexpression.

Conclusion. These findings indicated the significant role of oxidative stress in the development of diabetic nephropathy and a potential inhibition of progression of nephropathy by TRX1.

Keywords: diabetic nephropathy; oxidative stress; thioredoxin1

Introduction

Diabetic nephropathy is one of the leading causes of end-stage renal disease. The features characteristic of this disease consist of persistent albuminuria and, histopathologically, mesangial matrix expansion followed by glomerulosclerosis. Transforming growth factor-β (TGF-β) has been indicated to play an important role in establishing the pathological changes in diabetic renal tissues, principally by the enhancement of extracellular matrix production. In fact, the increased expression of TGF-β in the renal glomeruli has been reported in both human and experimental diabetes [1,2]. Furthermore, hyperglycaemia-derived oxidative stress has been implicated in the up-regulation of various cytokines including TGF-β to accelerate the development of diabetic nephropathy [3]. Local oxidative stress, especially, has been postulated to play an important role in the pathogenesis of human diabetic glomerular lesions [4].

Oxidative stress is induced by a variety of mechanisms including glycation reaction, polyl pathway and protein kinase C-dependent activation of membranous NADPH oxidase under diabetic conditions. Recent study shows that enhanced production of superoxide anion by mitochondria is also one of the major sources of reactive oxygen species (ROS) in a milieu of...
hypoglycaemia [5]. However, cells possess protective mechanisms against oxidative stress. As one of the major intracellular antioxidant mechanisms, thioredoxin (TRX1), a 12-kDa small protein with a redox-active dithiol/disulphide in the conserved active site (-Cys-Gly-Pro-Cys-), has been suggested to play an important role in the regulation of intracellular redox balance [6]. Therefore, it has been investigated whether enhancement of TRX1 activity would prevent the oxidative stress-related disorders. For example, overexpression of human TRX1 in transgenic mice (TRX1-Tg) has been reported to have a protective activity against oxidative stress-related disorders such as postischaemic reperfusion injury in the brain [7] and kidney [8] and adriamycin-induced cardiotoxicity [9]. However, the effect of TRX1 overexpression on the development of diabetic nephropathy has remained unknown. Thus, this issue was investigated using TRX1-Tg, which were rendered diabetic with streptozotocin (STZ), to determine the role of oxidative stress in the development of diabetic nephropathy, and to evaluate a potential inhibition of the nephropathy by TRX1.

Subjects and methods

Experimental animals

The generation and maintenance of TRX1-Tg were described previously [7]. TRX1-Tg were C57BL/6 mice that carry the human TRX1 (hTRX1) transgene under the control of β-actin promoter and express the hTRX1 throughout the body including the kidneys [7]. There were no differences in the expression of Mn-superoxide dismutase, CuZn-superoxide dismutase and glutathione peroxidase between wild-type mice (WT) and TRX1-Tg by immunohistochemistry and western blotting [7]. The genotype of each animal was confirmed by polymerase chain reaction (PCR) analysis on purified tail DNA.

Eight-week-old male TRX1-Tg and WT weighing 19–26 g at the onset of the experimental protocol were intraperitoneally injected with either STZ (Sigma Chemical Co., St. Louis, MO) (200 mg/kg body weight in 100 μl of sterile citrate buffer, pH 4.5) or citrate vehicle alone. Mice with venous blood glucose levels of over 17 mmol/l obtained from the tail, measured by Glutest-Ace (Sanwa Kagaku Kenkyusho, Nagoya, Japan), were included in the study as diabetic animals. The mice were classified into four groups: (i) non-diabetic WT, (ii) non-diabetic TRX1-Tg, (iii) diabetic WT and (iv) diabetic TRX1-Tg. They had free access to standard chow and tap water throughout the experiment. Body weight and haemoglobin A1c (HbA1c) levels were measured at 2-month intervals. HbA1c level was determined by DCA2000 analyzer (Bayer Medical, Tokyo, Japan) using venous blood from the tail.

Assessment of pancreatic β cell destruction by STZ treatment

To rule out the possibility that TRX1 over expression can modify β cell destruction by STZ treatment, histology of the pancreas and serum insulin levels were compared from each group of mice after 24 weeks of treatment. The pancreas was removed from each mouse, fixed with phosphate-buffered 4% paraformaldehyde solution and then prepared for haematoxylin and eosin staining. Serum insulin levels were determined using a mouse insulin ELISA kit (Shibayagi, Gunma, Japan).

Measurement of blood pressure

Blood pressure was measured by the tail-cuff method using the BP Monitor for Mice and Rats, MODEL MK-2000 (MuromachiKikai Co. Ltd, Tokyo, Japan) according to the manufacturer’s instructions. Ten consecutive measurements per day were averaged and the mean value for 3 days was calculated for each mouse.

Assessment of diabetic nephropathy and oxidative stress

After 24 weeks of STZ treatment, 24-h urine collection using metabolic cages from each mouse was done. The urine was kept at −20°C until urinary albumin excretion (UAE), 8-hydroxy-2′-deoxyguanosine (8-OHdG) and acrolein adduct were determined. The mice were then sacrificed under ether anaesthesia to obtain blood via cardiac puncture. Serum samples were stored at −80°C until analysis. Both kidneys were quickly removed from all the animals, cleaned of the surrounding fat, washed in sterile saline solution and weighed. They were immediately fixed by immersion in phosphate-buffered 4% paraformaldehyde solution and routinely embedded in paraffin for light microscopy, immunohistochemistry and in situ hybridization using conventional fixatives and techniques. The pancreas was also removed to evaluate the extent of β cell destruction after 24 weeks of STZ treatment. The procedures were approved by the Institutional Animal Care and Use Committee guidelines at Kobe University Graduate School of Medicine.

Determination of albumin, 8-OHdG and acrolein adduct in urine

Albuminuria was evaluated using Albwell M (Exocell, Inc., Philadelphia, PA). Urinary 8-OHdG and acrolein adduct levels were determined by a sandwich enzyme-linked immunosorbent assay kit (NOF Corporation, Tokyo, Japan).

Immunohistochemistry

The expression of hTRX1 was immunohistochemically determined using anti-hTRX1 monoclonal antibody (Redox Bioscience Inc, Kyoto, Japan). Formation of 8-OHdG and acrolein adduct in renal tissues was assessed with anti-8-OHdG and anti-acrolein adduct mouse monoclonal antibodies (NOF Corporation, Tokyo, Japan). In brief, cut sections of 5 μm thick were pre-incubated with blocking agent (Simple Stain mouse system, Nichirei, Tokyo, Japan), followed by incubation with primary antibodies described above for 60 min at room temperature. A universal Immunoperoxidase Polymer (Histofine™ Simple Stain MAX PO system, anti-mouse and rabbit, Nichirei, Tokyo, Japan) was used for immunostaining. We subsequently performed

"..."
quantitative analyses of the immunostaining to 8-OHdG and acrolein adduct by evaluating 50 independent glomeruli in each mouse in order to count the number of immunoreactive mesangial cells per glomerulus on the three mice in each group. Similarly, 50 high-power fields were evaluated in each mouse to count the number of positive cells in the renal tubules.

Renal histology and morphometric analyses

The entire kidney was cut into 5 μm thick sections and stained with periodic acid-silver methenamine (PAM) and Azan. At least 30 glomerular tuft profiles per sample were photographed with a digital camera (Olympus IX71, Olympus, Tokyo, Japan) and imported into Viewfinder Lite ver.1.0 (Pixera Japan, Kanagawa, Japan). Evaluation of the mesangial matrix expansion was performed as described elsewhere [10]. In brief, the mesangial matrix area identified by dense PAM staining and whole glomerular tuft area from each glomerulus were measured using Lumina Vision ver.1.13 (Mitani Corporation, Fukui, Japan). The mesangial matrix index was calculated using the formula [(mesangial matrix area/glomerular tuft area) × 100 (%)]. Forty glomeruli per mouse were evaluated on the same animals utilized for the analyses of formation of the oxidative stress markers as described above. Furthermore, the percentage of atrophic tubules in the renal cortex was determined by counting tubules with dilation, cell loss, or necrosis in 40 tubular cross-sections per kidney. Renal interstitial fibrosis was also assessed as percent area of blue collagen staining of the tubulointerstitium in Azan-stained renal cortex sections. All of the evaluation was performed in a blinded fashion.

In situ hybridization

In situ hybridization was performed as described previously [11] to evaluate the expression of TGF-β. The single-stranded antisense DNA probe specific for mouse TGF-β was prepared by PCR as described below. A 330-bp cDNA fragment from mouse TGF-β was obtained by reverse transcription–polymerase chain reaction (RT–PCR) with rTth reverse transcriptase (ASTEC, PC-700, Japan) using the following pairs of oligonucleotide primers: 5′-CTCCA CTCCGTGCTTCTAG-3′: sense; 5′-CATAGATGGCC GTGTTGCGGTGTC-3′: antisense.

After de-waxing and dehydration, tissue sections were treated with 2 μg/ml proteinase K (Sigma) for 10 min at room temperature and refixed with 4% PFA for another 10 min. They were then acetylated with 0.1 M triethanolamine (pH 8) for 3 min and with 0.1 M triethanolamine containing 0.25% acetic acid for 10 min and dehydrated through a series of graded ethanol. Incubation in a hybridization medium [10 mM Tris-HCl (pH 7.3), 1 mM EDTA, 600 mM NaCl, 0.25% sodium dodecyl sulphate, 1× Denhardt’s medium, 50% (v/v) deionized formamide/1 μl/ml probe DNA, 10% dextran sulphate] at 50°C in a moist chamber for 12 h followed. Afterwards, the slides were washed with 50% deionized formamide/2× SSC to remove the superfluous probe, and again with 2× SSC and 0.2× SSC. To visualize the hybridized probe, they were incubated with alkaline phosphatase-conjugated anti-DIG antibody (Boeringer Mannheim, Mannheim, Germany) for 60 min after blocking with 1.5% non-fat dry milk in PBS for 10 min. Colorimetric reaction with nitro blue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate solution (Boehringer Mannheim) in the dark for 12 h was performed, then stopped with 10 mM Tris-HCl (pH 8) containing 1 mM EDTA. When the cells were uniformly stained, the case was regarded as positive. Fifty independent glomeruli in each mouse were assessed for the calculation of the average positive cells per glomerulus on the same animals used for other histopathological analyses. Similarly, 50 high-power fields were evaluated in each mouse to count the number of positive cells in the renal tubules.

Statistical analysis

Results were expressed as mean ± SD, with n indicating the number of mice studied. Statistical analysis was performed using Statview 5.0 (Abacus Concepts, Berkeley, CA). Data were examined by one-way ANOVA followed by Tukey–Kramer test. P < 0.05 was considered statistically significant.

Results

Transgenic mice

TRX1-Tg were thoroughly characterized previously [7]. No abnormal difference in appearance or performance was found between TRX1-Tg and WT C57BL/6 mice. The hTRX1 expression was confirmed to be substantially higher (3- to 10-fold) in several tissues and around 5-fold higher in the whole kidney than endogenous murine TRX1 levels in the TRX1-Tg [7]. Immunohistochemical staining for hTRX1 was only evident in TRX1-Tg in the glomeruli and tubules (Figure 1). There was no difference between diabetic and non-diabetic groups (data not shown).

Animal profiles

Changes in body weight and HbA1c levels during the study were presented in Figure 2. Body weight steadily increased in non-diabetic mice throughout the 24-week observation period. In contrast, diabetic mice showed a little gain in weight and a tendency to rather decrease after 16 weeks. Thus, a difference in weight between non-diabetic and diabetic mice became statistically evident (P < 0.01) at 24 weeks (Figure 2A). Overexpression of TRX1, however, did not affect body weight in both non-diabetic and diabetic groups. HbA1c levels were significantly (P < 0.01) higher in diabetic groups than in non-diabetic groups during the experiment (Figure 2B). No significant difference in HbA1c between WT and TRX1-Tg was found in either non-diabetic or diabetic groups.

Histological analysis of the pancreas revealed a similar extent of β cell destruction between WT and TRX1-Tg even after 24 weeks of STZ-treatment (Figure 3A–D). Serum insulin levels of the diabetic animals were kept below the detectable range throughout the experimental period.
Blood pressure

Since hypertension has been a well-known risk factor for diabetic nephropathy, the blood pressure was measured. Although diabetic groups exhibited a slightly higher value, systolic blood pressure among the four groups (Table 1) showed no significant difference.

Urinary albumin excretion

The earliest manifestation of renal disorder in diabetes is an increase in the level of UAE. The 24-hour UAE was markedly higher in diabetic WT than in non-diabetic WT ($P < 0.01$). Diabetic TRX1-Tg showed tendency to slightly increase UAE compared with the corresponding non-diabetic group, however,
Fig. 3. Pancreatic β cell destruction. The pancreas of each mouse was removed after 24 weeks (A–D) of STZ or vehicle treatment. Pancreatic tissues were stained with haematoxylin and eosin. After 24 weeks of treatment, the diabetic mice (C, D) had marked β cell destruction in comparison with non-diabetic mice (A, B). However, there was no difference between diabetic wild-type mice (C) and diabetic TRX1 transgenic mice (D) in the severity of β cell destruction. A: non-diabetic wild-type mice. B: non-diabetic TRX1 transgenic mice. C: diabetic wild-type mice. D: diabetic TRX1 transgenic mice. Magnification is 400×.

Table 1. Blood pressure, kidney weight and urinary data

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<td></td>
<td>N-WT</td>
<td>N-TRX</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122±10</td>
<td>123±11</td>
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<tr>
<td>Kidney weight/body weight (mg/g)</td>
<td>7.1±0.8</td>
<td>7.0±1.6</td>
</tr>
<tr>
<td>Albuminuria (µg/day)</td>
<td>51.7±20.7</td>
<td>34.7±20.1</td>
</tr>
<tr>
<td>Urinary 8-OHdG (ng/day)</td>
<td>21.5±8.4</td>
<td>14.9±10.9</td>
</tr>
<tr>
<td>Urinary acrolein (nmol/day)</td>
<td>1126±798</td>
<td>887±430</td>
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Data were obtained after 24 weeks of STZ or citrate vehicle treatment.
N-WT, non-diabetic wild-type mice; N-TRX, non-diabetic TRX1 transgenic mice; D-WT, diabetic wild-type mice; D-TRX, diabetic TRX1 transgenic mice.

Data are means ± SD. *P<0.01 vs non-diabetic wild-type mice. **P<0.01 vs diabetic wild-type mice. ***P<0.05 vs diabetic wild-type mice.
its magnitude was significantly \((P < 0.01)\) less than that of diabetic WT (Table 1).

**Oxidative stress makers**

To evaluate the extent of oxidative stress in the animals, urinary 8-OHdG, a sensitive indicator of oxidative DNA damage, and urinary acrolein adduct, a sensitive indicator of lipid peroxidation, were determined. Both markers were remarkably \((P < 0.01)\) higher in diabetic WT than those of non-diabetic mice. On the other hand, diabetic TRX1-Tg revealed a suppressed excretion of the markers, resulting in absence of significant difference with the values from non-diabetic mice (Table 1).

**Immunohistochemistry**

To assess the presence of oxidative stress in the renal tissues, immunohistochemical staining for 8-OHdG (Figure 4A–D) and acrolein adduct (Figure 4E-H) was conducted. Staining to 8-OHdG was clearly intensified in the renal tissues of diabetic WT (Figure 4C) compared with non-diabetic WT (Figure 4A). Especially, augmentation of staining in the tubules was prominent in the diabetic WT. In contrast, staining was significantly attenuated in both non-diabetic (Figure 4B) and diabetic TRX1-Tg (Figure 4D) compared with corresponding WT groups. Immunohistochemical study on acrolein adduct produced a similar outcome. That is, the enhanced staining to acrolein adduct, observed in both glomeruli and tubules of diabetic WT (Figure 4G), was remarkably attenuated in diabetic TRX1-Tg (Figure 4H). We additionally performed quantitative analyses on these observations (Table 2).

**Morphological changes of the kidney**

The kidney weight/body weight ratio was significantly \((P < 0.01)\) increased in diabetic WT compared with the non-diabetic WT at the 24th week after STZ treatment (Table 1). On the other hand, the ratio in diabetic TRX1-Tg was not significantly higher than the non-diabetic TRX1-Tg, though it showed tendency to slightly increase. The renal tissue from non-diabetic mice irrespective of WT (Figure 5A) or TRX1-Tg (Figure 5B) demonstrated a normal appearance under light microscope. Conversely, diabetic WT showed extensive mesangial matrix expansion and slight tubular atrophy (Figure 5C), whereas no apparent change was observed in diabetic TRX1-Tg (Figure 5D). Quantitative analysis revealed that diabetic WT demonstrated a significant \((P < 0.01)\) increase in the area occupied by matrix per glomerulus, in comparison with non-diabetic WT (Table 3). On the contrary, diabetic TRX1-Tg had significant \((P < 0.05)\) reduction of the index compared with diabetic WT.

As regards the tubulointerstitial changes, atrophic tubules were found in the kidneys of diabetic WT to a certain extent, whereas non-diabetic mice showed little changes in the renal tubules (Table 3). In contrast, the percentage of atrophic tubules in diabetic TRX1-Tg was markedly diminished compared with diabetic WT. Histopathological analysis by Azan stain revealed a significant augmentation of fibrotic changes in the renal interstitium of diabetic WT compared with non-diabetic mice (Figure 6A and C, Table 3). This interstitial damage exhibited a tendency to be attenuated in diabetic TRX1-Tg, though it was not statistically significant partly due to the limited number of animals examined (Figure 6D, Table 3).

**TGF-\(\beta\) mRNA expression in renal tissue**

*In situ* hybridization revealed only a weak signal of TGF-\(\beta\) mRNA present in the renal cortex tissue of both non-diabetic groups (Figure 7A, B, E and F, Table 2). In contrast, diabetic WT showed a

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Fig. 4. Immunohistochemical detection of oxidative stress markers in the kidneys. The kidneys were removed after 24 weeks of STZ or vehicle treatment. Renal tissues were immunostained with anti-8-OHdG monoclonal antibody (A–D) and anti-acrolein adduct monoclonal antibody (E–H). Intensities of immunostaining of the oxidative markers were enhanced in the kidney of diabetic wild-type mice (C, G) and were suppressed in the diabetic TRX1 transgenic mice (D, H). A and E: non-diabetic wild-type mice. B and F: non-diabetic TRX1 transgenic mice. C and G: diabetic wild-type mice. D and H: diabetic TRX1 transgenic mice. Magnification is 400×.
Table 2. Quantitative analysis of immunostaining and in situ hybridization

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<th>Diabetic mice</th>
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<tr>
<td></td>
<td>N-WT</td>
<td>N-TRX</td>
</tr>
<tr>
<td>8-OHdG Glomeruli</td>
<td>0.48 ± 0.11</td>
<td>0.50 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>40.55 ± 5.33</td>
<td>18.80 ± 3.36</td>
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<tr>
<td>Acrolein adduct</td>
<td>1.62 ± 0.33</td>
<td>1.58 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>41.20 ± 5.60</td>
<td>33.8 ± 6.20</td>
</tr>
<tr>
<td>TGF-β Glomeruli</td>
<td>4.20 ± 1.86</td>
<td>3.88 ± 2.06</td>
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<td></td>
<td>10.55 ± 3.20</td>
<td>9.08 ± 3.13</td>
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Data were obtained after 24 weeks of STZ or citrate vehicle treatment. The number of positive cells per glomerulus (Glomeruli) and the number of positive cells in the renal tubule per high power field (Tubules) are expressed. Data are mean ± SD of 3 mice per group. N-WT, non-diabetic wild-type mice; N-TRX, non-diabetic TRX1 transgenic mice; D-WT, diabetic wild-type mice; D-TRX, diabetic TRX1 transgenic mice. *P < 0.05 vs non-diabetic wild-type mice. **P < 0.05 vs diabetic wild-type mice.

Fig. 5. PAM staining of kidney sections. The kidneys were removed after 24 weeks of STZ or vehicle treatment. The renal tissue from non-diabetic mice demonstrated a normal appearance (A, B). Diabetic wild-type mice showed extensive mesangial matrix expansion (C), whereas no apparent change was observed in diabetic TRX1 transgenic mice (D). A: non-diabetic wild type mice. B: non-diabetic TRX1 transgenic mice. C: diabetic wild-type mice. D: diabetic TRX1 transgenic mice. Magnification is 400×.
**Table 3.** Mesangial matrix index, tubular injury and interstitial damage

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<th>Non-diabetic mice</th>
<th>Diabetic mice</th>
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<tr>
<td></td>
<td>N-WT</td>
<td>N-TRX</td>
</tr>
<tr>
<td>Mesangial matrix index (%)</td>
<td>7.79±0.80</td>
<td>7.08±0.59</td>
</tr>
<tr>
<td>Atrophic tubules (%)</td>
<td>0.06±0.02</td>
<td>0.06±0.02</td>
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<tr>
<td>Azan stain (% area)</td>
<td>3.33±1.33</td>
<td>2.79±1.37</td>
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</table>

Data were obtained after 24 weeks of STZ or citrate vehicle treatment. N-WT, non-diabetic wild-type mice; N-TRX, non-diabetic TRX1 transgenic mice; D-WT, diabetic wild-type mice; D-TRX, diabetic TRX1 transgenic mice.

Data are mean±SD of 3 mice per group. *P<0.01 vs non-diabetic wild-type mice. **P<0.01 vs diabetic wild-type mice. ***P<0.05 vs diabetic wild-type mice.

**Fig. 6.** Azan staining of kidney sections. The kidneys were removed after 24 weeks of STZ or vehicle treatment. Compared with non-diabetic mice (A, B), marked collagen deposition was identified in the kidneys of diabetic wild-type mice (C). The deposition showed a tendency to be attenuated in diabetic TRX1 transgenic mice (D). A: non-diabetic wild-type mice. B: non-diabetic TRX1 transgenic mice. C: diabetic wild-type mice. D: diabetic TRX1 transgenic mice. Magnification is 400×.
Discussion

In the present study, the hallmarks of diabetic nephropathy including albuminuria, expansion of mesangial area and tubular injury were efficiently prevented in the diabetic TRX1-Tg. Urinary excretion of 8-OHdG, a marker of oxidative DNA damage, and acrolein adduct, a marker of lipid peroxidation, were elevated in STZ-induced diabetic WT, while both markers were significantly suppressed in diabetic TRX1-Tg. The observed attenuation of oxidative stress in TRX1-Tg appeared not to be attributable to the improvement of glycaemic control since glycated haemoglobin levels were not different between WT and TRX1-Tg under diabetic conditions. In contrast to the pancreatic β cell-specific TRX1-Tg, which have successfully prevented the induction of diabetes by STZ [12], TRX1-Tg used in the present study failed to ameliorate blood glucose level and to prevent β cell destruction. The probable cause could be the too little increase in transfected hTRX1 in the pancreas (data not shown) in order to suppress STZ-induced β cell damage. This difference seemed to be accredited to a difference in the promoter used for construction of the hTRX1 transgene. On the other hand, the transfected hTRX1 was distinctly overexpressed in the kidneys of TRX1-Tg (Figure 1B).

We further investigated the intensity and localization of 8-OHdG and acrolein adduct in the renal tissues (Figure 4). As a result, we found the intensified immunostaining to these oxidative stress markers in the renal tissues of diabetic WT, whereas, it was diminished in diabetic TRX1-Tg. In addition, the oxidative stress markers were relatively predominant in the renal tubules compared with the glomeruli in diabetic WT. These findings are consistent with the recent report, which signifies that nuclear factor-κB (NF-κB), an oxidative stress-related tissue factor, is markedly activated in the tubular cells rather than the glomerular cells [13]. Our subsequent quantitative analyses of immunostaining confirmed the same tendency. Since it has been postulated that tubulointerstitial damage is involved in the pathogenesis of diabetic nephropathy [14], the enhanced oxidative stress in the tubules seems to be associated with its development. Although the augmented formation of both oxidative stress markers were significantly suppressed in the diabetic TRX1-Tg, the suppression of 8-OHdG formation in the renal tubules of diabetic TRX1 was not enough to reach to control level. This data may provide an explanation for the insufficient suppression of TGF-β expression in renal tubular cells in the diabetic TRX1, since hyperglycaemia-induced ROS formation has been postulated to be strongly associated with up-regulation of TGF-β mRNA expression. Since the role of TGF-β in the progression of renal fibrosis is established, its insufficient suppression may interpret a slight remaining renal fibrosis as evaluated by Azan stain in the diabetic TRX-1. On the other hand, the mesangial matrix expansion induced by the enhanced extracellular matrix production in the glomeruli was significantly suppressed in the diabetic TRX1, which was accompanied by the attenuation of TGF-β expression in the glomeruli.

TRX1 suppresses the oxidative stress by a variety of mechanisms. First, it directly scavenges ROS such as hydroxyl radical and singlet oxygen by reversible oxidation of its redox-active cysteine residues [15]. Second, it indirectly enhances the reduction of hydroperoxides by activating peroxiredoxin, a family of peroxidases, by providing electrons to it [16]. Thus, TRX1 possesses a potent antioxidirical-quenching capability. In addition, it plays a crucial role as a redox-regulator of intracellular signal transduction [17]. For example, a reduced form of TRX1 is known.

Fig. 7. TGF-β mRNA expression. Renal tissues were obtained after 24 weeks of STZ or vehicle treatment. TGF-β mRNA expression was evaluated by in situ hybridization. It was increased in glomeruli and tubules of diabetic wild-type mice (C, G). The expression showed a tendency to be attenuated in diabetic TRX1 transgenic mice (D, H). A and E: non-diabetic wild-type mice. B and F: non-diabetic TRX1 transgenic mice. C and G: diabetic wild-type mice. D and H: diabetic TRX1 transgenic mice. Magnification is 400×.
to suppress an activity of apoptosis signal-regulating kinase 1 (ASK1) which is a common upstream molecule of both p38 MAPK and c-Jun N-terminal kinase (JNK) pathways [17]. These signal transductions are known to be activated in response to a variety of cellular stresses including oxidative stress. They contribute to the establishment of stress-related pathological changes by modulating the transcription activity in the cells. It was previously shown that the p38 MAPK pathway was also activated by methylglyoxal (MG), a highly reactive glucose-derived dicarbonyl compound, in rat mesangial cells [18]. Since hyperglycaemia accelerates formation of both ROS and MG, the stress-responding signal transductions are likely to be markedly activated under diabetic conditions.

Thus, a reduced form of TRX1 seems to play an important function in eliminating intracellular ROS and in suppressing stress-mediating signal transductions, which are induced by hyperglycaemia. However, it has been postulated that the activity of TRX1 is per se suppressed under diabetic conditions by several mechanisms. First, NADPH required for reversing an oxidized form of TRX1 to a reduced form is decreased because it is consumed in the first step of the polyol pathway, which is also accelerated under diabetic conditions. Second, hyperglycaemia inhibits TRX1 antioxidative function through p38 MAPK-mediated induction of thioredoxin-interacting protein [19], an endogenous inhibitor of thioredoxin also known as vitamin D3 up-regulated protein-1 or thioredoxin-binding protein-2 (TBP-2) [20]. It was also demonstrated that expression of TRX1 was down-regulated in COS-7 cells transiently transfected with TBP-2 expression vector [20]. Therefore, supplementation and/or induction of TRX1 are/is useful to restore its decreased capacity as a buffer against intracellular stresses. In addition, the present study suggested the importance of adequate and efficient inhibition of oxidative stress not only in the glomeruli but also in the renal tubules for complete normalization of the pathological changes in diabetic animals, since oxidative stress appeared to be substantially generated in the renal tubules.

In conclusion, TRX1-Tg showed a marked suppression of oxidative stress in diabetic renal tissue, accompanied by amelioration of characteristic pathophysiological changes of diabetic nephropathy. These findings indicated relation of oxidative stress in the development of diabetic nephropathy and a potential inhibition by TRX1 of the progression of diabetic nephropathy.

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Conflict of interest statement. None declared.

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