Kidney

Xanthine Oxidoreductase Depletion Induces Renal Interstitial Fibrosis Through Aberrant Lipid and Purine Accumulation in Renal Tubules

Toshio Ohtsubo, Kiyoshi Matsumura, Kanae Sakagami, Koji Fujii, Kazuhiko Tsuruya, Hideko Noguchi, Ilsa I. Rovira, Toren Finkel, Mitsuo Iida

Abstract—Xanthine oxidoreductase (XOR) is an enzyme responsible for purine degradation, reactive oxygen species production, and adipogenesis. XOR gene-disrupted (XOR−/−) mice demonstrate renal failure and early death within several months. The aim of this study was to elucidate the mechanism of renal damage in XOR−/− mice and to determine the physiological role of XOR in the kidney. Histological analysis revealed that renal tubular damage in XOR−/− mice was accompanied by deposition of crystals and lipid-rich substances. Triglyceride content in renal homogenates was significantly increased in XOR−/− mice. The level of lipogenesis-related gene expression was comparable in XOR+/+ and XOR−/− mice, whereas the expression of adipogenesis-related gene expression was significantly elevated in XOR−/− mice. Urinary excretions of xanthine and hypoxanthine were markedly elevated in XOR−/− mice. Immunohistochemical analysis, Western blotting, and real time RT-PCR revealed that various markers of fibrosis, inflammation, ischemia, and oxidative stress were increased in XOR−/− mice. Finally, we demonstrate that primary renal epithelial cells from XOR−/− mice are more readily transformed to myofibroblasts, which is a marker of increased epithelial mesenchymal transition. These results suggest that XOR gene disruption induced the depletion of uric acid and the accumulation of triglyceride-rich substances, xanthine, and hypoxanthine in the renal tubules. We believe that these changes contribute to a complex cellular milieu characterized by inflammation, tissue hypoxia, and reactive oxygen species production, ultimately resulting in renal failure through increased renal interstitial fibrosis.

Key Words: xanthine oxidoreductase ■ lipid ■ uric acid ■ xanthine ■ renal interstitial fibrosis ■ epithelial mesenchymal transition ■ oxidative stress

Xanthine oxidoreductase (XOR) was initially identified as the substance in milk that could decolorize methylene blue in 1902. XOR is the enzyme that catalyzes the final 2 steps in purine catabolism by converting hypoxanthine to xanthine and xanthine to uric acid. Therefore, XOR is a key regulator for the production of uric acid.

In addition, XOR has been identified as a critical source of reactive oxygen species (ROS) in a variety of pathophysiological conditions, such as ischemia/reperfusion injury, hypertension, endothelial dysfunction, atherosclerosis, and innate immunity. Furthermore, XOR was found to provide a pivotal role in the maturation of antigen-presenting dendritic cells as a source of uric acid and in the biology of the mammary glands, where XOR is a structural component of the membrane-encapsulated milk fat globule. Recently it was shown that XOR is also a novel regulator of adipogenesis and peroxisome proliferator-activated receptor (PPAR)-γ activity. Thus, XOR has many crucial roles in physiological and pathophysiological functions. However, only approximately half of all patients with XOR deficiency show xanthine calculi, and even less develop associated arthropathy and myopathy.

To elucidate the precise function of the XOR gene in vivo, we developed an XOR gene-disrupted mouse. XOR knockout mice failed to thrive after 10 to 14 days, and most died within the first month. Morphological and histological examination in XOR−/− mice revealed that obvious changes were detected only in the kidney. Hematoxylin and eosin staining showed that the renal parenchyma was immature with ectatic-to-cystic tubules. Similar observations have been made in mice with disruption of the cyclooxygenase-2 gene and in other mice models of obstructive nephropathy. Serum chemistries revealed that blood urea nitrogen in 3-week-old XOR−/− mice was elevated 3 times compared to those in XOR+/+ mice. These and other results suggest that our XOR−/− mice die of renal failure.

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In this study we have examined the detailed mechanism of renal injury in XOR−/− mice, and we discuss the physiological role of the XOR gene in the kidney. Disruption of the XOR gene induced the depletion of uric acid and the accumulation of fat-rich deposits and crystals in the renal tubules. Furthermore, fat-rich deposits accumulated in the renal tubules with the increased expression of adipogenesis-related genes. These changes might subsequently induce renal tubular dilatation, inflammation, ROS generation, and hypoxia of tubular cells. We further demonstrate that these changes ultimately result in renal failure through the expansion of renal interstitial fibrosis.

Methods

Animals
Handling of all animals was done in accordance with prescribed guidelines and ethical approval from the animal care and use committee of Kyushu University. Experiments were conducted under protocols approved by the committee of ethics in animal experimentation of the Faculty of Medicine, Kyushu University. Before the animals were euthanized, they were anesthetized with isoflurane (Abbott Japan). Subsequently, blood, urine, and tissues were removed and stored at −80°C. Kidney tissues were fixed in alcohol or 4% paraformaldehyde solution.

Blood and Urine
Because of their small size, blood samples from 2-week–old mice were pooled from 2 to 6 mice, and 5 independent samples per group were analyzed. Similarly, urine samples from 1-week–old mice were pooled from 4 to 6 mice, and 4 independent samples per group were analyzed. Blood and urinary concentrations of xanthine and hypoxanthine were determined using high-performance liquid chromatography.14 Urinary xanthine and hypoxanthine were analyzed. Similarly, urine samples from 1-week–old mice were pooled from 2 to 6 mice, and 5 independent samples per group were analyzed. Because of their small size, blood samples from 2-week–old mice were prepared as described previously.10

Preparation of F6 XOR Gene-Disrupted Mice and Genotyping
XOR gene-disrupted mice were prepared as described previously.10 To reduce the renal damage in XOR−/− mice, XOR+/− F1 male mice were mated with C57BL/6J female mice, which are resistant to various renal toxic reagents. Approximately half of the XOR−/− F6 mice were able to survive until 2 months, but they still remained runted.

Oil Red O Staining
Kidney samples were fixed with 4% paraformaldehyde; dehydrated with 10%, 15%, and 20% sucrose; subsequently embedded into OCT compound; and then stained in Oil Red O solution for 2 minutes and the counterstained with hematoxylin solution.

Lipid Contents in Kidney
Total lipids from kidney were extracted as described previously. The content was measured using a Triglyceride Quantification kit (Wako Chemicals).

Real-Time RT-PCR
Total RNA was prepared from the cortex of kidney or primary renal epithelial cells using RNeasy Protect Mini kit (Qiagen). RT-PCR was performed using specific primers (Table S1, please see the online Data Supplement at http://hyper.ahajournals.org). One Step SYBR RT-PCR Kit (Takara-Biomedicals) and LightCycler 2.0 System (Roche Diagnostics KK).

Immunohistochemistry
Immunohistochemistry was performed as described previously.16,17 After blocking, kidney sections were subsequently incubated with transforming growth factor-β (TGF-β; 1:500; Santa Cruz Biotechnology), connective tissue growth factor (CTGF; 1:5000; Abcam), α-smooth muscle actin (α-SMA; 1:20; Nichirei), osteopontin (1:100; Santa Cruz Biotechnology), F4/80 (1:1000; Serotec), hypoxia-inducible factor 1α (1:200; Santa Cruz Biotechnology), 4-hydroxy-
Results

Accumulation of Triglyceride-Rich Deposits in the Renal Tubules of XOR+/− Mice

The amount of visceral fat appeared to decrease in XOR+/− mice, whereas many yellow deposits were found in cross-sectional regions of kidney in 4-week–old XOR−/− mice (Figure 1A). To determine whether the yellow deposits contained lipids, Oil Red O staining was performed (Figure 1B). Positive staining was detected in the renal tubules from the corticomedullary-to-cortical region of XOR−/− mice, which corresponded with the location of the observed yellow deposits. No staining was detected in XOR+/− mice. The ratios of triglyceride:phospholipid levels in kidney homogenates were significantly elevated in 4-week–old XOR−/− mice (Figure 1C).

Expression of Lipogenesis- and Adipogenesis-Related Genes In kidney

To further clarify the mechanism of renal lipid accumulation in XOR−/− mice, the expressions of lipogenesis-related genes and adipogenesis-related genes were examined (Table). The amounts of 3-hydroxy-3-methylglutaryl coenzyme A synthase, fatty acid synthase, and sterol regulatory element-binding protein-1c mRNA were comparable in XOR+/− and XOR−/− mice. On the other hand, the amount of CCAAT/enhancer binding protein-α and CCAAT/enhancer binding protein-β mRNA expressions was ∼20- and 50-fold higher in XOR−/− mice.

Table. Relative mRNA Expression to GAPDH in the Kidney of 4-Week-Old XOR Mice

<table>
<thead>
<tr>
<th>Gene</th>
<th>XOR+/−</th>
<th>XOR−/−</th>
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<tbody>
<tr>
<td>Lipogenesis</td>
<td></td>
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</tr>
<tr>
<td>HMG-CoA</td>
<td>1 ± 0.54</td>
<td>2.58 ± 0.10</td>
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<tr>
<td>FAS</td>
<td>1 ± 0.21</td>
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<tr>
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<td>1 ± 0.39</td>
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<tr>
<td>Adipogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEBP-α</td>
<td>1 ± 0.12</td>
<td>21.0 ± 8.45*</td>
</tr>
<tr>
<td>CEBP-β</td>
<td>1 ± 0.45</td>
<td>49.8 ± 16.4*</td>
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<tr>
<td>PPAR-α</td>
<td>1 ± 0.29</td>
<td>7.49 ± 1.49*</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>1 ± 0.16</td>
<td>5.73 ± 1.23*</td>
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<tr>
<td>Fibrosis</td>
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<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td>1 ± 0.08</td>
<td>25.2 ± 2.12*</td>
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<tr>
<td>α-SMA</td>
<td>1 ± 0.27</td>
<td>123.29 ± 9.9*</td>
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<tr>
<td>Vimentin</td>
<td>1 ± 0.21</td>
<td>172.32 ± 3.1*</td>
</tr>
<tr>
<td>PAI-1</td>
<td>1 ± 0.21</td>
<td>283.26 ± 8.8*</td>
</tr>
<tr>
<td>Inflammation</td>
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<tr>
<td>TNF-α</td>
<td>1 ± 0.35</td>
<td>24.2 ± 7.99*</td>
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<tr>
<td>MCP-1</td>
<td>1 ± 0.20</td>
<td>453 ± 48.8*</td>
</tr>
<tr>
<td>gp91phox</td>
<td>1 ± 0.32</td>
<td>15.8 ± 1.99*</td>
</tr>
</tbody>
</table>

Expression of mRNA was measured by RT-PCR method. Values are mean±SEM of 5 to 8 samples per each group. HMG-CoA indicates 3-hydroxy-3-methylglutaryl coenzyme A synthase; FAS, fatty acid synthase; SREBP-1c, sterol regulatory element-binding protein-1c; CEBP, CCAAT/enhancer binding protein; PAI-1, plasminogen activator inhibitor-1; TGF-α, tumor necrosis factor-α; MCP-1, monocyte chemotactic protein 1.

*P<0.01 vs XOR+/− mice.

Immunohistochemical Detection of Hypoxic Region

Pimonidazole hydrochloride (hypoxyprobe-1; Chemicon International) was injected IP into XOR mice (60 mg/kg; n=3 per group). Thirty minutes after injection, kidneys were quickly removed, fixed in 4% paraformaldehyde, and embedded in paraffin. After sectioning, sections were deparaffinized and rehydrated through a graded series of ethanol. The sections were incubated in 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity, and then blocked with 10% normal goat serum for 1 hour. Sections were then incubated with nitrotyrosine (1:100; Santa Cruz Biotechnology) primary antibodies for 1 hour, followed by incubation with horseradish peroxidase–conjugated streptavidin (1:1000; Santa Cruz Biotechnology, Santa Cruz, California) for 30 minutes. Reaction was visualized by incubating with 3,3′-diaminobenzidine (DAB) and H2O2.

Western Blot Analysis

Renal cortex was resuspended in radioimmunoprecipitation assay buffer and homogenized using the Mixer MM 300 (Qiagen). Western blotting was performed as described previously. The blocked nitrocellulose filter was incubated with nitrotyrosine (1:100; Santa Cruz Biotechnology), 4-HNE (1:100), or malondialdehyde (MDA) (1:100; NOF Corporation) primary antibodies. The membranes were incubated with the horseradish peroxidase–conjugated secondary antibodies and visualized. Blots were stripped in Restore Western Blot Stripping buffer (Pierce Biotechnology Inc) and reprobed with GAPDH antibody (1:100; Santa Cruz Biotechnology). The density of the signal was analyzed with Image J software. Relative expression of nitrotyrosine and MDA for 1-week–old XOR+/− mice was analyzed. Results are presented from 4 mice per group.

Primary Culture of Renal Tubular Epithelial Cells

Primary renal epithelial cells were prepared by modifying a method described previously. Kidneys from 1-week–old XOR mice were dissected to obtain cortical tissue, which was digested with 1% type II collagenase (Worthington Biochemical) and 0.5 mg/mL of soybean trypsin inhibitor (Invitrogen Japan) at 37°C for 45 minutes. The tissue was further dissociated by trituration with a sterile pipette and then passed through a 100-μm cell strainer (Invitrogen Japan). Cells were grown in a 1:1 serum-free mixture of DMEM and Ham F-12 containing 15.0 mmol/L of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 2.5 mmol/L of l-glutamate, 50 ng/mL of prostaglandin E1, 50 nM hydrocortisone, 40 nM sodium selenite, 10 μg/mL of insulin, 5.5 μg/mL of transferrin, 50 nM hydrocortisone, 10 ng/mL of recombinant mouse epidermal growth factor (BioSource, Camarillo, California), 5 μM 3,3′,5-triodo-L-thyronine (reverse T3), 50 μM of penicillin, and 50.0 μg/mL of streptomycin at 37°C. Prostaglandin E1, hydrocortisone, and reverse T3 were purchased from Invitrogen Japan. Cells were grown in 4% paraformaldehyde, treated with 0.5% Triton-X100, and blocked with 3% BSA. The sections were subsequently incubated with TGF-β (1:100), vimentin (1:50; Neo-Markers), or E-cadherin (1:1000; BD Transduction Laboratories) primary antibodies; probed with secondary antibody; and visualized by incubating with 3,3′-diaminobenzidine and H2O2.

Immunostaining for Primary Cultured Cells

Primary cultures were fixed in 4% paraformaldehyde, treated with 0.5% Triton-X100, and blocked with 3% BSA. The sections were subsequently incubated with TGF-β (1:100), vimentin (1:50; Neo-Markers), or E-cadherin (1:1000; BD Transduction Laboratories) primary antibodies; probed with secondary antibody; and visualized by incubating with 3,3′-diaminobenzidine and H2O2.

Statistical Analysis

Comparisons among age-matched mouse groups were made by the Student t test, and differences between the same types of 2-1-week–old mice were analyzed by 1-way ANOVA followed by the Bonferroni/Dunn test. Values are expressed as mean±SEM. Differences were considered statistically significant for P<0.05.
Tubules of XOR showed that many brown deposits existed in the dilated renal hypoxanthine. *P<0.001 vs age-matched XOR and the nucleus of tubular cells in 4-week–old XOR performed, and positive staining was detected in the cytosol by SEM. C, Microscopic view of dilated renal tubules containing brown material (top) and white deposit (bottom) by polarization in 4-week–old XOR mice, and 4 independent samples were analyzed. Seven samples of 4-week–old mice were used. Values are mean±SEM.

Accumulation of Crystal Deposits in the Renal Tubules of XOR−/− Mice

Blood concentrations of xanthine and hypoxanthine rapidly decreased after birth and reached the lower level of measurement sensitivity (<0.05 μg/mL) at 4 weeks of age in XOR+/+ mice but were markedly and persistently elevated in XOR−/− mice (Figure 2A). Urinary excretion ratios of xanthine and hypoxanthine to creatinine were statistically significant compared with those in age-matched XOR+/+ mice. The expression levels of TGF-β, α-SMA, vimentin, and plasminogen activator inhibitor 1 mRNA also significantly increased in XOR−/− mice when compared with age-matched XOR+/+ mice (Table). In addition, immunohistochemical analysis revealed that, for α-SMA, which is a marker of epithelial-mesenchymal transition (EMT), positive staining was detected in both tubulointerstitial and interstitial cells (Figure 3B). These positive stainings in XOR−/− mice were statistically significant compared with those in age-matched XOR+/+ mice. The expression levels of TGF-β, α-SMA, vimentin, and plasminogen activator inhibitor 1 mRNA also significantly increased in XOR−/− mice when compared with age-matched XOR+/+ mice (Figure 4A). On the other hand, F4/80 positive cells, which indicate macrophage infiltration, invaded the interstitial spaces between the renal tubules in 4-week–old XOR−/− mice. These changes were not observed in 4-week–old XOR+/+ mice when compared with age-matched XOR+/+ mice (Table).

**XOR Gene Deletion Induced Inflammatory Responses, Tissue Hypoxia, and Oxidative Stress in Kidney**

Immunoreactivity for osteopontin, an extracellular matrix glycoprophosphoprotein that induces macrophage infiltration, was detected in the renal epithelial cells of XOR−/− mice at 4 weeks of age (Figure 4A). On the other hand, F4/80 positive cells, which indicate macrophage infiltration, invaded the interstitial spaces between the renal tubules in 4-week–old XOR−/− mice. These changes were not observed in 4-week–old XOR+/+ mice. The amounts of tumor necrosis factor-α, monocyte chemoattractant protein 1, and gp91phox (1 of the NADPH oxidase subunits) mRNA expression were also significantly increased in 4-week–old XOR−/− mice when compared with age-matched counterparts (P<0.01; Table). To elucidate whether ischemia is involved in renal damage in XOR−/− mice, pimonidazole staining (a marker for tissue hypoxia) was performed in XOR mice. Pimonidazole-positive cells were observed in the segmental region of proximal tubular cells of 4-week–old XOR−/− mice. Moreover, hypoxia-inducible factor 1α–positive staining was de-
tected in the nucleus of proximal tubular cells of 4-week–old XOR−/− mice. Consistent with an increase in oxidative damage, 4-HNE–positive staining in interstitial cells and 8-oxo-7,8-dihydro-2′-deoxyguanosine–positive staining in the nucleus of interstitial and epithelial cells were detected in 4-week–old XOR−/− mice. The amounts of nitrotyrosine, 4-HNE, and MDA detected by Western blot analysis were decreased after birth until XOR−/− and XOR+/− mice were 2 weeks old. However, accumulations of nitrotyrosine and MDA in 4- and 8-week–old XOR−/− mice were significantly increased compared with age-matched XOR+/+ mice (Figure 4B).

**XOR Gene Deletion Facilitates Renal EMT**

Our results suggest that XOR gene deletion might potentiate EMT (see Figure 3B). To assess whether this is a cell-autonomous effect, primary renal epithelial cells were prepared. Histological examination revealed that primary renal epithelial cells from XOR−/− mice changed morphology from a cuboidal to a fibroblastic shape. Immunohistochemical analysis also showed that positive staining for TGF-β and vimentin was only detected in primary cells isolated from XOR−/− mice, whereas the regions of fibroblastic shaped cells in XOR−/− mice were not stained with anti-E-cadherin.

![Figure 3. Disruption of the XOR gene induces renal interstitial fibrosis. A, Masson trichrome staining in the kidney of 1- and 4-week–old XOR+/+ and XOR−/− mice (n=3 per each group). Arrows show positive regions. B, Immunohistochemical analysis of TGF-β, CTGF, and α-SMA expression in 4-week–old XOR+/+ and XOR−/− mouse kidneys (n=3 per group; top). Arrows show positive regions. Percentage of positive stained area for TGF-β and α-SMA and positive stained cells for CTGF (bottom). Each bar represents the mean±SEM from 3 mice per group. For each mouse, 10 randomly selected fields were analyzed. *P<0.01 vs XOR+/+ mice.](http://hyper.ahajournals.org/)

![A 1-week old B 4-week old Positive stained area (%) XOR+/+ XOR-/− Positive stained cells (%) XOR+/+ XOR-/− Positive stained area (%) XOR+/+ XOR-/−](http://hyper.ahajournals.org/)
antibody (Figure 5A). Furthermore, the amounts of TGF-β, vimentin, and α-SMA mRNA expression were also significantly increased in XOR−/− mice (P<0.01; Figure 5B).

Discussion

In the present study we describe the possible pathways involved in renal injury in XOR−/− mice. Disruption of the XOR gene induced the accumulation of triglyceride-rich lipids in the renal tubules with the increased expression of adipogenesis-related genes. Moreover, XOR gene deletion inhibits the conversion from hypoxanthine to xanthine and xanthine to uric acid, resulting in increases in hypoxanthine and xanthine concentrations in blood and urine and subsequent deposition of insoluble crystals in the renal tubules. We believe that these deposits in the renal tubule can trigger inflammation, decreased renal blood flow, and increased ROS production in spite of XOR gene disruption. In fact, the amounts of oxidative products were similar in both XOR+/+ and XOR−/− mice until they were 2 week old. However, accumulation of oxidative products were significantly increased in 4-week-old XOR−/− mice (Figure 4B), accompanying the increase in xanthine and hypoxanthine concentrations in blood (Figure 2A) and the augmented excretion of xanthine in urine (Figure 2B). Furthermore, because uric acid is known to possess strong antioxidant properties, the absence of uric acid might also accelerate the accumulation of ROS and aggravate the renal injury. As such, it would seem that these observed alterations in XOR−/− mice might combine to induce interstitial fibrosis after EMT and ultimately lead to renal failure.

Xanthinuria is a rare hereditary disorder of purine metabolism resulting from a deficiency of the XOR enzyme in humans. Patients with XOR gene deficiency rarely suffer from renal failure. In contrast, XOR−/− mice die of renal failure several weeks after birth, indicating that XOR plays a pivotal role during postnatal renal maturation in mice. Three reasons may explain the different progression of renal damage observed in humans and mice with XOR gene deficiencies. First, the role of the XOR protein in mice might be more important than in humans, because XOR enzyme activity in mice is 100 times higher than that in humans. Second, urinary concentration of xanthine, which is less soluble than uric acid, is 100-fold higher in XOR−/− mice than in xanthinuria patients, suggesting that high levels of xanthine might contribute to renal damage in mice. There might also be differences in purine catabolism between mice and humans with XOR gene deletion. For example, mice express the uricase enzyme to catalyze the metabolism of uric acid to
usually present in the peritubular region, but in the case of the renal tubule. It has been reported that XOR protein is
ular cells, or tubulointerstitial cells but was detected within deposition was not found in the renal epithelial cells, glomer-
hand, the hypoxanthine salvage pathway in hereditary xan-
activity. Third, humans with XOR gene deletions can use the XOR protein derived from their mother during pregnancy, because mice
And their kidneys are fully matured at birth. Meanwhile, mouse fetuses with XOR gene deletions are also capable of using XOR derived from their mother during pregnancy but cannot use the XOR protein to mature kidney, because mice need 16 days after birth for kidney maturation. Despite these important differences, we believe that our model provides a useful genetic system to explore the physiological role of xanthine oxidase.
It has been reported that renal triglyceride contents increase and deposit in the renal tubular and glomerular cells to protect the renal tissue against various types of renal damage. A high-fat diet also increases plasma triglycerides and cholesterol and induces marked neutral lipid accumulation in both the glomerular and tubulointerstitial lesions. In this study, the kidney homogenates of XOR−/− mice contained significantly higher triglyceride levels when compared with their counterparts (Figure 1C). On the other hand, lipid deposition was not found in the renal epithelial cells, glomerular cells, or tubulointerstitial cells but was detected within the renal tubule. It has been reported that XOR protein is usually present in the peritubular region, but in the case of hyperlipidemia, XOR is detected in the tubular epithelium. There is evidence that XOR is one of the regulators of adipogenesis and PPAR-γ activity in preadipocytes, and inhibition of XOR expression and/or enzymatic activity blocks both PPAR-γ activation and adipocyte differentiation. These results suggest that XOR or its products in renal epithelial cells may play a role in protecting them from damage by regulating adipogenesis and the redox state. Additional experiments will be required to clarify the detailed mechanism by which XOR regulates adipogenesis in the kidney.
EMT plays an important role in the progression of interstitial fibrosis in the kidney. Immunostaining for the α-SMA antibody demonstrated positive staining consistent with the accumulation of myofibroblasts (Figure 3). Increased expression of both TGF-β and CTGF protein, which are the main inducers of EMT, was also detected in XOR−/− mice. These results suggest that EMT participates in the progression of renal damage in XOR−/− mice. Furthermore, primary renal epithelial cells prepared from XOR−/− mice transformed to myofibroblasts and demonstrate increased expression of TGF-β, vimentin, and α-SMA (Figure 5). Together these observations suggest that XOR gene disruption in renal epithelial cells facilitates the EMT. The reason for this phenomenon remain unclear, but a decrease in antioxidant capacity with loss of uric acid or alteration of renal lipid homeostasis in XOR−/− mice might be involved in this alteration in the EMT.
In the past several years, evidence has been accumulated that hyperuricemia is associated with metabolic syndrome,
cardiovascular events, hypertension, stroke, diabetes mellitus, and chronic kidney disease. Furthermore, lowering uric acid with the use of XOR inhibitor can improve the progression of renal disease, ischemic injuries, inflammatory diseases, and chronic heart failure.

On the other hand, several large cohort studies showed that hypouricemia, as well as hyperuricemia, was also associated with cardiovascular disease, stroke, and chronic kidney disease in subjects with hypertension or diabetes mellitus, in addition to the general population. It has been reported that uric acid has antioxidant activity against a variety of oxidants. These results suggest that hyperuricemia may be, in part, a compensatory mechanism to counteract oxidative damage. There must be an optimal concentration of uric acid in blood, and the role of uric acid may be different in various physiological and pathophysiological conditions. In addition, it has been assumed that XOR protein is one of the major sources of ROS and worsens the oxidative injury, but complete deletion of XOR protein induced accumulation of oxidative products and renal injury. In some tissues under pathophysiological conditions, increased XOR activity may, therefore, be an adaptive response to produce uric acid and may reduce oxidative stress.

**Perspectives**

We examined the physiological role of XOR in the kidney using an XOR knockout mouse model and showed possible roles for this protein. The XOR gene might be essential for kidney maturation and survival of mice during the postnatal period via the regulation of fat and purine metabolism. The XOR gene may regulate adipogenesis in the kidney and have a pivotal role in preventing the epithelial cells from transforming to mesenchymal cells.

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**Disclosures**

None.

**References**


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Xanthine oxidoreductase depletion induces renal interstitial fibrosis through aberrant lipid and purine accumulation in renal tubules

Short title: Physiological role of XOR in kidney

Toshio Ohtsubo, Kiyoshi Matsumura, Kanae Sakagami, Koji Fujii, Kazuhiko Tsuruya, Hideko Noguchi, Ilsa I. Rovira, Toren Finkel and Mitsuo Iida

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Physiological role of XOR in kidney

Online Supplement Table

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<td>SREBP-1c</td>
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<td>C/EBP-α</td>
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<td>PPAR-γ</td>
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<td>GCGAGCCCATTGTTTGACAGAAGA</td>
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<tr>
<td>α-SMA</td>
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<td>TGTCAAGCAGTGCCGTAGTGTGTAGTC</td>
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<td>PAI-1</td>
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<td>TNF-α</td>
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<td>gp91phox</td>
<td>TTGGTCAGCAGCTGGCTCTG</td>
<td>TGGCCGCTGTCAGGCTATAC</td>
</tr>
<tr>
<td>GAPDH</td>
<td>AAATGGTGAGGGTCGGGTTG</td>
<td>TGAAGGGTGTCCGGATGAGG</td>
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HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A synthase, FAS: fatty acid synthase, SREBP-1c: sterol regulatory element-binding protein-1c, C/EBP: CCAAT/enhancer binding protein, PPAR: peroxisome proliferator activated receptor, TGF-β1: transforming growth factor-β1, α-SMA: α-smooth muscle actin, PAI-1: plasminogen activator inhibitor-1, TNF-α: tumor necrosis factor-α, MCP-1: monocyte chemoattractant protein-1, PCNA: proliferative cell nuclear antigen, GAPDH: glyceraldehyde-3-phosphate dehydrogenase