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Proximal tubule PPARα attenuates renal fibrosis and inflammation caused by unilateral ureteral obstruction

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1 Division of Nephrology, University of Arkansas for Medical Sciences, Little Rock, Arkansas; 2 Division of Rheumatology, Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas; 3 Central Arkansas Veterans Healthcare System, Little Rock, Arkansas; and 4 Division of Nephrology, Departments of Internal Medicine and Pathology, Center for Lung Biology, Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, Washington

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Li S, Mariappan N, Megyesi J, Shank B, Kannan K, Theus S, Price PM, Duffield JS, Portilla D. Proximal tubule PPARα attenuates renal fibrosis and inflammation caused by unilateral ureteral obstruction. Am J Physiol Renal Physiol 305: F618–F627, 2013. First published June 26, 2013; doi:10.1152/ajprenal.00309.2013.—We examined the effects of increased expression of proximal tubule peroxisome proliferator-activated receptor (PPARα) in a mouse model of renal fibrosis. After 5 days of unilateral ureteral obstruction (UUO), PPARα expression was significantly reduced in kidney tissue of wild-type mice but this downregulation was attenuated in proximal tubules of PPARα transgenic (Tg) mice. When compared with wild-type mice subjected to UUO, PPARα Tg mice had reduced mRNA and protein expression of proximal tubule transforming growth factor (TGF)-β1, with reduced production of extracellular matrix proteins including collagen 1, fibronectin, α-smooth muscle actin, and reduced tubulointerstitial fibrosis. UUO-mediated increased expression of microRNA 21 in kidney tissue was also reduced in PPARα Tg mice. Overexpression of PPARα in cultured proximal tubular cells by adenoviral transduction reduced aristolochic acid-mediated increased production of TGF-β, demonstrating PPARα signaling reduces epithelial TGF-β production. Flow cytometry studies of dissociated whole kidneys demonstrated reduced macrophage infiltration to kidney tissue in PPARα Tg mice after UUO. Increased expression of proinflammatory cytokines including IL-1β, IL-6, and TNF-α in wild-type mice was also significantly reduced in kidney tissue of PPARα Tg mice. In contrast, the expression of anti-inflammatory cytokines IL-10 and arginase-1 was significantly increased in kidney tissue of PPARα Tg mice when compared with wild-type mice subjected to UUO. Our studies demonstrate several mechanisms by which preserved expression of proximal tubule PPARα reduces tubulointerstitial fibrosis and inflammation associated with obstructive uropathy.

peroxisome proliferator-activated receptor; transforming growth factor-β; interleukin-10

RECENT STUDIES SUPPORT the notion that patients who develop acute kidney injury (AKI) are at higher risk of developing chronic kidney disease (CKD) (15, 26). The mechanisms that lead to progression from AKI to CKD in humans are not entirely clear, but available studies using numerous animal models suggest that the development of progressive interstitial fibrosis, diminished capillary density, and increased levels of angiotensin II, among other factors, represent mechanistic pathways by which AKI can lead to CKD (6). There is increased evidence suggesting that a sub-family of nuclear receptor transcription factors, known as peroxisome proliferator-activated receptors (PPARs), reduces inflammation and ameliorates tissue fibrosis, but the mechanisms involved in this cytoprotective response have not been elucidated (16, 39, 42, 44). PPARs are metabolic regulators with anti-inflammatory properties in various organs including the kidney (41). Our previous work using PPARα ligands and PPARα transgenic (Tg) mice demonstrated that preserving intact function of proximal tubule epithelial PPARα is cytoprotective during AKI (22–24, 32). PPARα is a nuclear receptor transcription factor expressed predominantly in the proximal tubule and the thick ascending limb of Henle that plays an important role in the modulation of energy utilization through the regulation of fatty acid oxidation (FAO) and oxidant production in mitochondria and peroxisomes (17, 27, 36). The importance of PPARα in modulating FAO by peroxisomes and mitochondria in kidney tissue is underscored by the response of renal PPARα to dietary lipids and by the cytoprotective effects of increasing its expression in the proximal tubule during AKI (23, 24, 35).

In the present study, we extended previous observations made in our laboratory and compared wild-type mice with PPARα Tg mice using the model of unilateral ureteral obstruction (UUO)-mediated renal fibrosis to define potential cellular mechanisms by which increased expression of proximal tubule PPARα reduces tubulointerstitial fibrosis. Our studies suggest that activation of proximal tubule PPARα may represent a novel therapeutic target to reduce renal fibrosis and ameliorate progression to CKD.

METHODS

Generation of proximal tubule PPARα Tg mice. Proximal tubule-specific expression of PPARα under the control of the androgen-sensitive KAP (kidney androgen-regulated protein) promoter was achieved with KAP2-PPARα Tg mice, which were generated and characterized as previously described (23). Eight- to ten-week-old, age- and weight-matched PPARα Tg and wild-type (C57 BL/6) female mice were used for the studies. To investigate the effects of
increased expression of proximal tubule PPARα, wild-type and PPARα Tg mice received a 5-mg subcutaneous testosterone pellet (Innovative Research of America, Sarasota, FL) and after 9 days were subjected to surgery (see scheme of experimental time course in Fig. 1A). The animals were housed at the Veterinary Medical Unit at the Central Arkansas Veterans Healthcare System (Little Rock, AR). When appropriate, animals were painlessly euthanized according to methods of euthanasia approved by the Panel on Euthanasia of the American Veterinary Medical Association. Our animal study protocols were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the Central Arkansas Veterans Healthcare System.

Renal fibrosis model of UUO. KAP2-PPARα Tg and wild-type mice, 8–10 wk old, were assigned to four treatment groups. Either UUO or sham surgeries were performed on both wild-type and PPARα Tg mice. All four groups of animals received 5-mg (21-day release) subcutaneous testosterone pellets 9 days before surgery. The left kidney was exposed through a midline incision under sterile conditions; the ureter was dissected and securely tied at two places with 6–0 silk sutures. Volume depletion was prevented by administration of ~0.1 ml saline into the peritoneal cavity. The midline incision was closed; the mice were returned to their cages and allowed free access to food and water for 5 days. As control, sham surgery was performed the same way as UUO without tying the ureter. After 5 days, the mice were euthanized and the left kidneys from UUO and sham mice were collected for protein, RNA isolation, and histological evaluation.

Gene expression studies. PPARα, transforming growth factor-β1 (TGF-β1), α-smooth muscle actin (α-SMA), collagen type I, α1 (Col1A1), fibronectin (Fn), collagen type IV α1 (Col4A1), laminin β, interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), arginine 1, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), CD86 mRNA levels, and miRNA21 (miR21) mRNA levels were determined by quantitative RT-PCR. Total RNA was extracted from cells or mouse kidney tissue and treated with RNase-free DNase before RT reaction. Real-time PCR was carried out using the StepOnePlus real-time PCR system (Invitrogen, Foster City, CA) with iTaqSYBR Green Supermix with Rox (Bio-Rad, Hercules, CA). In each experiment, triplicates of 50-ng cDNA (total RNA equivalent) samples were amplified in a 20-μl reaction. Specificity of the amplified product was confirmed by melting curve analysis and agarose gel electrophoresis. For relative quantification, a standard curve was generated from a six-step cDNA dilution series. Samples were amplified with primers for PPARα, TGF-β1, α-SMA, Col1A1, Fn, Col4A1, laminin β, IL-1β, IL-6, IL-10, TNF-α, arginine 1, MCP-1, MIP-1α, CD86, miR21, and 18S RNA. The relative expression of genes was calculated from the standard curve. Relative quantity was calculated by the ratio of the gene-specific and the appropriate 18S rRNA expression. The primer sequences in the RT-PCR were the following: for PPARα, 5′-AAA GAG GCA GAG GTC CGA TT-3′ (forward), 5′-AGC AGG GTG ACT TGG TCG TT-3′ (reverse); for TGF-β1, 5′-CGA GGC GGT GCT GTT CGT-3′ (forward), 5′-CAT AGA TGG CTT TGC GGT CCA-3′ (reverse); for α-SMA, 5′-TCG CTG TCA GGA ACC ATG CGG CC-3′ (forward), 5′-GTG CAC CCG CAC CCG GTA GC-3′ (reverse); for Col1A1, 5′-GCA GCA AGG GTC CTT CCG GTT-3′ (forward), 5′-AGG ACC AGG GCT GCC AGG AC-3′ (reverse); for Col4A1, 5′-GGT ATT CAG GGA GAC CGT GG-3′ (forward), 5′-ACC CTT GTG CAC CCC TAG AT-3′ (reverse); for laminin β, 5′-CTA CGT TAA GGG CCT GGT GA-3′ (forward), 5′-CCT CGG AGC AGC TAT TGT TGG CAC-3′ (reverse); for IL-1β, 5′-GAC CCC AAA AAG TGA GGC CCT-3′ (forward), 5′-TGG TGT GCT GGC AGA TTG GA-3′ (reverse); for IL-6, 5′-TCC GGA GAG GAG ACT TCA CA-3′ (forward), 5′-TTG

Fig. 1. A, top: schematic diagram showing the experimental time course. Animals received a subcutaneous pellet of 5 mg testosterone for 9 days before unilateral ureteral obstruction (UUO) surgery. Kidneys were harvested 5 days after UUO. A, bottom: effect of UUO on renal peroxisome proliferator-activated receptor (PPARα) mRNA expression in both wild-type (WT) and transgenic (Tg) mice. Level of PPARα was determined by qPCR. Data are expressed as means ± SE. *P < 0.005 when comparing WT sham vs. UUO mice; **P < 0.05 when comparing WT UUO vs. PPARα Tg UUO mice by unpaired Student’s t-test. B: representative photographs of periodic acid Schiff (PAS)-stained sections of sham-operated and 5 days after UUO kidneys from WT and PPARα Tg animals. The sham-operated WT and PPARα Tg mice showed normal kidney architecture, whereas WT mice subjected to UUO showed dilated distal nephron segments, with casts (*), interstitial expansion (circled area), and thickening of basement membrane (arrow head). In PPARα Tg mice 5 days after UUO only some tubular dilation was observed (*). PT, proximal tubule; TAL, thick ascending limbs; G, glomerulus. Magnification: ×488.
CCA TTG CAC AAC TCT TTT CT-3' (reverse); for IL-10, 5'-GCA TGG CCC AGA AAT CAA GG-3' (forward), 5'-AGG GGA GAA ATC GAT GAC AGC-3' (reverse); for TNF-α, 5'-ATG GCC TCC TCT TCA TCA GT-3' (forward), 5'-CTT GGT GGT TTT CTA CGA CG-3' (reverse); for arginase 1, 5'-GGC GGT CAT TTT CCT GAC ATC GGA-3' (forward), 5'-GCC GAT TCA CCT GAC CTG TCT TGT-3' (reverse); for PPARα, a final multiplicity of infection of 100, which resulted in an approx-
imately threefold increase of PPARα protein expression. Where indicated, cultures were treated with AA at 0.5 μg/ml concentration and grown for an additional 24 h. At that time, cells were harvested and RNA was isolated using TRIzol reagent (Invitrogen). RNA samples were used for RT-PCR to analyze TGF-β1, Col1A1, Col4A1, and laminin β as described above.

Kidney tissue processing and flow cytometry analysis of inflammatory cells. To study the kidney CD45+/CD11b+/F4/80+ macrophage population in UUO and sham-operated wild-type and PPARα Tg mice, we prepared a cell suspension of kidney tissue following the protocols published by Li et al. (20, 21). Briefly, kidneys were perfused with PBS, weighed, minced, and incubated with collagenase enzyme type IA (10 μg/ml; Sigma, St. Louis, MO, catalog no. C9891) in cold Dulbecco’s PBS buffer containing 2 mM EDTA for 15 min at 37°C. After digestion, cell suspension was passed through a 100-μm BD Falcon cell-strainer (Fisher Scientific, Pittsburgh, PA) and pellet by centrifugation at 1,200 rpm for 10 min. The cell pellet was washed in FACS buffer (1% BSA in PBS containing 0.1% sodium azide) and suspended again in FACS buffer. Individual sample volume was adjusted based on the kidney weight. After nonspecific Fc binding using an anti-mouse CD16/32 antibodies was blocked, an aliquot of cells was immunostained with CD45-PE alone or in combination with CD11b-FITC, F4/80-APC eFluor 780. Cells were also stained with 7-aminoactinomycin D (7-AAD; 2 μg/ml; Invitrogen Life Technologies-Molecular Probes, Grand Island, NY) to gate out dead cells. Antibodies were purchased from eBioscience (San Diego, CA) and immunostaining protocols were carried out on ice and in the dark.

Multicolor flow cytometry analysis was carried out using FACS Calibur. Total leukocytes in each sample were estimated by using Caltag counting beads (Invitrogen) followed by CD45+ absolute count per kidney using the method and formula by the manufacturer’s recommendation (21). Total leukocyte count and macrophage subset analyses were carried out by using lineage-specific markers. Leukocytes and macrophages within the kidney were quantified by analyzing at least 200,000 cells from each sample. Our results were confirmed by using ImageStreamX flow cytometry and IDEAS software (Ammis, Seattle, WA).

Statistical analysis. Results are presented as means ± SE. Statistical analysis was performed using an unpaired Student’s t-test. A P value of <0.05 was considered to be statistically significant.

RESULTS

PPARα expression downregulated after UUO is attenuated in PPARα Tg mice. PPARα expression was increased in the Tg mice by 2.3-fold (Fig. 1A) in sham-operated mice when compared with wild-type mice. Five days after UUO, PPARα expression was reduced by ~75% in wild-type mice. Although it was also reduced in PPARα Tg mice subjected to UUO, the level of expression was similar to that observed in sham-operated wild-type mice.

Increased proximal tubule PPARα expression ameliorates UUO-induced morphological damage. Sham-operated kidneys from wild-type and Tg animals showed normal architecture with no tubular dilation or interstitial changes. Five days after UUO, kidneys from wild-type mice showed significant dilation with no tubular dilation or interstitial changes. Five days after UUO, kidneys from PPARα Tg mice subjected to UUO also revealed tubular dilatation and some brush-border loss; however, the interstitial changes were rather similar to those seen in the sham-operated groups (Fig. 1B).
Increased proximal tubule PPARα expression reduces UUO-mediated increased expression of fibrogenic genes. We found that Tgfβ1, Coll1a1, Acta2 (α-SMA), and Fn1 transcripts were upregulated by UUO in wild-type mice. There was a 4.1-, 10.4-, 12.6-, and 10.1-fold increased level of mRNA expression for Tgfβ1, Coll1a1, Acta2, and Fn1, respectively (P < 0.001), after 5 days of UUO compared with sham-operated mice, as shown in Fig. 2A. Prior overexpression of PPARα in the Tg mice significantly attenuated the UUO-induced upregulation of Tgfβ1, Coll1a1, Acta2, and Fn1 transcripts when compared with wild-type mice (P < 0.05).

TGF-β1 protein expression increased sevenfold in wild-type mice 5 days after UUO. This increased expression was significantly reduced in PPARα Tg mice when compared with wild-type mice (Fig. 2B). Formalin-fixed, paraffin-embedded kidney sections were also used for the in situ localization of TGF-β1. Indistinguishable positive immune reaction could be detected in medullary and cortical thick ascending limbs in both wild-type and Tg kidneys. Five days after UUO showed a strong immunostaining for TGF-β in cortical thick ascending limbs, but also a positive staining could be seen in the basolateral surfaces of convoluted proximal tubules (S1-S2 segments) of the wild-type kidneys. The positive TGF-β1 immunoreaction was almost completely diminished from the PPARα Tg mouse kidneys 5 days after UUO. Only occasional weak staining could be found in some cortical thick ascending limbs. There was no obvious staining in proximal tubule segments in the Tg UUO group (Fig. 2C).

Proximal tubule PPARα reduces UUO-mediated interstitial fibrosis. To quantify the amount of collagen produced in the interstitium in wild-type and PPARα Tg mice subjected to UUO, kidney sections were stained with picro-sirius red staining. Positive red stain in the interstitium was increased 5 days after UUO in wild-type kidneys compared with sham ones (P < 0.05), and it was significantly reduced (P < 0.05) in

Fig. 2. A: effect of UUO on renal fibrogenic gene transcripts transforming growth factor (TGF)-β1, collagen type I, α1 (Coll1A1), α-smooth muscle actin (SMA), and fibronectin. Bars represent means ± SE mRNA levels for at least 4 mice in each group. *P < 0.001 when comparing WT sham vs. WT-UUO mice. ‡P < 0.05 when comparing WT UUO vs. PPARα Tg UUO mice. B: TGF-β1 protein expression in kidney tissue of WT and PPARα Tg mice subjected to sham and 5 days UUO. B: densitometry and quantification of TGF-β1 signals, normalized to GAPDH levels from Western blot analysis; GAPDH was used as a loading control. Data are expressed as means ± SE. *P < 0.005 when comparing WT sham vs. WT UUO mice. ‡P < 0.005 when comparing WT UUO vs. PPARα Tg UUO mice by unpaired Student’s t-test. C: representative photomicrographs of TGF-β1 immunostaining in WT and Tg mice 5 days after UUO. Positive staining is obvious in the cortical thick ascending limbs and proximal convoluted tubules of WT animals. The positive staining is reduced from Tg UUO kidney. *, thick ascending limb of loop of Henle; PT, proximal convoluted tubules. Magnification: ×244.
PPARα Tg mice as shown in Fig. 3, A and B, indicating that PPARα upregulation ameliorated UUO-induced kidney fibrosis.

Increased proximal tubule PPARα reduces UUO-mediated increased miR21 expression. miR21 directly suppresses expression of PPARα and silences other genes in the PPARα downstream signaling pathway in mitochondria and peroxisomes (5). As expected, miR21 expression determined by qPCR was significantly upregulated after UUO (3.5-fold) in wild-type mice \( (P < 0.05) \). Overexpression of PPARα in the kidney tubule after UUO, however, reduced miR21 levels close to those in normal kidneys (Fig. 4A). This observation was recapitulated in vitro in TKPTS cells injured by the tubular cell toxin AA (see Fig. 4B).

Overexpression of PPARα in proximal tubule (TKPTS) cells reduces TGF-β1 production and collagen 1α1, collagen 4, and laminin expression. Release of active TGF-β by injured epithelial cells is implicated in the pathogenesis of epithelial injury and interstitial fibrogenesis in the kidney. Moreover,
injured epithelial cells are believed to secrete increased tubule basement membrane proteins including collagen IV and laminin. To evaluate the effect of PPARα on cultured epithelial cells, we overexpressed PPARα in TKPTS cells by adenoviral transduction and injured epithelial cells with AA. Whereas AA stimulated epithelial TGF-β production, as well as degradation of collagen I, collagen IV, and laminin, PPARα overexpression blunted these effects. These results suggest that PPARα reduces tubular epithelial cell injury by preventing AA-mediated degradation of tubule basement membrane (Fig. 5).

Increased proximal tubule PPARα reduces infiltration of kidney tissue macrophages. Single cell preparations from whole kidney were initially analyzed by flow cytometry for CD45+, 7-AAD-negative cells to identify the total live leukocyte population. A significant increase in the total CD45+ population indicated a major influx of leukocytes into kidneys was seen as early as 48 h but persistent accumulation was seen after 5 days of UUO in wild-type mice (Fig. 6, A–B), whereas the sham-operated mice did not show similar perturbation. These results were confirmed by Image StreamX flow cytometry (data not included). After ascertaining that the mononuclear phagocytes were one of the major infiltrating cells in the UUO kidney, we investigated the role of PPARα-mediated protection by flow cytometry analysis of inflammatory mononuclear cells. CD45+/CD11b+/F4/80+ cells were considered proinflammatory monocyte/macrophages based on previous studies (11). Tg overexpression of PPARα in proximal tubules markedly blunted this response such that leukocyte recruitment was reduced by more than 50% as shown in Fig. 6, A and B.

PPARα increases IL-10 expression and suppresses renal UUO-induced proinflammatory cytokines/chemokines IL-1β, IL-6, TNF-α, MCP-1, and MIP-1α. To investigate the mechanisms by which increased expression of proximal tubule PPARα ameliorated UUO-mediated renal fibrosis, mRNA expression levels of pro- and anti-inflammation cytokines/chemokines were investigated. As shown in Fig. 7, there were 5.2-, 9.7-, 46.9-, 21.1-, 10.6-, and 8.2-fold increases of mRNA expression for IL-1β, TNF-α, IL-6, IL-10, MCP-1, and MIP-1α, respectively (P < 0.001), 5 days after UUO surgery in wild-type mice when compared with sham-operated mice. Overexpression of PPARα in Tg mice significantly attenuated the UUO-induced upregulation of proinflammation cytokines/chemokines IL-1β, IL-6, TNF-α, MCP-1, and MIP-1α mRNA expression when compared with wild-type mice (P < 0.05). Anti-inflammatory cytokine IL-10 was further increased (7-fold) in Tg mice 5 days after UUO surgery compared with wild-type sham mice and was also significantly elevated (P < 0.05) when compared with wild-type UUO mice (Fig. 7B).

PPARα increases expression of arginase-1 and repressed expression of macrophage marker CD86. To study the roles of PPARα on macrophages in UUO-induced renal fibrosis, we examined the mRNA expression of surface markers/receptors of M1/M2 macrophages. As shown in Fig. 7, B and C, there were 9.1- and 3.4-fold increases of mRNA expression for the macrophage markers arginase-1 and CD86, respectively (P < 0.001), 5 days after UUO surgery in wild-type mice when compared with sham mice. Overexpression of PPARα in Tg mice significantly suppressed the UUO-induced upregulation of CD86 mRNA expression when compared with wild-type mice (P < 0.05). In contrast, arginase-1 was further increased (22.5-fold) in Tg mice 5 days after UUO surgery when compared with wild-type sham mice, but arginase-1 levels were further increased when compared with wild-type UUO mice (P < 0.05).

Fig. 5. PPARα inhibited AA-stimulated TGF-β1, Col1A1, Col4A1, and laminin-β mRNA expression in TKPTS cells. Levels of mRNA were determined by qPCR. TKPTS cells were incubated with PPARα adenovirus for 18 h before being treated with AA and grown for an additional 24 h. *P < 0.05 when comparing untreated cells (control) vs. AA-treated cells. †P < 0.05 when comparing AA-treated cells vs. cells treated with AA in the presence of PPARα (AA+PPARα) in unpaired Student’s t-test.

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DISCUSSION

Our study is the first to evaluate the role of and mechanisms by which increased expression of proximal tubule PPARα attenuates renal fibrosis. Several important findings are supported by our data. Our studies confirm previous observations demonstrating increased expression of TGF-β in cortical proximal tubules (PCT and PST) of UUO-treated wild-type mice, although there was some level of expression of TGF-β in the thick ascending limb, collecting duct, and also in the interstitial compartment of the obstructed kidney (10). The observed peritubular fibrosis present after 5 days of UUO in wild-type mice was almost absent in the kidney tissue of PPARα Tg mice. Importantly, PPARα Tg mice also produced less proximal tubule TGF-β, which was demonstrated by reduced mRNA, protein, and reduced immunostaining in the basolateral domain of proximal tubules. TGF-β is a pleiotropic cytokine that plays a major role in stimulating extracellular matrix production after UUO, and blocking TGF-β has been shown to reduce tubular apoptosis in several models of chronic kidney injury (25, 30). It contributes to renal fibrosis by several mechanisms including induced loss of phosphatase tensin homolog that contributes to the failure of regenerating epithelial cells to redifferentiate, thereby causing the retention of proliferative signaling and giving rise to profibrotic peptides (19). TGF-β also increases renal fibrosis not only by a direct effect on myofibroblasts but by inducing the production of Notch (1), CTGF (34), and PDGF (40). TGF-β also mediates upregulation of osteopontin in injured epithelial cells and triggers a focal inflammatory process with migration of monocytes and macrophages (28). We find that the UUO-mediated increased expression of fibrogenic gene transcripts including Col1A1, α-SMA, and fibronectin in wild-type mice was significantly reduced in PPAR-α Tg mice subjected to UUO. These results indicate that PPARα overexpression in the proximal tubule alone effectively reduces interstitial fibrosis in the model of UUO.

Similar to the in vivo model of UUO, in the in vitro model of fibrosis using AA to injure proximal tubule epithelial cells in

Fig. 6. Reduced infiltration of inflammatory mononuclear phagocytes after UUO in PPARα Tg mice. Kidney cell suspensions obtained from mice subjected to 4 experimental conditions were analyzed by flow cytometry as described in METHODS. Inflammatory macrophages were identified as F4/80+/CD11b+ cells. Comparisons were made between WT and PPARα Tg mice. A: flow histogram of inflammatory macrophages identified as F4/80+/CD11b+ cells. B: quantification of macrophage accumulation in kidney tissue that was significantly reduced in PPARα Tg mice subjected to UUO when compared with WT mice. Data represent means ± SE of 3 independent experiments. *P < 0.05 when comparing WT sham vs. WT UUO mice. †P < 0.05 when comparing WT UUO vs. Tg UUO mice using unpaired Student’s t-test.
culture, increased expression of PPARα directly reduces production of TGF-β and prevented AA-mediated degradation of collagen 4 and laminin, the major components of tubular basement membrane. In addition to injured tubule epithelium, TGF-β can be produced also by interstitial kidney fibroblasts, macrophages, and endothelium (12). Our findings as well as recently published data (3, 43) support the concept that, during injury, the induction of tubulointerstitial fibrosis occurs as a result of altered cross talk mechanisms between tubular epithelial cells and interstitial fibroblasts. We demonstrated in AKI models (23) that proximal tubule PPARα could increase mitochondrial FAO and reduce oxidant production, and we speculate that these cellular mechanisms could affect the production of TGF-β or its effect on transcription of fibrotic genes. Our in vivo studies do not allow us to examine the cellular mechanisms by which PPARα reduces TGF-β expression; however, a recent study suggests that mitochondrial reactive oxygen species derived from complex III of the mitochondrial electron transport chain are required for TGF-β-mediated transcription of profibrotic genes (18).

Another important observation in the present study is the significant inhibition of UUO-induced miR21 expression in kidney tissue of proximal tubule PPARα Tg mice that is also accompanied by reduced fibrosis. miRNAs are a class of ~20-nucleotide single-strand endogenous RNAs that control the translation of mRNAs by promoting the degradation of target mRNAs or preventing their translation (4, 33). MiRNAs have recently been demonstrated to regulate cell proliferation, differentiation, and apoptosis (29). In a recent study, we described that PPARα expression and activity measured as FAO in kidney tissue were significantly reduced in models of renal fibrosis. In addition, miR21 expression was significantly elevated in mice subjected to an ischemic fibrosing model or UUO (5). Reducing miR21 expression in kidney tissue by using anti-miR21 oligonucleotides or miR21 knockout mice resulted in epithelial cell protection and less interstitial fibrosis in response to kidney injury (5). Moreover, using an array assay we demonstrated that PPARα and many genes regulating fatty acid metabolism are seed-matched targets for miR21 in the kidney indicating that miR21 directly silences PPARα and the downstream signaling pathways mediated by PPARα. The findings here of reduced expression of miR21 when proximal tubule PPARα was increased in Tg mice lend support to the notion of a feedback circuit that modulates miR21 expression in kidney tissue at the transcriptional level via the PPARα/AP1 signaling cascade (45). The reduced expression of miR21...
during UUO also could be explained by reduced expression of TGF-β in PPARα Tg mice. TGF-β receptor-mediated signaling has been shown to directly regulate miRNA biogenesis by several mechanisms including direct gene transcription of miRNA genes, direct binding of TGF-β receptor-induced SMAD proteins to specific miRNAs, and by stabilization of miRNA processing machinery (7, 14). The net effect of TGF-β signaling is to upregulate miRNA biogenesis.

Our studies also show that increased expression of proximal tubule PPARα during UUO is accompanied by reduced infiltration of macrophages. In addition, kidney tissues of PPARα Tg mice had reduced expression of inflammatory cytokines including MCP-1 and MIP-1α, which help recruit macrophages to kidney tissue during UUO. Macrophages and dendritic cells derived from monocytes are abundant in kidney injury and play an important role in inflammation, tissue repair, and fibrosis. Ablative studies in several models of kidney interstitial disease indicate that macrophages significantly contribute to the development of fibrosis (8, 13, 31, 38). Several mechanisms have been proposed to explain monocyte/macrophage function. One is that the way monocytes are activated upon entering the diseased kidney dictates their differentiation and function. Another is that monocytes exist as functionally discrete subsets. We also find that increased expression of proximal tubule PPARα markedly reduced production of pro-inflammatory cytokines (TNFα, IL-6, and IL-1β) and increased the expression of anti-inflammatory cytokine IL-10. Thus, the increased expression of proximal tubule PPARα seems to affect gene expression of CD11b+/F4/80+ cell subpopulations suggesting that renal inflammation involves at least two phenotypically different monocyte/macrophage subpopulations: CD11b+/F4/80+ monocytes showing M1-type activation and CD11b+/F4/80+ IL-10 and arginase-1 producing M2-type macrophages. In a previous study, we described that the anti-inflammatory effect of PPARα ligand in the cisplatin model of AKI was mediated by inhibition of kidney tissue NF-κB and this could be an additional mechanism for the anti-inflammatory effect of PPARα on renal fibrosis (22). Our results are different from the ones published by a group of investigators that showed that using a PPARα agonist.

In summary, we describe several mechanisms in this study by which increased expression of proximal tubule PPARα reduces inflammation and interstitial fibrosis including reduced production of TGF-β, reduced expression of miR21, and reduced influx of inflammatory macrophages. Together, these mechanisms contribute to reduced extracellular matrix production. Additional studies are needed to further examine the mechanisms by which PPARα affects production of TGF-β in renal epithelium, inflammatory cells, and/or pericytes, as well as the effects of increased expression of PPARα on UUO-mediated macrophage activation, production of IL-10, and the activation of pericytes to myofibroblasts.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
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