Mechanism and Treatment of Renal Fibrosis

SREBP inhibition ameliorates renal injury after unilateral ureteral obstruction

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Mustafa M, Wang TN, Chen X, Gao B, Krepinsky JC. SREBP inhibition ameliorates renal injury after unilateral ureteral obstruction. Am J Physiol Renal Physiol 311: F614–F625, 2016. First published July 6, 2016; doi:10.1152/ajprenal.00140.2016.—Tubulointerstitial fibrosis is a major feature associated with declining kidney function in chronic kidney disease of diverse etiology. No effective means as yet exists to prevent the progression of fibrosis. We have shown that the transcription factor sterol-regulatory element-binding protein 1 (SREBP-1) is an important mediator of the profibrotic response to transforming growth factor-β (TGF-β) and angiotensin II, both key cytokines in the fibrotic process. Here, we examined the role of SREBP in renal interstitial fibrosis in the unilateral ureteral obstruction (UUO) model. The two isoforms of SREBP (-1 and -2) were activated by 3 days after UUO, with SREBP-1 showing a more sustained activation to 21 days. We then examined whether SREBP1/2 inhibition with the small-molecule inhibitor fatostatin could attenuate fibrosis after 14 days of UUO. SREBP activation was confirmed to be inhibited by fatostatin. Treatment decreased interstitial fibrosis, TGF-β signaling, and upregulation of α-smooth muscle actin (SMA), a marker of fibroblast activation. Fatostatin also attenuated inflammatory cell infiltrate and apoptosis. Associated with this, fatostatin preserved proximal tubular mass. The significant increase in atubular glomeruli observed after UUO, known to correlate with irreversible renal functional decline, was also decreased by treatment. In cultured primary fibroblasts, TGF-β1 induced the activation of SREBP-1 and -2. Fatostatin blocked TGF-β1-induced α-SMA and matrix protein upregulation. The inhibition of SREBP is thus a potential novel therapeutic target in the treatment of fibrosis in chronic kidney disease.

unilateral ureteral obstruction; interstitial fibrosis; sterol regulatory element-binding protein (SREBP); transforming growth factor-β (TGF-β); inflammation

Chronic kidney disease (CKD) is estimated to affect ~11% of the population in developed countries (17). Kidney fibrosis is the hallmark of almost all forms of progressive kidney disease. While interruption of renin-angiotensin II signaling is currently our most effective treatment for CKD, particularly in the presence of proteinuria, disease progression generally occurs. There is thus a need to identify new strategies to prevent the progression of kidney fibrosis.

Sterol-regulatory element-binding proteins (SREBPs) are transcription factors most well known for their regulation of lipid homeostasis, but data have recently shown an additional important direct role in matrix regulation. Renal overexpression of active SREBP-1a or -1c induced glomerular sclerosis with upregulation of transforming growth factor-β (TGF-β), fibronectin, and collagen (16, 38). In glomerular mesangial cells, we have shown that high glucose, TGF-β1, and angiotensin II activate SREBP-1 and mediate their profibrotic response (4, 41, 44). In vivo, SREBP inhibition with the small-molecule inhibitor fatostatin reduced glomerular fibrosis induced by angiotensin II infusion (44). While these data suggest an important role for SREBP in glomerular fibrosis, the progression of renal insufficiency to end-stage renal disease is most closely correlated with interstitial fibrosis (19). Interestingly, SREBP-1 activation was recently observed in tubular epithelium of angiotensin II-infused rats (35). We thus postulated that SREBPs are key mediators of CKD progression through their contribution to interstitial fibrosis.

SREBP resides in the endoplasmic reticulum (ER) membrane in its inactive precursor form. In response to low intracellular cholesterol levels, SREBP cleavage-activating protein escorts SREBP to the Golgi for sequential cleavage by proteases to release the N-terminal mature transcription factor, which then translocates to the nucleus as a dimer and binds promoters of target genes (1, 8). Non-sterol-mediated SREBP activation, such as by shear stress, glucose, TGF-β1, and angiotensin II, was shown to proceed by a similar process (4, 27, 41, 44). There are three isoforms of SREBP: SREBP-1a/1c, generated from alternate transcription start sites, and SREBP-2 (8). Thus far, only SREBP-1 has been implicated in glomerular fibrosis. SREBP-2 has not as yet been assessed. However, SREBP-2 was shown to mediate renal proximal tubular epithelial cell (PTEC) apoptosis in response to ER stress or cyclosporin (25), and apoptosis is an important contributor to the development of fibrosis (40). Whether SREBP-1 and -2 are implicated in the development of interstitial fibrosis is as yet unknown and addressed by these studies.

The unilateral ureteral obstruction (UO) model is a well-established CKD model marked by significant tubular injury and tubulointerstitial fibrosis (6, 40). Multiple factors contribute to the development of fibrosis, among which are the SREBP-1 activators TGF-β and angiotensin II (11, 28, 40). Interestingly, increased lipid accumulation has been shown in this model (31), suggesting activation of SREBPs. We thus studied the role of SREBP in the development of fibrosis after UUO using the SREBP-1/2 inhibitor fatostatin. Our data identify the activation of both isoforms and an important role for SREBPs in the development of interstitial fibrosis and loss of proximal tubular cell mass.

Materials and Methods

In vivo studies. Animal studies were carried out in accordance with principles of laboratory animal care and McMaster University and Canadian Council on Animal Care guidelines. Male 8-wk-old C57BL/6 mice (Jackson Laboratory), housed under standard condi-
tions with free access to regular chow and water, underwent left ureteral ligation at the ureteropelvic junction using a posterior approach. Control mice underwent sham surgery in which the ureter was exposed but not ligated. Use of a separate control group, rather than the unligated contralateral kidney as a control, is recommended given the compensatory changes which occur in the contralateral kidney in response to UUO (6). Three days before surgery, treatment with fatostatin (ChemBridge) was initiated at 30 mg·kg⁻¹·day⁻¹ intraperitoneally (ip) in 3.75% DMSO/2/3 and 1/3 solution, with controls given vehicle injections. There were 5 vehicle-treated control mice, 3 fatostatin-treated control mice, 15 vehicle-treated UUO mice, and 7 fatostatin-treated UUO mice. Mice were euthanized 14 days after UUO. A separate group of untreated mice were analyzed after 1, 3, 7, 14, and 21 days of UUO (n = 4/group). At death, mice were perfused with cold saline, and kidneys were harvested for analysis by immunoblotting, mRNA analysis, and immunohistochemistry (IHC).

Imaging. Formalin-fixed sections (4 µm) were stained with Mason’s trichrome (Sigma). The Picrosirius Red stain (Polysciences) was used to assess collagens I and III. For IHC, sections were deparaffinized and heat-induced epitope retrieval was performed. Primary antisera used were SREBP-1 (1:3,100, Abcam), SREBP-2 (1:20, Santa Cruz Biotechnology), fibronectin (1:200, Sigma), α-smooth muscle actin (α-SMA; 1:1,500, Pierce), cleaved caspase-3 (1:200, Cell Signaling), phospho-Smad3 S423/S425 (1:100, Cell Signaling), CD3 (1:200, Dako), and F4/80 (1:200, Abcam). Apoptotic cells were detected using a TACS-XL Blue Label In Situ Apoptosis Detection Kit, based on the terminal transferase-mediated dUTP nick-end labeling (TUNEL) reaction (Trevena). This generates blue staining in cells with DNA fragmentation with Nuclear Fast Red to identify nuclei. Biotinylated Lotus tetragonolobus lectin (Lotus lectin; 1:200, Vector Labs) was used to identify PTEC. Five fields restricted to the subcapsular cortex were imaged at ×40. ImagePro 6.2 was used to measure the proximal tubular area (lectin positive), expressed as a percentage of the total parenchymal area. This fraction reflects the functional proximal tubular mass of the kidney (3). Lectin staining was also used to assess atubular glomeruli

Fig. 1. Sterol-regulatory element-binding protein 1 (SREBP-1) and -2 are increased after unilateral ureteral obstruction (UUO). A and C: kidneys were assessed for SREBP-1 and -2 by immunohistochemistry (IHC) after UUO from 1 to 21 days. Quantification of staining is shown in B and D (*P < 0.05, †P < 0.01, ‡P < 0.001 vs. con). E: immunoblotting confirms an increase in the mature, active SREBP (mSREBP) for both isoforms, which persists to 21 days (d) for SREBP-1. SREBP-2 activation, decreased by 14d, is no longer seen at 21d.
Glomeruli were scored as lectin positive if any degree of staining was present in Bowman’s capsule and negative if no staining was observed. The latter were counted as ATG, represented as the percentage of total glomeruli present in sections. For caspase-3 and TUNEL staining, positive cells were counted in 10 random fields at ×40. For other IHC, positive staining was quantified using ImagePro 6.2 from five different fields at a magnification of ×20.

Quantitative real-time PCR. Total RNA was extracted from snap-frozen kidneys using TRIzol according to the manufacturer’s instructions (Life Technologies). RT was performed using standard methods, and cDNA was analyzed by real-time PCR using a SYBR Green PCR Master Mix kit (Applied Biosystems). Amplification using primers for specific genes (sequences available on request) with 18S as an internal control was measured continuously using an ABI 7500 Sequence Detector (Applied Biosystems). Fold-change over control was calculated using the ΔΔCt method.

Cell culture. Primary rat kidney fibroblasts (Cell Biologics) were cultured in DMEM/F12 supplemented with 0.5% NEAA and 10% fetal calf serum, streptomycin (100 μg/ml), and penicillin (100 U/ml, Invitrogen, Burlington, ON) on collagen I-coated plates. Experiments used cells between passages 4 and 10. Cells were serum deprived for 24 h before treatment. TGF-β1 (10 ng, R&D) was added for 24 h, with fatostatin pretreatment for 4 h at 20 μM.

Fig. 2. Fatostatin inhibits the activation of SREBP-1 and -2 in vivo. Control and 14d UUO mice were treated with fatostatin as described in MATERIALS AND METHODS. A: increased expression of SREBP-1 and -2 was inhibited by fatostatin, as seen by IHC with quantification of staining shown in B and C (*P < 0.01 vs. con, Fato; †P < 0.01 vs. UUO+Fato). D: immunoblotting confirms inhibition of SREBP-1 activation as assessed by appearance of its mature form (mSREBP) by fatostatin, with quantification shown in E and F (*P < 0.05 vs. UUO). Control and fatostatin groups are not shown, as mature active SREBP was not detected by immunoblotting in these groups at the exposures used to detect signal in the UUO group.
Protein analysis. Cells were lysed and protein extracted as published, with the addition of ALLN to the lysis buffer at 25 μg/ml (Calbiochem) (23). Tissue was additionally homogenized in lysis buffer. Lysates were centrifuged at 4°C, 14,000 rpm for 10 min. The supernatant (50 μg) was separated by SDS-PAGE, and Western blotting was performed. Antibodies used were SREBP-1 (1:1,000, Santa Cruz Biotechnology), SREBP-2 (BD Pharmingen), collagen I (1:1,000, Abcam), fibronectin (1:5,000, BD Transduction), phospho-Smad3 (1:1,000, Cell Signaling), α-SMA (1:10,000, Pierce), active caspase 3 (1:1,000, Cell Signaling), F4/80 (1:2,000, Serotec), CD3 (1:2,000, Serotec), vimentin (2 μg/ml, Boster Immunoleader), and tubulin (1:1,000, Sigma).

Statistical analyses. Statistical analyses were performed with SPSS22 for Windows using one-way ANOVA, with Tukey’s honestly significant difference for post hoc analysis for more than two groups and a t-test for analysis of two groups. A P value <0.05 (2-tailed) was considered significant. Values are means ± SE, and the number of repetitions is denoted as n.

**RESULTS**

SREBP-1 and -2 are activated by UUO. Two major SREBP isoforms exist, SREBP-1 and -2, with alternate transcription start sites further generating SREBP-1a and -1c. In most cell lines, 1a is the predominant isoform, while in most animal tissues it is 1c (8). We first sought to determine whether SREBP was activated after UUO and whether both isoforms

Fig. 3. Fatostratin inhibits UUO-induced fibrosis. A: interstitial fibrosis was assessed by IHC using the trichrome stain. Collagens 1 and 3 were visualized with Picrosirius Red, and fibronectin using a specific antibody. B–D: quantification (*P < 0.01 vs. con, Fato; †P < 0.01 vs. UUO+Fato in B and †P < 0.05 vs. UUO+Fato in C and D). E: collagen 1α1 and 3α1 mRNA was assessed by real-time PCR. (⁎P < 0.05 vs. con, Fato; †P < 0.05 vs. UUO+Fato). F: fibronectin mRNA was also assessed (⁎P < 0.01 vs. con, Fato; †P < 0.05 vs. UUO+Fato). G: immunoblotting was used to determine collagen 1 expression, which is quantified in H (⁎P < 0.05 vs. con, Fato; †P < 0.05 vs. UUO+Fato). I: fibronectin was also assessed by immunoblotting, quantified in J (⁎P < 0.05 vs. UUO). Fibronectin was not seen in the control groups at the exposures used to detect signal in the UUO group, and was thus not shown or quantified.
were affected. We thus conducted a time course study to assess SREBP following 1–21 days of UUO. Figure 1 shows an increase in both SREBP-1 and -2 by IHC, seen 3 days after UUO. While SREBP-1 upregulation is more sustained, still seen at 21 days, that of SREBP-2 returns to baseline minimal expression by 21 days.

SREBP exists as an inactive precursor (∼120 kDa) in the ER membrane. The mature form, or active transcription factor, constitutes the N terminus of its precursor (∼68 kDa). This is generated after precursor SREBP is transported to the Golgi and cleaved by proteases. This mature N-terminal form can then translocate to the nucleus as a dimer to bind to target promoters (1, 8). Since the antibodies used for either SREBP-1 or SREBP-2 each recognize the N terminus of the respective protein, IHC cannot distinguish between precursor and active SREBP. We thus used immunoblotting, with which we can assess the size of the protein being detected, to determine whether SREBP is activated by UUO. As shown in Fig. 1E, activation of both SREBP-1 and -2 is induced by UUO.

**SREBP inhibition suppresses UUO-induced fibrosis.** We next assessed the therapeutic effects of the SREBP inhibitor fatostatin. This small-molecule inhibitor blocks the transport of SREBP from the ER to the Golgi, thereby preventing its cleavage to the mature active transcription factor (20). We chose 14 days of UUO since at this time point both isoforms are still activated, and fibrosis is readily detected. Mice were treated for 3 days with fatostatin before UUO. Figure 2 shows that fatostatin inhibited the increase in SREBP-1 and -2 seen by IHC. The activation of SREBP-1 was also inhibited, as detected by immunoblotting for the mature transcription factor. SREBP-2 activation also appeared to be inhibited, but this did not reach statistical significance with the number of mice included in this study (Fig. 2D).

Interstitial fibrosis is readily observed in the UUO model at 14 days (26). To determine whether fatostatin could inhibit fibrosis, we assessed its development using Masson’s trichrome and by evaluating the upregulation of collagen and fibronectin. Figure 3, A–D, shows the development of signifi-

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**Fig. 3.** continued.
significant fibrosis in UUO kidneys, seen by trichrome staining, Picrosirius Red to assess collagens 1 and 3 and by fibronectin IHC. Fatostatin inhibited all of these. The upregulation of collagen 1 and 3 and fibronectin transcripts after UUO were similarly inhibited by fatostatin (Fig. 3, A and B). Inhibition of collagen 1 and fibronectin protein upregulation by UUO was confirmed by immunoblotting (Fig. 3, C and D).

TGF-β1 is a major profibrotic regulator shown to be important in the development of fibrosis after UUO (11, 36). Smad3 is an essential mediator of TGF-β-induced matrix synthesis, with its deletion preventing fibrosis after UUO (36). Figure 4, A and B, shows that Smad3 activation, assessed by IHC for its activated C-terminal phosphorylated form, is significantly increased by UUO, and this is attenuated by fatostatin. Inhibition of Smad3 activation was confirmed by immunoblotting (Fig. 4, C and D). Since baseline phosphorylation of Smad3 was undetectable by immunoblotting, only UUO groups are shown.

Interstitial fibroblasts are a major contributor to the fibrotic process, and their activation is readily assessed by the de novo expression of α-SMA (6, 24). Figure 5, A and B, shows the upregulation of α-SMA by IHC in UUO kidneys. This was inhibited by fatostatin. Upregulation of the α-SMA transcript was also inhibited by fatostatin (Fig. 5C). Immunoblotting confirmed that fatostatin prevented α-SMA protein expression (Fig. 5D and E). Control groups are not shown, as they had undetectable α-SMA at exposures used to detect signal in the UUO groups. The increase in vimentin, a second marker of interstitial fibroblasts, in UUO kidneys was similarly attenuated by fatostatin (Fig. 5, F and G).

Thus fatostatin inhibits UUO-induced SREBP activation, and this is associated with inhibition of TGF-β signaling, fibroblast activation, and the development of interstitial fibrosis.

**SREBP inhibition preserves proximal tubular mass and integrity of the glomerular tubular junction.** UUO is characterized by PTEC injury with subsequent loss of proximal tubular mass and interstitial fibrosis (6, 45). Indeed, proximal tubular mass reduction accounts for the majority of renal parenchymal loss following ureteral ligation (10). To determine whether fatostatin was protective for PTEC, proximal tubules were identified by their positive stain for *Lotus* lectin, which binds to carbohydrates on PTEC and can thereby be used to assess the functional proximal tubular mass of the kidney (3). Figure 6, A and B, shows a significant reduction in PTEC mass by UUO. Fatostatin attenuated this loss, increasing proximal tubular mass by 11.1% (from 37.3 ± 0.11 in the UUO group to 48.4 ± 0.16% in the UUO+Fato group).

*Lotus* lectin-staining cuboidal epithelial cells normally extend from the proximal tubule to line the urinary pole of Bowman’s capsule in mice. These cells are identical to proximal tubular cells and normally found in roughly 80% of adult male mouse glomeruli (9). After UUO, the proximal tubular cells and glomerulotubular junction undergo injury with cell death, marked by a reduction in *Lotus* positivity, resulting in the formation of atubular glomeruli which are sealed off from their atrophic proximal tubular segment (9, 12). The lack of reversibility of this process contributes importantly to a decline in renal function (7). These glomeruli can be detected by the loss of *Lotus* lectin staining. It was shown that lack of *Lotus* lectin staining in glomeruli in a single median sagittal section correlated well with glomerulotubular degeneration as identified by serial sectioning (9). We thus assessed sections for atubular glomeruli. Figure 6C shows a notable reduction in lectin-positive glomeruli after UUO, with significant rescue by fatostatin. Inhibition of SREBP thus protects against tubular...
cell loss and helps to maintain the glomerular tubular connection.

Apoptosis contributes to the loss of PTEC after UUO (10, 40). We used TUNEL to assess for apoptotic cells, and as shown in Fig. 7, A and B, the increased apoptotic cell number seen after UUO was reduced by fatostatin. Activation of caspase 3 by its cleavage is also a reliable indicator of apoptosis. This was significantly increased after UUO, as assessed by both IHC and immunoblotting, and was also reduced by fatostatin (Fig. 7, A, C–E).

UUO-induced inflammation is attenuated by SREBP inhibition. Inflammatory cell infiltration is important to the progression of fibrotic injury, with infiltration of predominantly T lymphocytes and macrophages into the UUO kidney. This contributes to fibrosis through secretion of cytokines and activation of local interstitial fibroblasts (2, 14, 34, 39, 40). To determine whether fatostatin attenuates this inflammatory influx, we assessed for T lymphocytes by detection of the CD3 antigen and for macrophages by detection of the F4/80 antigen. Figure 8 shows the expected infiltration of UUO kidneys by both T lymphocytes and macrophages, with significant reduction in both by fatostatin.

*SREBP inhibition prevents TGF-β1-induced renal fibroblast activation and matrix synthesis. Renal fibroblasts contribute significantly to interstitial fibrosis and can be activated by TGF-β1 secreted by various sources, including PTEC and infiltrating inflammatory cells (6, 24, 32). Since we observed a significant reduction in α-SMA upregulation and fibrosis by fatostatin in vivo, suggesting decreased fibroblast activation, we sought to determine whether this would also occur in vitro. We thus cultured primary renal fibroblasts and first determined whether TGF-β1 would induce activation of SREBP in these cells. Figure 9, A and B, shows that after 24 h, both SREBP-1 and -2 were activated by TGF-β1, as assessed by the appearance of their mature active forms. As expected, activation was blocked by fatostatin pretreatment. We next assessed the effect of fatostatin on the activation and profibrotic response of these cells. Figure 9, C and D, shows that the upregulation of α-SMA and matrix proteins collagen I and fibronectin induced by TGF-β1 was pre-
vented by fatostatin. SREBP is thus a major mediator of fibroblast profibrotic responses.

DISCUSSION

Interstitial fibrosis is an important determinant of a progressive decline in renal function (19), with currently limited therapeutic options. We now show that SREBP inhibition with a small-molecule inhibitor is effective at attenuating the development of interstitial fibrosis in mice with UUO, a well-characterized model of interstitial fibrosis and CKD. The efficacy of SREBP inhibition appears to be mediated through multiple mechanisms, including attenuation of matrix production, at least in part through inhibition of fibroblast activation, attenuation of apoptosis, and inhibition of inflammatory cell infiltration. A protective effect on PTEC integrity was also observed. SREBP inhibition thus provides a potential novel therapy for prevention of renal fibrosis in CKD.

Recent studies have shown that SREBPs have a direct role in matrix regulation. In glomerular mesangial cells, SREBP-1 regulates TGF-β1 transcription through direct binding to its promoter in response to both high glucose and angiotensin II (42, 44). Interestingly, TGF-β1 itself activates SREBP-1, which works cooperatively with Smad3 to regulate TGF-β1 profibrotic transcriptional responses (4). SREBP-1 is thus an important mediator of both TGF-β1 upregulation and signaling in mesangial cells. This effect is supported by in vivo observations showing that renal overexpression of active SREBP-1a or 1c leads to glomerulosclerosis, with elevated TGF-β1, fibronectin, and collagen expression (16, 38). There are also some data to suggest a role for SREBPs in interstitial fibrosis. In models of CKD including diabetic nephropathy, 5/6 nephrectomy, angiotensin II infusion, and the UUO model, increased tubular lipid deposition was observed, suggesting activation of SREBPs (18, 21, 31, 35, 38). Furthermore, two major mediators of fibrosis in the UUO model, angiotensin II and TGF-β, are also strong regulators of SREBP activation (4, 11, 40, 44).

To formally address the question of whether SREBP activation contributes to interstitial fibrosis, we used the well-characterized UUO model in which tubular injury and interstitial fibrosis are the prevailing pathological findings (6, 40). Our data now show the upregulation and activation of both SREBP-1 and -2 in this model, with a more sustained response...
seen with SREBP-1. Fatostatin, which inhibits both isoforms, significantly decreased interstitial fibrosis. TGF-β and its downstream Smad3 signaling is a major mediator of fibrosis in UUO (36). The inhibition of Smad3 activation by fatostatin suggests an important role for SREBP in mediating the effects of TGF-β in cells other than mesangial cells. Indeed, our observation that fatostatin also inhibits the upregulation of α-SMA indicates a potential regulatory effect of SREBPs on the activation of interstitial fibroblasts, the major source of interstitial matrix production (32, 40). Consistent with an inhibitory effect of fatostatin on fibroblast activation, our data in cultured renal fibroblasts showed that TGF-β1 activates both SREBP-1 and -2 isoforms, with fatostatin preventing the TGF-β1-induced upregulation of both α-SMA and matrix proteins. These data support an important role for SREBPs in directly regulating matrix production in varied cell types.

Inflammatory cell infiltration is important in the progression of tubular injury and fibrosis (14, 34). PTEC produce chemokines that recruit inflammatory cells, with an important profibrotic role shown for both macrophages and T lymphocytes through their secretion of a variety of cytokines, including TGF-β (2, 39, 40). Our study shows that SREBP inhibition reduced the influx of both T lymphocytes and macrophages after UUO. Interestingly, SREBP-1a deletion in macrophages protected against LPS endotoxic shock through a blunted proinflammatory response (15), suggesting a direct effect of SREBP on regulating the immune response. Conversely, inflammatory stress increased SREBP-2 activation and lipid accumulation in the kidneys of diabetic mice (30), and SREBP1/2 activation in pulmonary alveolar cells increased lipid deposition and inflammation (33). Thus, whether SREBP activation in UUO is an early event leading to inflammation, or

Fig. 7. Fatostatin attenuates apoptosis after UUO. Apoptosis was assessed by terminal transferase-mediated dUTP nick-end labeling (TUNEL) staining and IHC for caspase 3 using an antibody that detects the cleaved, active form of the enzyme. Arrows point to TUNEL- and caspase 3-positive cells in A. The percentage of total cells staining positive for TUNEL is shown in B (*P < 0.05 vs. Con, Fato; †P < 0.001 vs. UUO+Fato) and for caspase 3 in C (*P < 0.01 vs. Con, Fato; †P < 0.05 vs. UUO+Fato). D: activated, cleaved caspase 3 was assessed by immunoblotting, with quantification shown in E (*P < 0.05 vs. all others).
its activation is a secondary response to inflammatory cell infiltration, remains to be definitively addressed.

UUO is known to cause extensive proximal tubule degeneration, with a 65% reduction in proximal tubular mass at 14 days (10). Fatostatin reduced this loss in PTEC mass. The PTEC response to injury includes expression of a number of chemokines and cytokines, including TGF-β1, which lead to the activation of local fibroblasts and the recruitment of inflammatory cells (19, 22). Indeed, the precise spatial correlation of affected tubular cells and the interstitial response suggests a primary role for injured tubular cells in generating inflammation and fibrosis in their immediate surroundings (19). The prominent role of PTEC in renal fibrosis was supported recently in a genetic model in which diphtheria toxin-mediated injury was targeted most prominently to the proximal tubule, with no morphological change in glomeruli, endothelial cells, and collecting ducts. Proximal tubular injury led to infiltration of T lymphocytes and macrophages and interstitial fibrosis (14). The beneficial effect of fatostatin on maintaining PTEC integrity may thus be an important factor in its attenuation of both fibrosis and inflammation.

Proximal tubular injury may also lead to glomerular degeneration, thereby impairing glomerular function (14, 22). In the UUO model, proximal tubular injury leads to atrophy of the tubuloglomerular junction, resulting in atubular and thus nonfunctional glomeruli (3, 10). This is of significant functional importance given that 75–80% of glomeruli ultimately either atrophy or become atubular in this model (9). Our data show that fatostatin exerted significant beneficial effects on preservation of the glomerular tubular junction, with a 21% reduction

Fig. 8. Fatostatin attenuates inflammation in response to UUO. A: T cell infiltration was assessed by IHC for CD3 and macrophage inflammation by IHC for F4/80. As cells were too numerous to count, quantification was done by assessing the percent positively stained area, shown in B (*P < 0.001 vs. Con, Fato; †P < 0.001 vs. UUO+Fato) and C (*P < 0.001 vs. Con, Fato; †P < 0.05 vs. UUO+Fato). D: immunoblotting for these markers was also done, with quantification for F4/80 shown in E (*P < 0.001) and for CD3 shown in F (*P < 0.01).
in atubular glomeruli. Although not tested in this study, these observations would indicate a potential beneficial effect of fatostatin on preserving renal function after UUO reversal.

The mechanism by which fatostatin preserves proximal tubular mass and integrity of the tubuloglomerular junction has yet to be elucidated. Tubular cell loss has been ascribed to various processes, including apoptosis, necrosis, and autophagy (7, 10, 46). SREBP has been most strongly linked to various processes, including apoptosis, necrosis, and autophagy (7, 10, 46). SREBP has been most strongly linked to further studies will be required to identify whether this process is regulated by SREBPs in UUO.

Our studies have thus identified SREBP activation as a novel mediator of interstitial fibrosis and preservation of proximal tubular integrity through its effects on several pathways promoting injury. Both SREBP-1 and -2 appear to be important but may mediate different aspects of the response. Assessment of their relative contribution and importance would require genetic studies. Future studies will determine whether SREBP inhibition can preserve renal function in this model, and whether treatment after the initiation of injury, to better model the clinical scenario, will be equally effective.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.M., T.N.W., X.C., and B.G. performed experiments; M.M. and J.C.K. analyzed data; M.M., T.N.W., X.C., B.G., and J.C.K. prepared figures; M.M., T.N.W., X.C., B.G., and J.C.K. approved final version of manuscript; J.C.K. provided conception and design of research; J.C.K. interpreted results of experiments; J.C.K. drafted manuscript; J.C.K. edited and revised manuscript.

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