The Orphan Nuclear Receptor SHP Attenuates Renal Fibrosis

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ABSTRACT

The accumulation of extracellular matrix proteins is a common feature of fibrotic kidney diseases. Accumulating evidence suggests that TGF-β and plasminogen activator inhibitor type 1 (PAI-1) promote the development of renal fibrosis by stimulating the generation and inhibiting the removal of matrix proteins. The small heterodimer partner (SHP) represses PAI-1 expression in the liver by inhibiting TGF-β signaling, but whether SHP inhibits renal fibrosis is unknown. Here, unilateral ureteral obstruction (UUO) markedly increased the expression of PAI-1, type I collagen, and fibronectin but decreased SHP gene expression. Moreover, in kidneys of SHP−/− mice, the expression of PAI-1, type I collagen, fibronectin and α-smooth muscle actin (α-SMA) were higher compared with those in kidneys of wild-type mice. In addition, loss of SHP accelerated renal fibrosis after UUO. Adenovirus-mediated overexpression of SHP in cultured rat mesangial cells and renal tubular epithelial cells inhibited TGF-β-stimulated expression of PAI-1, type I collagen, and fibronectin. SHP inhibited TGF-β- and Smad3-stimulated PAI-1 promoter activities as well as TGF-β-stimulated binding of Smad3 to its consensus response element on the PAI-1 promoter. Similarly, in vivo, adenovirus-mediated overexpression of SHP in the kidney inhibited the expression of UUO-induced PAI-1, type I collagen, fibronectin, and α-SMA. In summary, SHP attenuates renal fibrosis in obstructive nephropathy, making its pathway a possible therapeutic target for chronic kidney disease.


The accumulation of extracellular matrix (ECM) proteins is the key feature of chronic fibrotic kidney disease. TGF-β is central to the development of renal fibrosis through its stimulating effect on matrix protein generation and its inhibitory effect on matrix protein removal.1,2 Expression of TGF-β is elevated in multiple forms of experimental and human kidney disease, ranging from diabetic nephropathy and GN to tubulointerstitial nephritis.3–6 Overexpression of TGF-β was reported in experimental GN and the progression of the glomerular disease.7–9 In the postobstructed kidney, TGF-β expression was increased to induce the transcription of genes involved in ECM protein accumulation, including type I collagen and fibronectin.9 In addition, TGF-β stabilizes ECM proteins by stimulating the expression of protease inhibitors, including plasminogen activator inhibitor 1 (PAI-1). Thus, suppression of TGF-β signaling has been included in several therapeutic approaches for preventing renal fibrosis.10,11

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PAI-1 is the main physiologic inhibitor of the tissue and urokinase plasminogen activator and is considered to be the most important inhibitor of fibrinolysis.12 However, plasminogen inhibition does not explain the action of PAI-1 in renal fibrosis.13,14 Recent studies suggest that PAI-1 directly promotes tissue fibrosis by promoting the migration of monocytes/macrophages, trans-differentiated tubular epithelia, and myofibroblasts.15–17 It has been implicated in experimental GN,18 chronic renal transplant rejection,19 and pulmonary fibrosis.20 Transgenic mice overexpressing PAI-1 develop significantly greater pulmonary fibrosis when administered bleomycin, whereas similarly treated PAI-1-deficient mice have substantially less fibrosis compared with wild-type mice.21 PAI-1 deficiency also protects against renal interstitial fibrosis induced by unilateral ureteral obstruction (UUO).16

Orphan nuclear receptor small heterodimer partner (SHP) is an atypical member of the orphan nuclear receptor superfamily because it lacks a conventional DNA-binding domain.22 It is a transcriptional repressor and exerts its regulatory functions through protein-protein interactions with other nuclear hormone receptors and possibly other transcription factors that can inhibit or even reverse their transactivation.23 A recent study has shown that the loss of SHP sensitizes mice to liver injury from obstructive cholestasis.24 Previously, we demonstrated that SHP represses hepatic PAI-1 expression by inhibiting TGF-β signaling through the repression of transactivation by Smad3.25 Moreover, Fiorucci et al. have reported that SHP attenuates liver fibrosis by inhibition of hepatic stellate cells by the farnesoid X receptor.26 These studies suggest that direct targeting of SHP may provide promising prospects for the prevention of fibrotic disease; however, the clinical significance of SHP in fibrotic kidney disease remains to be determined. Here, we examined whether SHP plays an important role in renal fibrosis in the UUO model and whether upregulation of SHP prevents renal fibrosis.

**RESULTS**

**SHP Expression in UUO Kidney Is Downregulated and Loss of SHP Increases PAI-1 Expression, ECM Protein Expression, and Renal Fibrosis after UUO**

We first examined whether the expression levels of SHP in kidney are altered by UUO. SHP expression in the kidney was examined by β-galactosidase staining (Supplemental Figure 1). A drastic increase in TGF-β expression is a key feature of the UUO kidney. Levels of TGF-β mRNA expression in UUO kidneys were increased at day 7 after ureteral ligation compared with sham-operated animals. As expected, the expression of TGF-β target fibrotic genes including PAI-1, type I collagen, and fibronectin were increased in the kidneys of rats with UUO compared with those of control kidneys. Interestingly, SHP mRNA expression was abundant in control kidneys, but its expression was markedly decreased in UUO kidneys (Figure 1).

We next examined whether the loss of SHP influences renal expression of PAI-1, type I collagen, fibronectin, and α-SMA as well as renal fibrosis by using SHP−/− mice. Indeed, in the kidneys of SHP−/− mice, PAI-1, type I collagen, and fibronectin mRNA levels were increased compared with those from the kidneys of wild-type mice (Figure 2, A and B). PAI-1 and α-SMA protein expression in the kidneys of SHP−/− mice was examined by Western blot analysis (Figure 2, C and D). Moreover, loss of SHP accelerated renal fibrosis after UUO. On day 7 after UUO, wild-type mice were characterized by widespread renal tubulointerstitial damage and fibrosis, as evidenced by Sirius red and Masson’s trichrome staining. In comparison with wild-type mice, the SHP−/− mice exhibited more markedly increased tubulointerstitial damage and fibrosis 7 d after UUO. The differences of tubulointerstitial damage and renal fibrosis between wild-type mice and SHP−/− mice were more evident at day 14 after UUO (Figure 2, E through H). Taken together, these data suggest that SHP plays an important role in ECM accumulation in obstructive nephropathy.

**SHP Inhibits TGF-β-Stimulated PAI-1 and ECM Protein Expression**

Next, we examined whether SHP inhibits TGF-β-stimulated fibrotic gene expression in cultured renal cells. As shown in Figure 3A, adenovirus-mediated overexpression of SHP in rat mesangial cells (RMCs) and NRK-52E cells inhibited TGF-β-stimulated PAI-1, type I collagen, and fibronectin mRNA expression in a dose-dependent manner (Figure 3A). Transient transfection showed that SHP inhibited the TGF-β-stimulated expression of RT-PCR results expressed as the mean ± SEM of three independent experiments (n = 9 in each group). β-actin protein levels were analyzed as an internal control. *P < 0.001 and **P < 0.01 compared with control.