Fyn deficiency attenuates renal fibrosis by inhibition of phospho-STAT3

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The hallmark of renal tubulointerstitial fibrosis is the accumulation of myofibroblasts and extracellular matrix proteins. Fyn, a member of the Src family of kinases, has diverse biological functions including regulation of mitogenic signaling and proliferation and integrin-mediated interaction. Src family proteins promote pulmonary fibrosis by augmenting transforming growth factor-β signaling, but their role in renal fibrosis is less understood. We observed upregulation of Fyn in a renal fibrosis model induced by unilateral ureteral obstruction. Upon ureteral obstruction, Fyn-deficient mice exhibited attenuated renal fibrosis relative to wild-type mice. Furthermore, obstruction-induced renal expression of type I collagen, fibronectin, α-smooth muscle actin, and plasminogen activator inhibitor-1 was suppressed. Pharmacologic inhibition of Fyn blocked induction of extracellular matrix proteins in kidney cell lines. Importantly, the attenuation of renal fibrosis by Fyn deficiency was not accompanied by changes in the Smad pathway. Rather, the antifibrotic effect of Fyn deficiency was associated with downregulation of signal transducer and activator of transcription 3 (STAT3). Small, interfering RNA targeting STAT3 in Fyn-deficient cells further suppressed α-smooth muscle actin expression, whereas a STAT3 activator partially restored plasminogen activator inhibitor-1 expression, indicating that STAT3 signaling is critically involved in this process. Thus, Fyn plays an important role in renal fibrosis. Hence, Fyn kinase inhibitors may be therapeutically useful against renal fibrosis.

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Renal tubulointerstitial fibrosis is thought to be the final common outcome of chronic kidney disease.[1] The main pathologic feature of tubulointerstitial fibrosis during chronic kidney disease progression is accumulation of myofibroblasts and extracellular matrix (ECM).[2] Evidence obtained in studies of both experimental and human kidney disease indicates that transforming growth factor-β (TGF-β) acts as a key mediator of tubulointerstitial fibrosis by stimulating production of ECM while inhibiting its degradation.[3,4] It is generally accepted that TGF-β activates downstream Smad signaling to regulate fibrosis-related gene expression.[5] However, emerging evidence suggests that 1 or more non-Smad signaling pathways are also involved in TGF-β–mediated fibrosis.[6]

The Src family kinases are a group of nonreceptor tyrosine kinases that regulate a variety of cell functions including migration, invasion, and growth.[7–9] Src kinases are activated in response to TGF-β stimulation, resulting in myofibroblast differentiation and activation, thereby promoting lung fibrosis.[10] More importantly, via its interaction with epidermal growth factor receptor (EGFR), Src contributes to TGF-β–induced plasminogen activator inhibitor 1 (PAI-1) expression in vascular smooth muscle cells,[11] although it remains unclear whether this is also true in the kidney. Thus, Src family kinases might also be involved in TGF-β–mediated, non-Smad signaling. In addition, Src activates the signal transducer and activator of transcription 3 (STAT3) and Ras/c-Raf mitogen-activated protein kinase pathways in podocytes.[12] Fyn, a member of the Src family originally identified in 1986,[13] has attracted special attention among Src family kinases because it interacts with nephrin in glomeruli, and its disruption causes proteinuria, suggesting that it plays a critical role in renal diseases.[14]

STAT3 mediates multiple cellular functions including cell survival, proliferation,[15–17] and fibrosis.[18] In addition, STAT3 induces expression of multiple genes, including TGF-β.[19,20] Elevated STAT3 activity results in activation of renal interstitial fibroblasts and progression of renal fibrosis.[21,22] STAT3 is activated in interstitial fibroblasts of fibrotic kidneys following unilateral ureteral obstruction (UUO),[23] and STAT3 inhibitors attenuate renal fibrosis, suggesting that they could be therapeutically useful against renal fibrosis.[21] In this report, we show that loss of Fyn in the kidney prevents UUO-induced tubulointerstitial fibrosis and further demonstrate

that this inhibitory action of TGF-β–stimulated ECM accumulation is mediated by a non-Smad signaling pathway, namely, by a reduction in STAT3 phosphorylation.

RESULTS

Expression of Fyn and Lyn is elevated in the kidney in a UUO-induced fibrosis model, and silencing of Fyn alleviates ECM protein expression in cultured renal cells

First, we investigated whether expression of Src family kinases in the kidney is altered by UUO. Expression of Fyn and Lyn was upregulated at the mRNA and protein level in kidneys of UUO mice (Figure 1a and b). As expected, profibrotic factors such as α-smooth muscle actin (α-SMA) and PAI-1 were highly expressed in kidneys of UUO mice, in accordance with Fyn and Lyn protein levels (Figure 1b). In addition, we confirmed that UUO increased phosphorylation of Fyn, as demonstrated by immunohistochemical (IHC) staining and Western blot (Figure 1c and d). Phosphorylation of Lyn was elevated on UUO as well (Supplementary Figure S1).

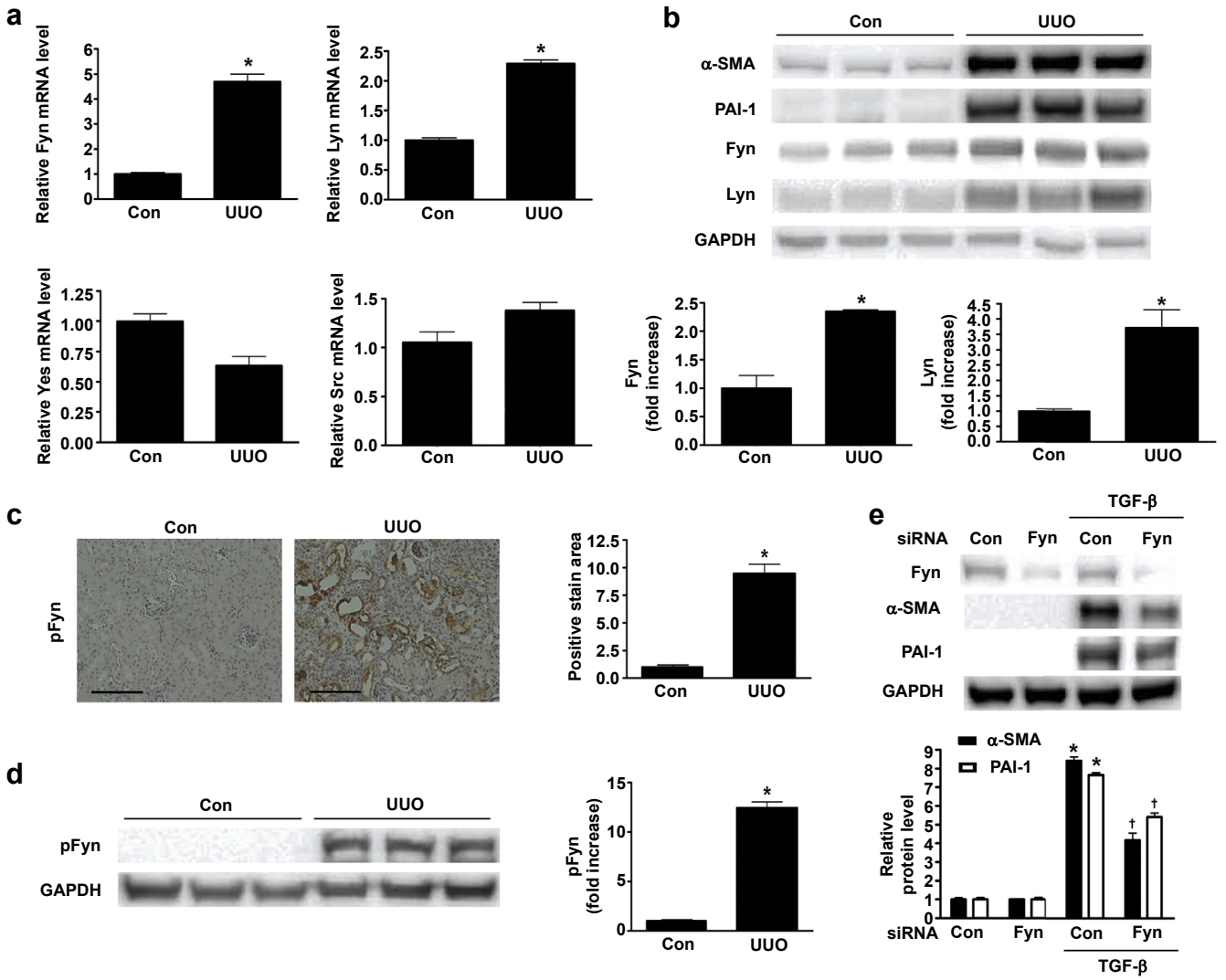

Figure 1 | Fyn is upregulated in UUO kidneys. Representative real-time reverse transcriptase polymerase chain reaction (**a**) and Western blot analysis (**b**) of Src family kinase mRNA (Fyn, Lyn, Yes, and Src) and protein (Fyn and Lyn) expression in the kidney after unilateral ureteral obstruction (UUO). Data in the bar graphs are the mean ± SEM. *$P < 0.05$ compared with control (Con). (**c**) Areas of positive phospho-Fyn (pFyn) immunostaining were quantitated by computer-based morphometric analysis. All morphometric data obtained in UUO kidneys were normalized against the corresponding values in control animals, and the data in all bar graphs are expressed as the fold increase relative to the control. Data are the mean ± SEM of 5 random fields from each kidney. Original magnification ×200. Bars = 100 μm. (**d**) Representative Western blot analysis of pFyn level in the kidney after UUO. Data in the bar graph are the mean ± SEM. *$P < 0.05$ compared with control. (**e**) Western blot analysis showing the effect of small, interfering RNA (siRNA)-Fyn on transforming growth factor-β (TGF-β)–stimulated α-smooth muscle actin (α-SMA) and plasminogen activator inhibitor 1 (PAI-1) protein expression. NRK49F cells were transfected with 100 nM siRNA-Fyn or control siRNA, and then treated with or without TGF-β. Data in the bar graph are the mean ± SEM of 3 independent measurements. *$P < 0.05$ compared with siCon and †$P < 0.05$ compared with TGF-β with control siRNA. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.