

Secreted Frizzled-related Protein 1 (Sfrp1) Regulates the Progression of Renal Fibrosis in a Mouse Model of Obstructive Nephropathy*

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Background: Secreted Frizzled-related protein 1 is a secreted Wnt antagonist.

Results: The kidneys from Sfrp1 knock-out mice showed significant increase in the renal fibrosis after unilateral ureteral obstruction.

Conclusion: Deletion of Sfrp1 makes mice more susceptible to renal damage through non-canonical Wnt/PCP pathway.

Significance: The relationship between kidney damage and Wnt/non-canonical pathway definitely opens a new field to study mechanisms of renal diseases.

Renal fibrosis is responsible for progressive renal diseases that cause chronic renal failure. Sfrp1 (secreted Frizzled-related protein 1) is highly expressed in kidney, although little is known about connection between the protein and renal diseases. Here, we focused on Sfrp1 to investigate its roles in renal fibrosis using a mouse model of unilateral ureteral obstruction (UUO). In wild-type mice, the expression of Sfrp1 protein was markedly increased after UUO. The kidneys from *Sfrp1* knock-out mice showed significant increase in expression of myofibroblast markers, α -smooth muscle actin (α SMA). *Sfrp1* deficiency also increased protein levels of the fibroblast genes, vimentin, and decreased those of the epithelial genes, E-cadherin, indicated that enhanced epithelial-to-mesenchymal transition. There was no difference in the levels of canonical Wnt signaling; rather, the levels of phosphorylated c-Jun and JNK were more increased in the *Sfrp1*^{-/-} obstructed kidney. Moreover, the apoptotic cell population was significantly elevated in the obstructed kidneys from *Sfrp1*^{-/-} mice following UUO but was slightly increased in those from wild-type mice. These results indicate that Sfrp1 is required for inhibition of renal damage through the non-canonical Wnt/PCP pathway.

Renal tubulointerstitial fibrosis is regarded as a final common pathway of progressive chronic kidney diseases (1–4). Fibrotic damage is characterized by the increase of interstitial fibroblasts and myofibroblasts (5, 6). In a recent study, epithelial-to-mesenchymal transition is essential for the development of renal fibrosis, in which tubular epithelial cells are transformed into interstitial fibroblasts and myofibroblasts by activating signaling pathways, including the Wnt and TGF- β pathway (7–11).

Wnt family members secrete glycoproteins that play crucial roles in various cellular functions (12, 13) (see the Wnt Home-

page, Nusse laboratory, Stanford University). Wnt proteins can signal through the canonical (Wnt/ β -catenin) pathway. The Wnt/ β -catenin signaling pathway is a regulator of cellular functions in embryonic development, homeostatic state, and tissue injury (14–16). The Wnt/ β -catenin pathway is also activated in the process of kidney development and renal fibrosis (17–20). Wnt signals can also be transmitted through some additional pathway: non-canonical Wnt pathway, such as planar cell polarity (PCP)² or Ca²⁺ pathway, which is β -catenin-independent (21, 22). The Wnt/PCP pathway transduces its signal by activating c-Jun N-terminal kinase (JNK). The disruption of the Wnt/PCP pathway is detrimental for oriented cell division and apicobasal polarity, but the function of non-canonical Wnt pathway is largely unknown in the process of kidney diseases.

The secreted Frizzled-related protein (Sfrp) is a secreted Wnt antagonist that interacts directly with Wnt ligand (23–28). Of the five Sfrp family members (Sfrp1 to Sfrp5), Sfrp1, Sfrp2, and Sfrp5 comprise Sfrp1 subfamily due to their sequence similarities (28). The phenotypes of single knock-out mice null for each Sfrp1, Sfrp2, or Sfrp5, were viable due to redundancy in their functions. The double or triple Sfrp knock-out mice were resulted in a lethal embryonic phenotype with reduction of anterior-posterior patterning (29, 30). Genetic and biochemical analyses revealed that Sfrp1/2/5 regulated Wnt/ β -catenin and the Wnt/PCP pathways (30–32). Recently, some reports showed that Sfrp1 functioned as a mediator of senescence (33) and dysregulated glucose metabolism (34). However, little is still known about Sfrp1 function in the pathological events.

In the present study, we investigated the Sfrp1 function in the injured kidney. In a model of kidney injury, using the unilateral ureteral obstruction (UUO) model, the expression of Sfrp1 protein was increased. The kidneys from Sfrp1 knock-out mice showed significant increase in the renal fibrosis. Our results suggest that Sfrp1 is required for inhibition of renal tubulointerstitial fibrosis.

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² The abbreviations used are: PCP, planar cell polarity; Sfrp, secreted Frizzled-related protein; UUO, unilateral ureteral obstruction; α SMA, α -smooth muscle actin; DKK-1, Dickkopf-related protein 1.

EXPERIMENTAL PROCEDURES

Mice—*Sfrp1*-deficient mice were maintained 129 and C57BL/6 mixed genetic background (29) and backcrossed for more than five generations onto C57BL/6 background.

Renal fibrosis was induced by ligation of the left ureter in male mice (35). *Sfrp1*^{+/+} and *Sfrp1*^{-/-} mice (*n* = 3 per group) were used at 3, 7, and 14 days after the operation. The obstructed (UUO) and non-obstructed (Sham) kidneys were collected carefully and subjected to the analyses.

Cell Culture and Transfection—293T cells were grown in DMEM supplemented with 10% FBS. Mammalian expression vectors for *Sfrp1*-FLAG was constructed by insertion into pcDNA3 vectors (Invitrogen). Transfection was performed using Lipofectamine reagent (Invitrogen).

Preparation of Recombinant Proteins—His-tagged (for production of mouse *Sfrp1*, *Sfrp2*, and *Sfrp5* antibodies) and MBP-tagged (for immunoblotting) *Sfrp1*–5 were expressed in BL21-CodonPlus-RP (Agilent Technologies, Santa Clara, CA) transformed with pET-28a (Invitrogen) and pMAL (New England Biolabs), respectively. Each His or MBP fusion protein was purified through affinity chromatography with TALON metal affinity resin (Clontech) or with amylose resin (New England Biolabs), respectively.

Antibody—We produced mouse monoclonal *Sfrp1*, rat monoclonal *Sfrp2*, and rat monoclonal *Sfrp5* antibodies, as described previously (36).

The antibodies against the following proteins: vimentin (Progen, Heidelberg, Germany); Ca²⁺/calmodulin-dependent kinase II (EP1829Y), phospho-Smad3 (EP823Y), actinin 4 (EPR2533, Epitomics, Burlingame, CA); E-cadherin (no. 3195), active- β -catenin (no. 8814), Cyclin D1 (DCS6; no. 2926), phospho-JNK (no. 4668), JNK (no. 9252), phospho-Ca²⁺/calmodulin-dependent kinase II (no. 12716), Smad3 (no. 9523) phospho-c-Jun (no. 9261), c-Jun (no. 9165), phospho-p38 (no. 4511), p38 (no. 8960; Cell Signaling Technology); β -catenin (BD Transduction Laboratories), FLAG (M2), α SMA (1A4), phospho-histone H3 (Ser-10; Sigma), c-Myc (sc-764; Santa Cruz Biotechnology, Santa Cruz, CA), and MBP (New England Biolabs).

Tissue Extract Preparation and Immunoblotting—Mouse kidneys were homogenized directly in a SDS-PAGE sample buffer. Protein concentrations for cell extracts were determined by the Coomassie Brilliant Blue staining by SDS-PAGE gels. The lysates were loaded, transferred, and subjected to Western blotting with specific antibodies.

Histology and Immunohistochemistry—Mouse kidneys were fixed with 4% paraformaldehyde/PBS overnight at 4 °C, and embedded in paraffin. Three- μ m-thick sections were prepared and mounted. Some slides were stained with hematoxylin and eosin. For immunohistochemistry, the slides were deparaffinized, and endogenous peroxidase was inactivated in 3% H₂O₂ in methanol for 30min, treated with 10 mM citrate buffer (pH 6.0) in a microwave for 15 min, and blocked in 5% serum in TBST for 1 h. Sections were incubated with primary antibodies overnight at 4 °C and then with appropriate biotinylated secondary antibodies (VECTOR Laboratories, Burlingame, CA) for 1 h at room temperature. The detection was carried out by

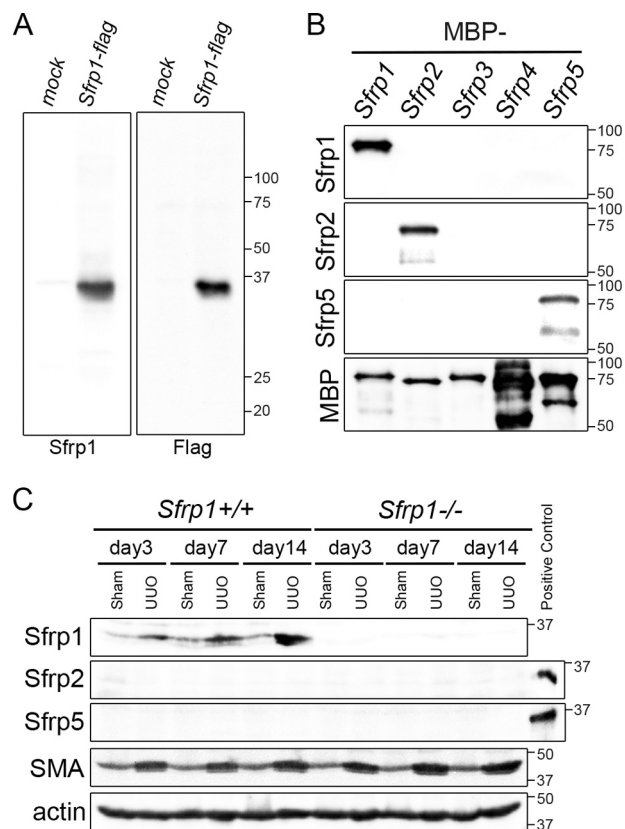


FIGURE 1. *Sfrp1* increases after unilateral ureteral obstruction (UUO). *A*, the *Sfrp1* antibody reacted specifically with a band corresponding to *Sfrp1* in lysates of *Sfrp1*-FLAG-expressed 293T cells, but not 293T (mock) cells by Western blotting. *B*, characterization of an antibody specifically recognized *Sfrp1*, *Sfrp2*, and *Sfrp5*. Immunoreactivity was impaired specifically with *Sfrp1*, but not with other *Sfrp* protein families (*Sfrp2*–5). The *Sfrp2* and *Sfrp5* antibodies were also immunoreacted specifically with *Sfrp2* and *Sfrp5* proteins, respectively. *C*, the levels of the *Sfrp1*, *Sfrp2*, and *Sfrp5* proteins were detected by Western blot analysis in the Sham and UUO kidneys in wild-type mice (*Sfrp1*^{+/+}) or *Sfrp1* knock-out mice (*Sfrp1*^{-/-}) at different time points after UUO. Actin was evaluated as an internal control. His-*Sfrp2* and His-*Sfrp5* were used as a positive control to detect *Sfrp2* and *Sfrp5* proteins, respectively.

using the VECTASTAIN ABC KIT and diaminobenzidine reagent (VECTOR Laboratories).

The areas positive for α SMA, vimentin, E-cadherin, and actinin 4 were quantified by ImageJ software. Statistical significance, which was evaluated using Welch's *t* test, was defined as *p* < 0.05. Error bars indicate S.D.

TUNEL Assay—Apoptosis in the sham and UUO kidneys was assayed using the ApopTag Plus peroxidase kit (Chemicon, Temecula, CA) as described previously (37).

Ethics Statement—All animals were handled in strict accordance with good animal practice as defined by the relevant national and/or local animal welfare bodies, and all animal works were approved appropriately by the Shigei Medical Research Institute.

RESULTS

***Sfrp1* Protein Is Increased in the Obstructed Kidney After UUO**—To investigate the *Sfrp1* function of the kidney disease, we produced monoclonal antibodies that specifically recognize *Sfrp1* protein. Immunoblot analysis revealed that the monoclonal anti-*Sfrp1* antibody immunoreacted specifically with a band

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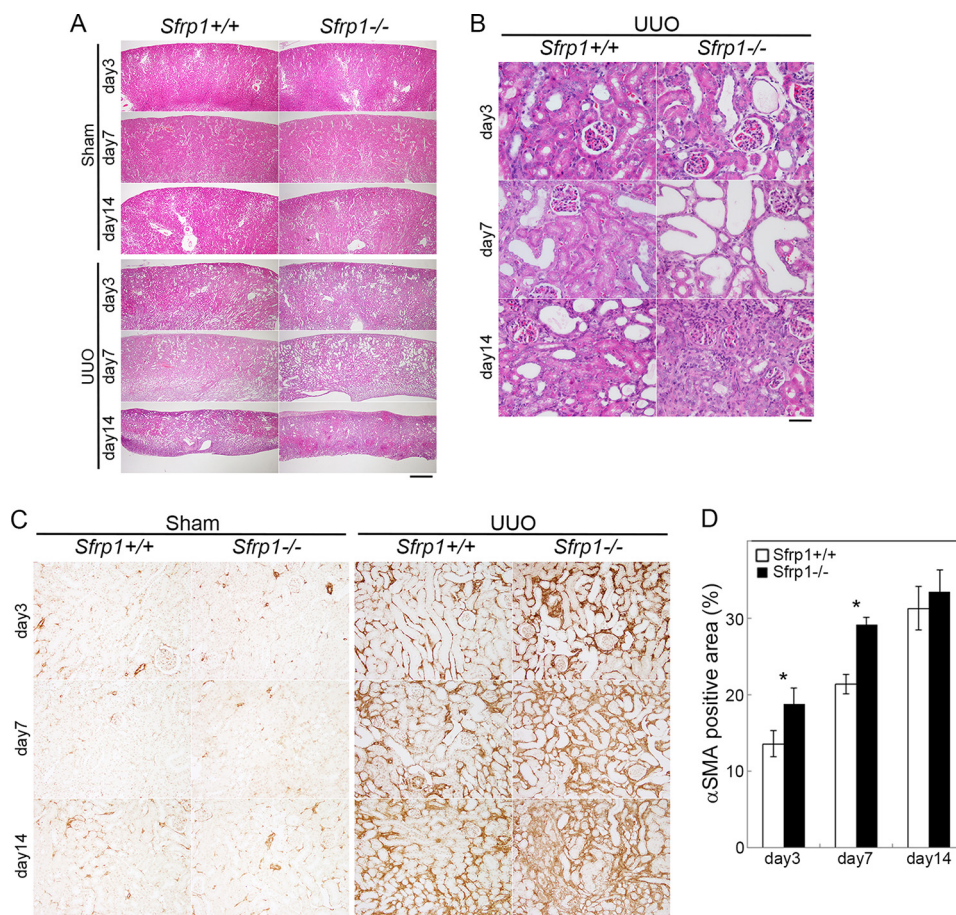


FIGURE 2. Loss of *Sfrp1* exacerbates the progression of renal fibrosis after UUO. *A* and *B*, representative microscopic images of the sham and UUO kidneys in wild-type (*Sfrp1*^{+/+}) and *Sfrp1*-deficient mice (*Sfrp1*^{-/-}). These tissue sections were prepared and stained with hematoxylin and eosin. *B*, higher magnification is shown in *A*. *C*, α SMA immunostaining in the obstructed kidneys. *D*, graph shows analysis of the percentage of α SMA-positive area in the UUO kidneys. Scale bars, 500 μ m (*A*), 50 μ m (*B*), 100 μ m (*C*). *, $p < 0.05$.

corresponding to the position of the similar molecular weights in 293T cell lysates expressing mouse *Sfrp1* (Fig. 1A). To explore the specificity of monoclonal anti-*Sfrp1* antibody, Western blotting was performed using recombinant *Sfrp* proteins (mouse *Sfrp1*–5). As shown in Fig. 1B, this antibody was only reacted with *Sfrp1*, but not with other *Sfrp* proteins.

It is previously reported that *Sfrp1* was highly expressed in the kidney of the newborn (38, 39). To determine whether *Sfrp1* protein was changed after kidney damage, we performed Western blot analyses in the obstructed kidneys of wild-type and *Sfrp1*-deficient mice. After UUO, we found that *Sfrp1* protein was increased at different time points in the obstructed kidneys (Fig. 1C). There were no *Sfrp1* bands detectable in the sham-operated and UUO *Sfrp1*^{-/-} kidney lysates when using the *Sfrp1* antibody (Fig. 1C).

In previous reports, *Sfrp1*, *Sfrp2*, and *Sfrp5* have been suggested on the basis of the similarity of their expression patterns during embryogenesis (29, 30). To examine the extent of functional redundancy between three *Sfrp* members, we investigated *Sfrp1*^{-/-} UUO kidneys with graded levels of *Sfrp2* and *Sfrp5* protein expressions. Deletion of *Sfrp1* did not result in a compensatory increase of the remaining gene product, *Sfrp2* and *Sfrp5* proteins (Fig. 1C).

Loss of Sfrp1 Is Associated with Increased Renal Fibrosis After UUO—In the UUO injury model, *Sfrp1* protein levels were significantly increased in the obstructed kidney. To analyze whether loss of *Sfrp1* exacerbated the progression of fibrosis, UUO in the *Sfrp1* knock-out mice was performed in the obstructed kidneys of *Sfrp1*^{+/+} and *Sfrp1*^{-/-} mice. The kidney sections from sham-operated and UUO mice were stained with hematoxylin and eosin. In the wild-type (*Sfrp1*^{+/+}) UUO-operated kidneys, fibrotic lesion in the cortex was observed after the surgery. In the heterozygous (*Sfrp1*^{+/-}) UUO kidneys, there was no detectable difference of renal fibrosis compared with that of wild-type (data not shown). In contrast, the development of tubulointerstitial injury was progressively deteriorated in the homozygous UUO kidney (Fig. 2, *A* and *B*). Immunohistochemical analyses also revealed that the expression of α SMA, a marker of myofibroblast, was increased in the UUO kidneys (Fig. 2C). The α SMA-positive area was significantly higher in the *Sfrp1*-deficient kidneys compared those with wild-type kidneys at days 3 and 7 after UUO (Fig. 2, *C* and *D*).

To further explore the effects of *Sfrp1* disruption in the obstructed kidney, we examined the expression of vimentin and E-cadherin after UUO. Immunohistochemical analyses showed the expression level of vimentin was increased in the damaged

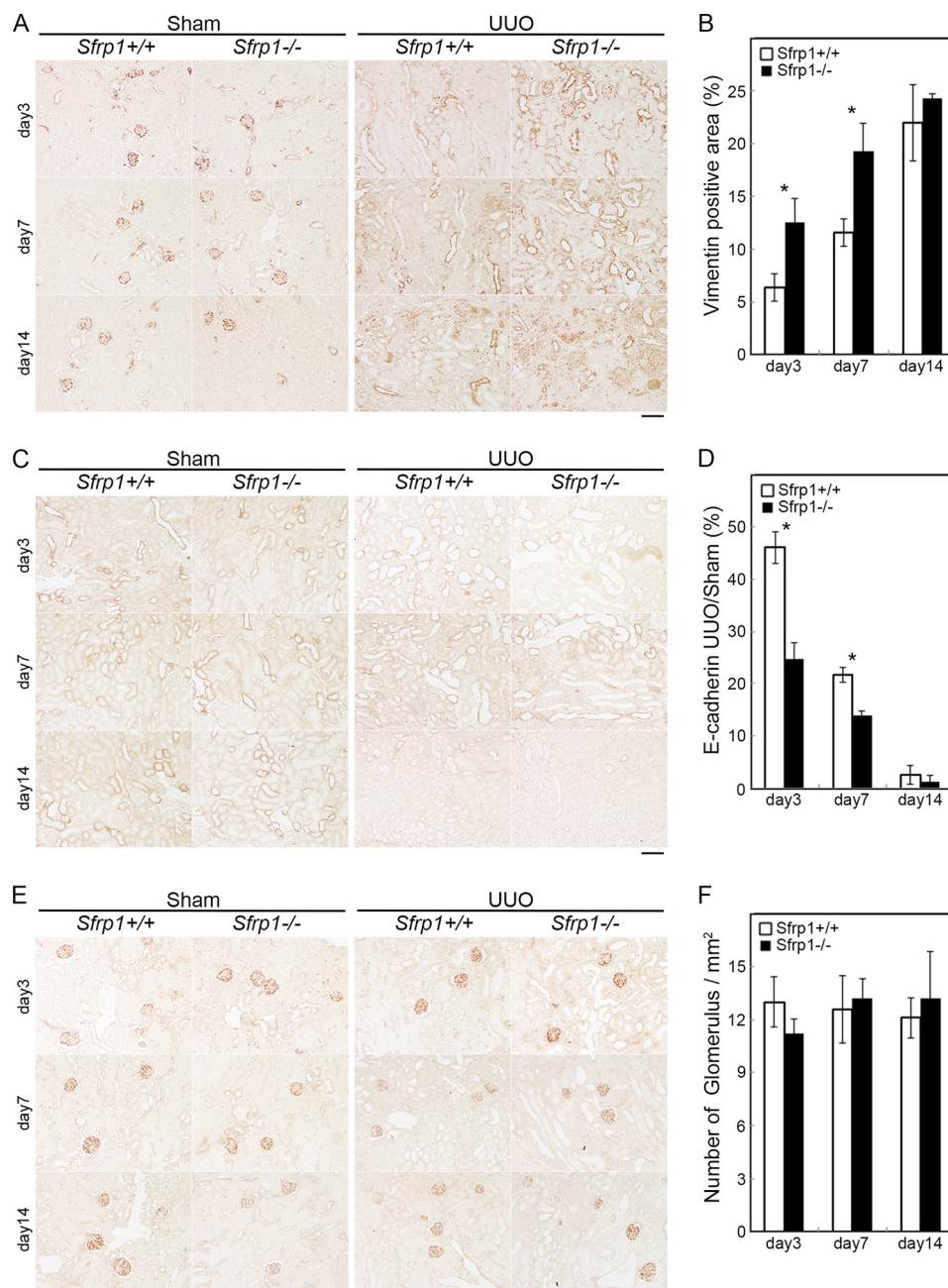


FIGURE 3. *Sfrp1*-deficient mice are enhanced epithelial-to-mesenchymal transition after UUO. *A*, *C*, and *E*, immunohistochemical analyses of the mouse kidney sections with antibodies against vimentin (*A*), E-cadherin (*C*), and actinin 4 (*E*) in the sham and UUO kidneys after UUO. *B*, graph shows analysis of the percentage of vimentin-positive area in the UUO kidneys. *D*, the E-cadherin expression levels are given as a percentage of the UUO to sham-operated kidneys. *F*, renal glomerulus is unchanged in the obstructed *Sfrp1*^{-/-} kidneys by counting actinin 4-positive glomerulus. Scale bars, 100 μ m. *, $p < 0.05$.

kidneys from *Sfrp1*^{-/-} mice when compared with those of *Sfrp1*^{+/+} littermates (Fig. 3, *A* and *B*). In contrast, the expression level of E-cadherin was decreased (Fig. 3, *C* and *D*). Actinin 4, a marker of renal glomerulus, the expression was not changed (Fig. 3, *E* and *F*). These results indicate that *Sfrp1* maintain renal tubular epithelial cells during the fibrosis.

Canonical Wnt/ β -catenin Pathway in the Obstructed *Sfrp1*^{-/-} Kidneys—In the previous study, *Sfrp1* regulated canonical and non-canonical Wnt pathway (30). We studied which Wnt pathway was regulated by *Sfrp1* in the UUO kidney. As described previously (20), the canonical Wnt/ β -catenin pathway was up-regulated after UUO. To investigate whether *Sfrp1* protein dur-

ing kidney damage-modulated canonical Wnt signaling, we performed Western blotting and immunohistochemistry of the UUO kidneys for measuring the amount of active β -catenin that is not phosphorylated on both Ser-37 and Thr-41 (40). The activity levels were not altered in the obstructed *Sfrp1*^{-/-} kidneys in comparison with those in the *Sfrp1*^{+/+} kidneys (Fig. 4, *A* and *B*).

We then observed the protein expression of several target genes of the Wnt/ β -catenin pathway in the obstructed kidney. Western blot analyses showed that expression levels of the target genes, *c-Myc* and *cyclin D1*, were not altered (Fig. 4*A*). Furthermore, we determined whether cell proliferation ratio was

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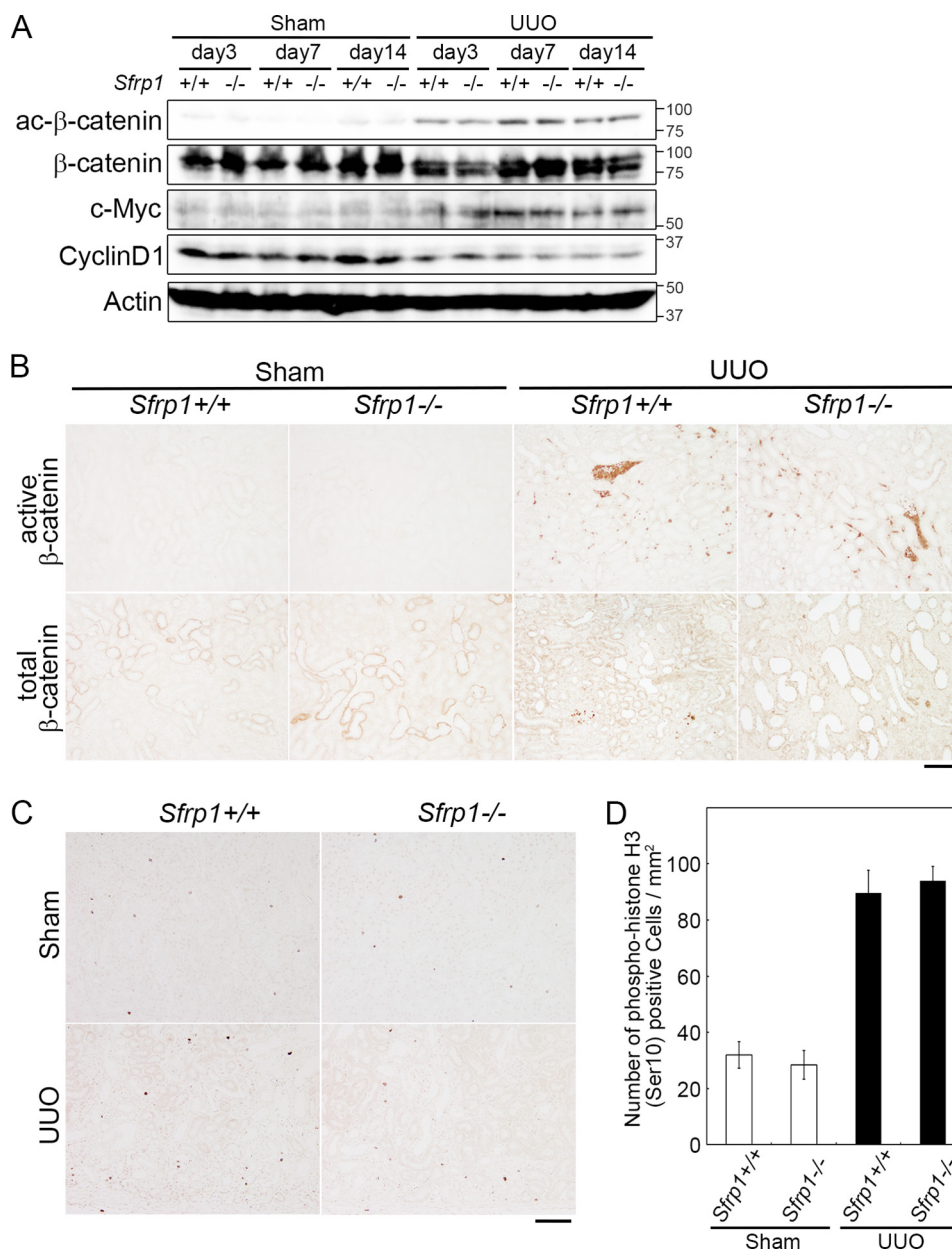


FIGURE 4. Wnt/ β -catenin pathway in the *Sfrp* deficient obstructed kidneys. *A*, detection of active- and total β -catenin, c-Myc, and cyclin D1 by Western blotting of the UUO kidneys. Actin was evaluated as an internal control. *B*, immunohistochemical analyses of mouse kidney sections with antibodies against active and total β -catenin in the sham and UUO kidneys at 7 days after the surgery. *C* and *D*, number of phospho-Histone H3 (Ser-10) cells in the obstructed kidneys at 7 days after UUO. *C*, section of the Sham and UUO kidneys in *Sfrp*^{+/+} and *Sfrp*^{-/-} mice were subjected to immunostaining with an anti-phospho-histone H3 (Ser-10) antibody. *D*, graph shows analysis of the percentage of phospho-histone H3 (Ser-10)-positive cells in the UUO kidneys. Scale bars, 100 μ m.

changed in the *Sfrp1*^{-/-} obstructed kidneys. No difference was immunohistochemically found in cell proliferation by counting phospho-histone H3-positive cells (Fig. 4, *C* and *D*). These data indicate that canonical Wnt/ β -catenin pathway did not alter in the obstructed *Sfrp1*^{-/-} kidneys.

Non-canonical Wnt/PCP Pathway Modulates the Maintenance of Renal Fibrosis—To study whether the non-canonical Wnt pathway was affected in the obstructed kidney of *Sfrp1*-deficient mice, we investigated activation of several putative mediators of the signaling (Fig. 5A). The JNK signaling pathway (Wnt/PCP pathway) is one branch of non-canonical Wnt pathway. We also performed Western blotting and immunohistochemistry of the UUO kidneys for measuring the levels of phos-

pho- and total c-Jun, a downstream of JNK. We found that the levels of phospho- and total c-Jun were increased in the obstructed *Sfrp1*^{-/-} kidneys (Fig. 5, *A* and *B*). In addition, we detected increased phospho-JNK levels by Western blotting (Fig. 5A). Surprisingly, the levels of total JNK were increased in Sham and UUO *Sfrp1*^{-/-} kidneys (Fig. 5A).

Next, the calcium-dependent signaling pathway (Wnt/ Ca^{2+} pathway) is a second branch of the non-canonical Wnt pathway. So, we asked whether the phosphorylation levels of Ca^{2+} /calmodulin-dependent kinase II, which was activated in Wnt/ Ca^{2+} pathway-mediated responses, was regulated. Western blot analyses indicated that this activity was unaltered in the obstructed *Sfrp1*^{-/-} kidneys (Fig. 5A).

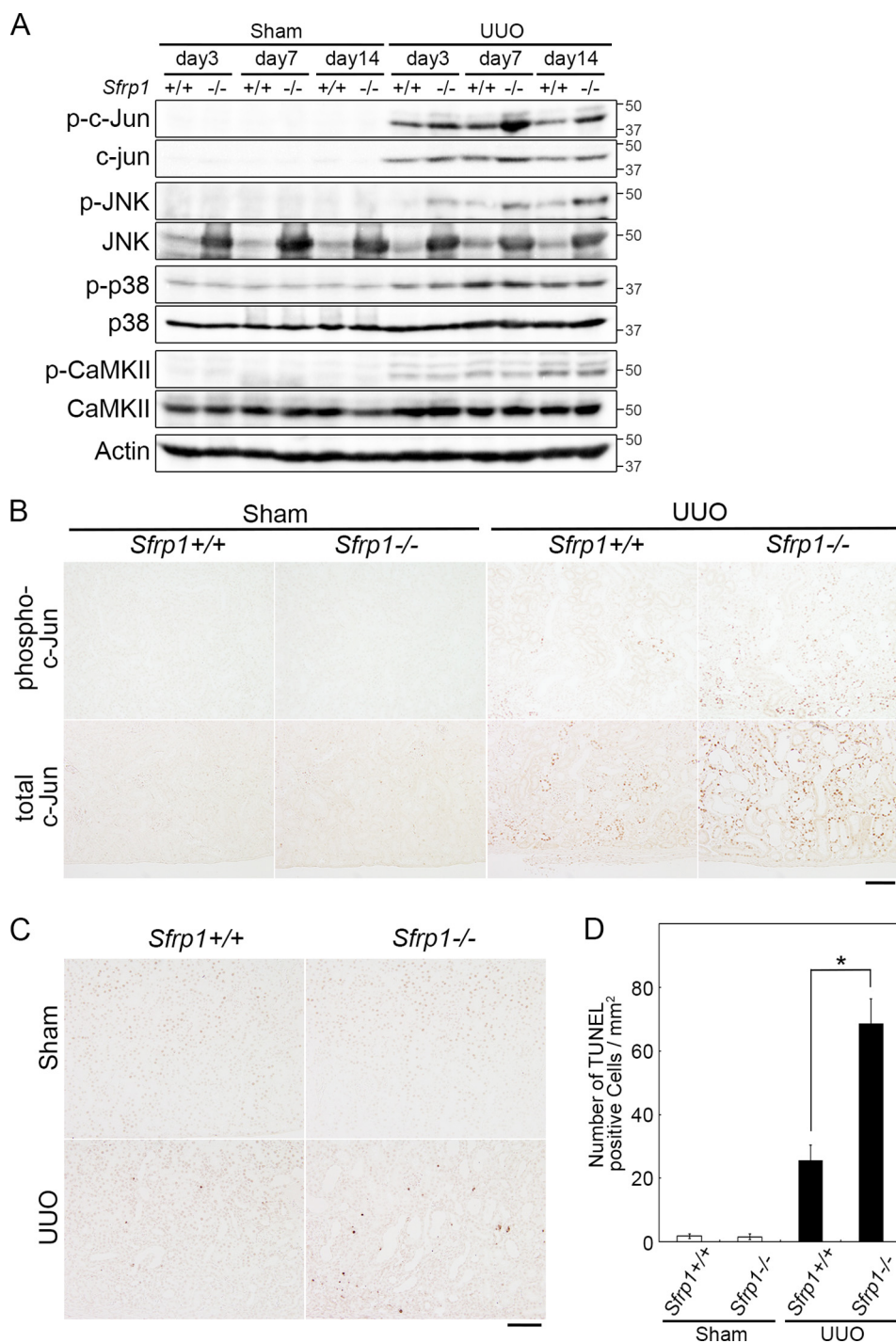


FIGURE 5. Non-canonical Wnt pathway in the *Sfrp1*-deficient obstructed kidneys. *A*, detection of phospho- and total c-Jun, phospho- and total JNK, phospho- and total p38, and phospho- and total Ca^{2+} /calmodulin-dependent kinase II (*CaMKII*) by Western blotting of the UUO kidneys. Actin was evaluated as an internal control. *B*, immunohistochemical analyses of mouse kidney sections with antibodies against phospho- and total c-Jun in the sham and UUO kidneys at 7 days after the surgery. *C* and *D*, number of TUNEL-positive cells in the obstructed kidneys at 7 days after UUO. *C*, section of the sham and UUO kidneys in *Sfrp1*^{+/+} and *Sfrp1*^{-/-} mice were subjected to TUNEL staining. *D*, graph shows analysis of the percentage of TUNEL-positive cells in the UUO kidneys. Scale bars, 100 μ m. *, $p < 0.05$.

The activation of c-Jun was reported to regulate the diverse biological functions, including apoptosis, invasion, and metastasis, and cell polarity as well as non-canonical Wnt signaling. To evaluate the effect of apoptosis, we performed a TUNEL assay in the *Sfrp1*^{-/-} obstructed kidneys. The numbers of TUNEL-positive apoptotic cells increased in the UUO kidneys of *Sfrp1*-deficient mice, compared with that of wild-type mice

(Fig. 5, *C* and *D*). However, the level of phospho-p38, which regulates the apoptosis signal similar to the c-Jun pathway, showed no difference between the wild-type and homozygous mutant (Fig. 5*A*).

Finally, we focused the relationship between Wnt and TGF- β signaling in the *Sfrp1*-mediated UUO kidneys. To explore whether the TGF- β was affected in the obstructed kidney of

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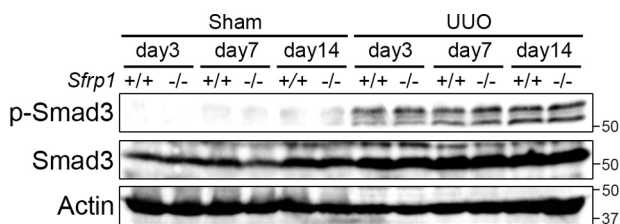


FIGURE 6. TGF- β signaling is not altered in the obstructed *Sfrp1*^{-/-} kidneys. Detection of phospho- and total Smad3 by Western blotting of the UUO kidneys. Actin was evaluated as an internal control.

Sfrp1-deficient mice, we examined Western blotting of the UUO kidneys for measuring the levels of phospho-Smad3, which are the most critical mediators in TGF- β signaling pathway. We found that the phosphorylation levels were not altered in the obstructed *Sfrp1*^{-/-} kidneys in comparison with those in the *Sfrp1*^{+/+} kidneys (Fig. 6). Thus, these observations suggest that the non-canonical Wnt/PCP pathway modulates the maintenance of renal fibrosis after kidney damage.

DISCUSSION

The present study demonstrated that *Sfrp1* protein was increased in the obstructed kidney, and inactivation of *Sfrp1* was associated with increased renal fibrosis during unilateral ureteral obstruction. *Sfrp1* knock-out mice indicated that the renal tubules were disrupted, and the numbers of apoptotic cells were increased after UUO. Furthermore, phosphorylated c-Jun levels were significantly elevated in the obstructed *Sfrp1*^{-/-} kidneys, indicated that *Sfrp1* controlled renal damage through the non-canonical Wnt/PCP pathway.

It is well established that Wnt signaling is silenced in adult tissues (41, 42); however, it is activated in the injured tissues due to the progression of diseases (19, 20). The recent study showed that Wnt4 might be dispensable for myofibroblast transformation in the damaged kidney (18). In addition, Dkk-1 (Dickkopf-related protein 1), a ligand for Wnt antagonist, controls the myofibroblast progression of renal fibrosis (17). These studies indicate that Wnt/ β -catenin signaling is important for the maintenance of kidney damage. Indeed, the amount of active β -catenin increases during progression of renal tubulointerstitial fibrosis (20). Dkk-1 blocks the increase of Wnt/ β -catenin signaling, resulted in the reduction of kidney fibrosis (17). In the previous reports, recombinant *Sfrp4*, a secreted antagonist of Wnt signaling, also reduced the number of myofibroblasts and active β -catenin, indicated that *Sfrp4* was able to interfere with kidney fibrosis through Wnt/ β -catenin signaling (20). In this study, we demonstrated that loss of *Sfrp1* led expansion of renal fibrosis in the obstructed kidney. However, the activity of β -catenin did not alter in the obstructed *Sfrp1*^{-/-} kidneys. Distinct difference in the responses of Wnt/ β -catenin signaling pathway might be due to the following possibilities.

Previous data of Ren *et al.* (17) suggested that Dkk-1 overexpression inhibited cell proliferation in the obstructed kidney after UUO. Notably, Wnt/ β -catenin pathway is implicated in regulating cell proliferation. In contrast, our results indicated that cell proliferation was not altered in the obstructed kidneys between mouse genotypes. Alternatively, the numbers of apoptotic cells were increased in the UUO kidneys of *Sfrp1*-defi-

cient mice. Renal tubular apoptosis causes progressive kidney disease such as fibrosis (43). The p38 mitogen-activated protein kinase (MAPK) cascade and JNK cascade play a critical role in renal fibrosis (44). Upon kidney damage, the levels of JNK and p38 phosphorylation are increased in UUO (43, 44).

In this study, we found that the levels of phosphorylated c-Jun and JNK were increased in the obstructed *Sfrp1*^{-/-} kidneys. This observation is consistent with a recent report that in small intestine of *Sfrp1*^{-/-}; *Sfrp2*^{-/-}; *Sfrp5*^{+/-} mice, phospho-c-Jun levels were elevated significantly in the epithelium in comparison with the control small intestine epithelium (31). Moreover, the Wnt/PCP pathway does not lead to β -catenin stabilization but activates JNK/c-Jun signaling through Frizzled and Dishevelled (31). Thus, it is understandable that *Sfrp1* regulation of Wnt/PCP signaling controls renal interstitial fibrosis among chronic renal diseases. In recent study, Wnt11 signaling by TGF- β was activated in the renal fibrosis through the non-canonical Wnt/PCP pathway (45). We found no evidence that the levels of phospho-Smad3 altered in the obstructed *Sfrp1*^{-/-} kidneys. However, further studies are needed to draw a definite conclusion concerning contribution of Wnt/PCP cascade to kidney pathology.

In conclusion, we identified that *Sfrp1* regulated the progression of renal fibrosis in mouse unilateral ureteral obstruction. The relationship between kidney damage and the Wnt/non-canonical pathway definitely opens a new field to study mechanisms of renal diseases. *Sfrp1* is a reliable candidate for the anti-fibrogenesis drug; however, further studies are needed to understand the underlying molecular mechanism(s).

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