Kindlin-2 regulates renal tubular cell plasticity by activation of Ras and its downstream signaling

Xiaofan Wei,1,2,3 Xiang Wang,1,2,3 Yang Xia,1,2,3 Yan Tang,1,2,3 Feng Li,1,2,3 Weigang Fang,1,4 and Hongquan Zhang1,2,3

1Key Laboratory of Carcinogenesis and Translational Research, Ministry of Education, Peking University Health Science Center, Beijing, China; 2State Key Laboratory of Natural and Biomimetic Drugs, Peking University Health Science Center, Beijing, China; 3Laboratory of Molecular Cell Biology and Tumor Biology, Department of Anatomy, Histology, and Embryology, Peking University Health Science Center, Beijing, China; and 4Department of Pathology, Peking University Health Science Center, Beijing, China

Submitted 12 September 2013; accepted in final form 10 November 2013

Kindlin-2 regulates renal tubular cell plasticity by activation of Ras and its downstream signaling. Am J Physiol Renal Physiol 306: F271–F278, 2014. First published November 13, 2013; doi:10.1152/ajprenal.00499.2013.—Kindlin-2 is an adaptor protein that contributes to renal tubulointerstitial fibrosis (TIF). Epithelial-to-mesenchymal transition (EMT) in tubular epithelial cells was regarded as one of the key events in TIF. To determine whether kindlin-2 is involved in the EMT process, we investigated its regulation of EMT in human kidney tubular epithelial cells (TECs) and explored the underlying mechanism. In this study, we found that overexpression of kindlin-2 suppressed epithelial marker E-cadherin and increased the expression of fibronectin and the myofibroblast marker α-smooth muscle actin (SMA). Kindlin-2 significantly activated ERK1/2 and Akt, and inhibition of ERK1/2 or Akt reversed kindlin-2-induced EMT in human kidney TECs. Mechanistically, kindlin-2 interacted with Ras and son of sevenless (Sos)-1. Furthermore, overexpression of kindlin-2 increased Ras activation through recruiting Sos-1. Treatment with a Ras inhibitor markedly repressed kindlin-2-induced ERK1/2 and Akt activation, leading to restraint of EMT. We further demonstrated that knockdown of kindlin-2 inhibited EGFR-induced Ras-Sos-1 interaction, resulting in reduction of Ras activation and suppression of EMT stimulated by EGF. Importantly, we found that depletion of kindlin-2 significantly inhibited activation of ERK1/2 and Akt signaling in mice with unilateral ureteral obstruction. We conclude that kindlin-2, through activating Ras and the downstream ERK1/2 and Akt signaling pathways, plays an important role in regulating renal tubular EMT and could be a potential therapeutic target for the treatment of fibrotic kidney diseases.

tubular cell plasticity; kindlin-2; Ras signaling

RENAL TUBULOINTERSTITIAL FIBROSIS (TIF) is the final common pathway of all chronic kidney diseases leading to end-stage renal failure (37, 53). The key feature of TIF is the accumulation and deposition of extracellular matrix (ECM), which is thought to be produced mainly by myofibroblasts (7, 31). Accumulating evidence has demonstrated that renal tubular epithelial cells (TECs) are one of the major sources of myofibroblasts (32). It is reported that more than one-third of the matrix-producing fibroblasts are derived from TECs through epithelial-mesenchymal transition (EMT) (23). However, the degree to which EMT contributes to kidney fibrosis remains a matter of intense debate because recent cell lineage tracking experiments do not support that EMT occurs in diseased kidneys in vivo (17, 25). At any rate, it is agreed that renal TECs can undergo EMT in vitro.

EMT is a biological process that allows a polarized epithelial cell to change its cell plasticity that enables it to assume a mesenchymal cell phenotype (24). TECs can be induced by EMT by multiple stimuli such as transformign growth factor (TGF)-β1 and EGF (12, 49). It is reported that small Ras GTPase is crucial for extracellular matrix (ECM) overproduction and is involved in the EMT process induced by TGF-β and EGF (18, 47). Ras proteins function as intracellular switches in signal transduction cascades that play a central role in the regulation of cell proliferation and differentiation. Upon activation, Ras will mainly activate Raf/EKR1/2 and phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathways (50). Previous studies demonstrated that increased activation of Ras and its downstream effectors ERK1/2 and Akt was detected in ligated kidneys (19, 40); moreover, they have been found to mediate certain pathological effects of the molecules involved in renal fibrogenesis (33).

Ras activation is induced by multiple growth factors through binding to several transmembrane receptors (33). It is well established that Ras activation is catalyzed by a specialized group of enzymes known as guanine nucleotide exchange factors (GEFs) (9). Son of sevenless (Sos)-1 is regarded as the major human GEF for Ras (6). Upon stimulation, Sos-1 is recruited to the plasma membrane through interaction with adaptor proteins such as Grb2 and catalyzes GTP/GDP exchange to activate Ras (9, 13).

Kindlins are a group of FERM domain-containing adaptor proteins. The kindlin protein family has recently attracted attention for their ability to bind to and activate integrins. Moreover, they have also been linked to inherited and acquired diseases such as Kindler syndrome and cancer (26, 34). Kindlin-2, a member of the kindlin protein family, is widely expressed and evolutionarily conserved and emerging as an important regulator of integrin-mediated cell-extracellular matrix (ECM) interaction (26, 34). Our previous study has demonstrated that kindlin-2 functions as an adaptor protein in mediating Ras activation by recruitment of Sos-1, resulting in activation of ERK1/2 and Akt signaling, and contributes to TEC EMT. Importantly, knockdown of kindlin-2
inhibits ERK1/2 and Akt activation in the kidneys of unilateral ureteral obstruction (UUO) mice. These findings suggest that kindlin-2 is an important component of the intracellular machinery that regulates Ras signaling in TECs, leading to tubular EMT, shedding new light on the mechanism underlying the pathogenesis of TIF.

MATERIALS AND METHODS

Cell culture and transfection. Human kidney proximal TECs (HKCs) were cultured as described (29). HKCs were grown in DMEM/F12 medium supplemented with 10% FBS (Invitrogen, Carlsbad, CA). The cells were seeded in complete medium. When the cells reached ∼70% confluence, they were serum-starved overnight and then treated with recombinant EGF (R&amp;D Systems, Minneapolis, MN) for various time periods as indicated. For kinase activity inhibition experiments, cells were pretreated with respective inhibitors for 1 h before transfection. After the cells were transfected with different plasmids for 6 h, the cells were refed with fresh growth media. At this time, the inhibitors were added again for another 48 h.

For transient transfection, cells at 50–80% confluence were transfected with indicated plasmids or small interfering (si) RNA using Lipofectamine 2000 according to the manufacturer’s instructions (Invitrogen).

Establishment of stable cell lines. The stable cell line overexpressing kindlin-2 was established as described previously (29). HKCs were transfected with Flag-tagged kindlin-2 expression plasmid using Lipofectamine 2000 according to the manufacturer’s directions (Invitrogen). The empty vector p3XFlag-CMV (Sigma) was used as a mock-transfection control. Twenty-four hours after transfection, the cells were trypsinized and refed with fresh selective medium containing 0.8 mg/ml G418 (Geneticin; Invitrogen). The change to fresh medium occurred every 3 days. Clones were first visible after 7 days and continuously cultured in selective medium for ∼14 days. The cells were then individually transferred into six-well plates for expansion. After two further passages in selective medium, expanded independent clones were cultured in standard medium. Ectopic expression of kindlin-2 in the stable cell lines was confirmed by Western blot analysis.

siRNA inhibition of kindlin-2 and Sos-1. Specific siRNA targeting human kindlin-2 was designed according to the human kindlin-2 cDNA sequence and synthesized by Qiagen (Hamburg, Germany). The sense targeting sequence was as follows (1): AAGCUUGUGGAGAAACUCUG. Sos-1 siRNA was designed and synthesized by RiboBio (RiboBio, Guangzhou, China). The sense targeting sequence was as follows: GCAATAACGCTTTGCATTTA. An irrelevant dsRNA with the sense sequence CGAGUGGCUAGUGGAGAA was used as a control.

Real-time PCR. Total RNA from HKCs or mice kidney tissue was extracted using TriZol (Invitrogen). Two micrograms of RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Promega). Real-time PCR was set up using SYBR Green mix (Applied Biosystems) with the PCR conditions: 95°C 3 min; 90°C 20 s, 60°C 1 min, for 40 cycles. Expression of various genes was determined by the comparative CT method (2ΔΔCT). The sequences of the primer sets were as follows: kindlin-2, TTGCTCCCOCTATCTAAAAGGT (sense), TGAGGGCGCTCCAGAGTTCT (antisense); E-cadherin, CCACCAAAGTCACTCAATACC (sense) and GGAATTTGGGAAAATGTGAGCAA (antisense); fibronectin, GGTGACACCTTGAGGCTCTAAA (sense) and AACATGTAACCCACGTCTCATGTT (antisense); and β-smooth muscle actin (SMa), GCTGCGCCAGACCCGTGT (target sequence) and TTATCAGTGATGCCAGACACT (antisense).

Western blot analysis. Cell or tissue lysates were prepared using PBSTDS lysis buffer containing 1× cocktail inhibitor (Boehringer Mannheim, Mannheim, Germany). Samples were heated at 95°C for 5 min and then separated on SDS-PAGE gels. Transfer membranes were immunoblotted with primary antibodies against kindlin-2 (Millipore, Billerica, MA), E-cadherin (Invitrogen), α-SMA (Abcam, Cambridge, MA), fibronectin (Abcam), p-ERK1/2 (Epitomics), p-Akt (Epitomics), p-Smad3 (Abcam), p-Smad2 (Abcam), Smad2 (Epitomics), Smad3 (Epitomics), Ras (Millipore), Actin (Santa Cruz Biotechnology, Santa Cruz, CA) or Flag (Sigma-Aldrich, St. Louis, MO) overnight at 4°C. After extensive washing in TBS buffer, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody or for 1 h at room temperature. Immobilized antibodies were then detected by enhanced chemiluminescence (Amersham Biosciences, Sunnyvale, CA). Quantification was performed by measurement of the intensity of the bands with the use of National Institutes of Health Image J analysis software.

Immunoprecipitation. Immunoprecipitation (IP) was performed according to the method described previously (54). Lysates were prepared in RIPA buffer (1× PBS, pH 7.4, 0.5% sodium deoxycholate, 1% Triton X-100, 1% SDS) with protease inhibitor cocktail, followed by centrifugation to remove cell debris. Protein complexes were obtained by incubating precleared lysates with indicated antibodies or normal IgG (as controls) overnight at 4°C, respectively. Immunoprecipitated complexes were washed three times with RIPA buffer and separated by SDS-PAGE gels. Transfer membranes were probed with indicated primary antibodies and HRP-conjugated antibody TrueBlot (eBioscience, San Diego, CA) as the secondary antibody (21). The membranes were detected by ECL, as mentioned above.

GST-(Raf)-Ras binding domain pulldown assay. A GST-(Raf)-Ras binding domain (RBD) pulldown assay was performed according to the method described previously (14). The RBD of Raf glutathione S-transferase (GST) fusion protein (GST-Raf-RBD) was coupled with glutathione-Sepharose 4B according to the manufacturer’s instructions and then incubated with HKC lysates for 2 h at 4°C to detect GTP-bound Ras-GTPases as described previously. The amount of bound, activated Ras-GTP in the pulldowns and total Ras in lysates was visualized by immunoblotting with Ras antibodies.

Immunofluorescence staining. Immunofluorescence staining was performed as described previously (28). Briefly, the cells cultured on coverslips were washed with cold PBS twice, fixed in 4% formaldehyde, permeabilized with 0.5% Triton X-100, and stained with the specific primary antibodies against E-cadherin, α-SMA, and fibronectin for 1 h at 4°C, followed by incubation with a secondary antibody conjugated with Alexa Fluor 488 or 588 (Invitrogen). Cells were also stained with 4,6-diamidino-2-phenylindole (DAPI) to visualize the nuclei. Intracellular localization was visualized using confocal microscopy (Leica Microsystems, Wetzlar, Germany).

Animal model and kindlin-2 siRNA treatment. Male ICR mice, weighing 20–25 g, were obtained from and housed in the Beijing University animal facility. The mice were divided into three groups (n = 5 in each group): 1) sham-operated mice receiving control siRNA, 2) UUO mice receiving control siRNA, and 3) UUO mice receiving kindlin-2 siRNA. UUO was performed using an established procedure as described (38, 51). Administration of kindlin-2 siRNA or negative control siRNA were synthesized by RiboBio. The target sequence used for knockdown of kindlin-2 in this study was 5′-AAGTTGTTGAAAACCTGAT-3′.
dilution peroxide (3%) was used to eliminate endogenous peroxidase. Sections were incubated overnight at 4°C with primary antibodies against kindlin-2 (Abcam), p-ERK1/2, and p-Akt. Following extensive washing in PBS buffer, sections were then incubated for 30 min with secondary antibodies (Dako, Carpinteria, CA). Control experiments included omission of the primary antibodies and substitution of the primary antibodies with nonimmune rabbit or mouse IgG. The immunostaining was examined with an Olympus BX51 microscope (Olympus, Tokyo, Japan). Positive stains were quantified using image-analysis software (Image Pro-Plus, Media Cybernetics, Silver Spring, MD).

Statistical analysis. Data are presented as means ± SD. Comparisons between two groups were made using two-tailed Student’s t-tests. Differences among more than two groups were compared using one-way ANOVA. Pairwise comparisons were evaluated by the Student-Newman-Keuls procedure or Dunnett’s T3 procedure when the assumption of equal variances did not hold. A P value <0.05 was considered statistically significant.

RESULTS

Ectopic expression of kindlin-2 induces tubular EMT. To investigate the functionality of kindlin-2 in EMT of HKCs, we established stable cell lines that overexpress kindlin-2 by transfecting with either the expression vector of the Flag-kindlin-2 or an empty vector. Overexpression of the Flag-kindlin-2 was confirmed by Western blotting with anti-kindlin-2 or anti-Flag and QRT-PCR (Fig. 1, A and B). We found overexpression of kindlin-2 significantly suppressed epithelial cell marker E-cadherin expression and upregulated myofibroblast marker α-SMA expression in TECs. In addition, expression of exogenous kindlin-2 induced extracellular matrix component fibronectin expression (Fig. 1, A and B). Furthermore, HKCs stably transfected with kindlin-2 altered their morphology and appeared more elongated (Fig. 1C). Immunofluorescence staining showed that compared with the empty vector controls, overexpression of kindlin-2 resulted in disappearance of E-cadherin in plasma membrane, whereas α-SMA and fibronectin expression was markedly induced in kindlin-2-overexpressing cells (Fig. 1D). These data indicate kindlin-2 may play a role in the development of renal fibrosis through stimulation of the EMT process.

Kindlin-2 induced EMT through activating ERK1/2 and Akt in HKCs. A multiple of signaling pathways were involved in regulating EMT, including TGF-β/Smads, PI3K/Akt, and MAPK signaling (10, 43). To investigate the mechanism of kindlin-2-induced EMT in HKCs, we first examined which signaling pathway kindlin-2 could activate. Activation of TGF-β/Smads, PI3K/Akt, and MAPK signaling in HKCs transfected with Flag-kindlin-2 for 48 h was examined. The results showed overexpression of kindlin-2 significantly promoted phosphorylation of ERK1/2 and Akt, while phosphorylation of p38 and JNK, the other two components of the MAPK family, cannot be activated by kindlin-2. In addition, consistent with our previous study, although kindlin-2 enhanced TGF-β-induced Smad3 phosphorylation, kindlin-2 alone cannot trigger Smad2 and Smad3 phosphorylation (Fig. 2, A–C). Moreover, phosphorylation of ERK1/2, Akt, p38, and JNK was detected in HKCs stably overexpressing kindlin-2. Consistently, phosphorylation of ERK1/2 and Akt increased in kindlin-2-overexpressing HKCs, while kindlin-2 had no effect on phosphorylation of p38 and JNK (Fig. 2, D and E).

To further confirm whether ERK1/2 and Akt were responsible for kindlin-2-induced EMT, specific inhibitors were used to block individual signaling pathways. First, we detected the effect of specific inhibitors on kinase activity. The results showed inhibitors of ERK1/2 (U0126), PI3K (wortmannin), p38 (SB203580), and JNK (SP600125) could significantly inhibited activation of ERK1/2, PI3K, p38, and JNK, respectively (Fig. 3, A and B). Next, we explored the role of the inhibitors in kindlin-2-induced EMT. As shown in Fig. 3, C and D, inhibitors of ERK1/2 and PI3K inhibited kindlin-2-induced upregulation of α-SMA and fibronectin at both the mRNA (Fig. 3C) and protein levels (Fig. 3D). Meanwhile, inhibitors of ERK1/2 and PI3K could restore E-cadherin expression. Other inhibitors of p38 and JNK could not block kindlin-2-induced EMT, suggesting kindlin-2-induced EMT through activation of the ERK1/2 and Akt pathways.

Kindlin-2 increases activation of Ras via recruiting Sos-1. Ras was activated through binding to several transmembrane receptors, resulting in the activation of several downstream signaling pathways including the Ras-ERK1/2 and Ras-Akt pathways (5, 44). Since kindlin-2 could activate ERK1/2 and Akt, we next investigated whether kindlin-2 induced Ras
activation. First, the interaction of kindlin-2 with Ras was examined by co-IP. As shown in Fig. 4A, exogenous kindlin-2 physically bound to Ras in HKCs. Moreover, we found kindlin-2 remarkably increased activation of Ras compared with the control group (Fig. 4B).

Ras activation is dependent on Sos-1. Upon growth factor treatment of cells, Sos-1 is translocated to the plasma membrane and interacts with Ras to activate Ras (13). In view of the adaptor protein character of kindlin-2, we hypothesize that kindlin-2 may recruit Sos-1 to Ras to enhance Ras activation. To this end, co-IP was performed to detect the association of kindlin-2 with Sos-1. The result showed kindlin-2 interacted with Sos-1 (Fig. 4C), and knockdown of Sos-1 could obviously inhibit kindin-2-induced Ras activation (Fig. 4D). These data suggest that kindlin-2 increases activation of Ras via recruiting Sos-1.

Ras inhibitor blocks kindlin-2-induced EMT in HKCs. To further establish the involvement of Ras in kindlin-2-induced EMT in HKCs, we investigated the impact of the inhibition of Ras activation in TECs. For this purpose, farnesylthiosalicylic acid (FTS), an effective Ras antagonist (2, 15), was used in HKCs. We found FTS significantly inhibited kindlin-2-induced ERK1/2 and Akt activation in HKCs (Fig. 5, A and B). Furthermore, FTS restored E-cadherin expression in the kindlin-2-overexpressing TECs. Similarly, FTS inhibited fibronectin and α-SMA expression induced by kindlin-2 at both the mRNA (Fig. 5C) and protein levels (Fig. 5D). These results suggest the role of Ras in kindlin-2-induced EMT in HKCs.

Knockdown of kindlin-2 inhibits EGF-induced Ras activation and EMT. It is well known that EGF induces Ras activation involving a Grb2/Sos-1 signaling protein complex (41). To further confirm that kindlin-2 plays an important role in regulating Ras activation, we examined the effects of loss of kindlin-2 on EGF-induced Ras activation. HKCs were transfected with control siRNA or kindlin-2 siRNA followed by incubation with EGF. EGF significantly induced Ras activation in 30 min, and knockdown of kindlin-2 reduced active Ras (Fig. 6A). To reveal mechanistic insights into kindlin-2 underlying the activation of Ras induced by EGF, the interaction of Sos-1 with Ras was examined in kindlin-2-silencing HKCs. The results showed EGF increased Ras-
The results presented in this study demonstrate that kindlin-2, a FERM domain-containing adaptor protein, plays a critical role in mediating tubular EMT, the mechanism by which kindlin-2 activates Ras via recruiting Sos-1, leading to activation of ERK1/2 and Akt signaling pathway. Inhibition of Ras, ERK1/2, or Akt significantly suppressed kindlin-2-induced EMT in TECs. Importantly, we found that knocking down kindlin-2 in UUO remarkably inhibited ERK1/2 and Akt activation. These results demonstrated for the first time that kindlin-2 mediates ERK1/2 and Akt activation in vivo.

**DISCUSSION**

The previous studies have demonstrated EGF promoted EMT in renal TECs through activation of ERK1/2 (3, 16). Since kindlin-2 is required for EGF-induced Ras activation, we next tested whether kindlin-2 was involved in EGF-induced EMT in HKCs. HKCs were transfected with control siRNA or kindlin-2 siRNA followed by incubation with EGF for 48 h. As shown in Fig. 6, C and D, knockdown of kindlin-2 significantly inhibited EGF-induced upregulation of α-SMA and fibronectin at both the mRNA and protein levels. Similarly, knockdown of kindlin-2 rescued E-cadherin expression. These data indicate an intimate signaling link for EMT via an EGF-kindlin-2-Sos-1-Ras axis.

Depletion of kindlin-2 inhibits activation of ERK1/2 and Akt in UUO mice. It is reported that ERK1/2 and Akt were activated in the kidney of UUO mice (40). Our previous study has demonstrated that depletion of kindlin-2 attenuates TIF in UUO mice (46). To further verify the activation of ERK1/2/Akt induced by kindlin-2, we detected the impact of kindlin-2 depletion on ERK1/2 and Akt activation in vivo. Immunofluorescence study was used to detect the alterations in kindlin-2, p-ERK1/2, and p-Akt in UUO for 7 days. In agreement with our previous study, kindlin-2 expression was detected predominately in TECs and kindlin-2 expression was upregulated in UUO mice (Fig. 7A). In control kidneys, the intensity of p-ERK1/2 and p-Akt in the tubule epithelium was very weak. After UUO, the intensity of p-ERK1/2 and p-Akt was markedly enhanced in the nuclei of TECs, consistent with the previous report. Knocking down kindlin-2 in vivo significantly reduced the renal expression of kindlin-2, p-ERK1/2, and p-Akt in the kidneys with ureteral ligation (Fig. 7A). Western blot analysis also showed depletion of kindlin-2 prevented activation of ERK1/2 and Akt (Fig. 7, B and C). These data suggest kindlin-2 mediates ERK1/2 and Akt activation in vivo.
kindlin-2 played a crucial role in mediating EMT through activation of Ras/ERK1/2 and Ras/Akt signaling in TECs and might be involved in the pathogenesis of TIF.

EMT has emerged as an important pathway leading to generation of matrix-producing fibroblasts and myofibroblasts in renal fibrosis (10). Accumulating studies have shown that activation of Ras and its effectors ERK1/2 and/or Akt may mediate certain pathological effects of the molecules involved in renal fibrogenesis (33). Ras/ERK1/2 activation seems to be a necessary step in the induction of EMT (22, 42, 48). Also, it is reported that ERK1/2 and/or Akt is an important mechanism in EMT induced by various mediators such as TGF-β, EGF, and FGF-2 (27, 48). Our study demonstrated that kindlin-2 induced tubular EMT through Ras/ERK1/2 and Ras/Akt pathways. The conclusion is supported by several lines of evidences. First, stable overexpression of kindlin-2 induced EMT in HKCs, including loss of E-cadherin and upregulation of α-SMA and fibronectin. Second, kindlin-2 effectively activated ERK1/2 and Akt mediated by Ras activation in HKCs. Third, ERK1/2 and Akt inhibitors significantly suppressed kindlin-2-induced EMT in HKCs. Furthermore, Ras inhibitors blocked ERK1/2 and Akt activation, thereby also inhibiting kindlin-2-induced EMT. Last, knockdown of kindlin-2 remarkably inhibited EGF-triggered EMT in HKCs. Taken together, our data demonstrated for the first time that kindlin-2 plays a critical role in tubular EMT through activating Ras and its effectors in renal TECs.

Our interesting finding in the present study is that depletion of kindlin-2 decreased the activation of ERK1/2 and Akt in UUO kidneys. Previous studies have found that ERK1/2 and Akt are activated in an experimental model of tubulointerstitial fibrosis (7 days after the operation). p-ERK1/2, t-ERK1/2, p-Akt, and t-Akt in the ligated kidneys from sham operation, UUO, and kindlin-2 knockdown groups 7 days after the operation. Scale bar = 50 μm. B: Western blot analysis showing the expression of kindlin-2, p-ERK1/2, and p-Akt in the ligated kidneys from sham operation, UUO, and kindlin-2 knockdown groups 7 days after the operation. Scale bar = 50 μm. C: Western blot analysis showing the expression of kindlin-2, p-ERK1/2, and p-Akt in the ligated kidneys from sham operation, UUO, and kindlin-2 knockdown groups 7 days after the operation. Scale bar = 50 μm. D: graphic presentation of the relative expression level of proteins in the ligated kidneys from sham operation, UUO, and kindlin-2 knockdown groups 7 days after the operation.
renal fibrosis induced by UUO (36, 39). Inhibition of ERK1/2 or Akt activation reduced fibroblast activation and ECM production (40). Our previous study found that knockdown of kindlin-2 attenuated TIF in UUO mice by inhibiting Smad3 activation. Combining this with present study, we presumed that kindlin-2 may promote renal fibrosis through activating multiple signaling pathways.

In summary, we demonstrated that kindlin-2 facilitated activation of Ras and its downstream signaling via recruiting Sos-1 and promoted EMT in renal TECs. In view of the role of Ras and its effectors ERK1/2 and Akt in fibrosis, our findings offer novel insights into the mechanism underlying the development of TIF. These studies are also instrumental for designing rational strategies for the treatment of fibrotic kidney diseases.

GRANTS

This work was supported by grants from the Ministry of Science and Technology of China (2013CB910501, 2010CB912203, and 2010CB529402), the National Natural Science Foundation of China (30830048, 81230051, and 31170711), the 111 Project of the Ministry of Education, Beijing Natural Science Foundation (7120002), and a Leading Academic Discipline Project of the Beijing Education Bureau to H. Zhang. This work was also supported by the National Natural Science Foundation of China (81300663 to X. Wei), China Postdoctoral Science Foundation (2013M540028 to X. Wei) and a Postdoctoral Fellowship from the Peking-Tsinghua Center for Life Sciences to X. Wei.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: X. Wei, W.F., and H.Z. provided conception and design of research; X. Wei, X. Wang, Y.X., Y.T., and F.L. performed experiments; X. Wei and X. Wang analyzed data; X. Wei and H.Z. interpreted results of experiments; X. Wei prepared figures; X. Wei drafted manuscript; X. Wei, X. Wang, Y.X., Y.T., F.L., W.F., and H.Z. approved final version of manuscript; H.Z. edited and revised manuscript.

REFERENCES


