Overexpression of Corin Ameliorates Kidney Fibrosis through Inhibition of Wnt/β-Catenin Signaling in Mice

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The Wnt/β-catenin pathway represents a promising therapeutic target for mitigating kidney fibrosis. Corin possesses the homologous ligand binding site (Frizzled—cysteine-rich domain (Fz-CRD)) similar to frizzled proteins, which act as receptors for Wnt. The Fz-CRD has been found in eight different proteins, all of which, except for corin, are known to bind Wnt and regulate its signal transmission. It is hypothesized that corin may inhibit the Wnt/β-catenin signaling pathway and thereby reduce fibrogenesis. Reduced expression of corin along with the increased activity of Wnt/β-catenin signaling was found in unilateral ureteral obstruction (UUO) and ureteral ischemia/reperfusion injury (UIRI) models. In vitro, corin can bind to the Wnt through its Fz-CRDs and inhibit the Wnt function responsible for activating β-catenin. Transforming growth factor-β1 inhibited corin expression, accompanied by activation of β-catenin; conversely, overexpression of corin attenuated the fibrotic effects of transforming growth factor-β1. In vivo, adenovirus-mediated overexpression of corin attenuated the progression of fibrosis, which was potentially associated with the inhibition of Wnt/β-catenin signaling and the down-regulation of its target genes after UUO and UIRI. These results suggest that corin acts as an antagonist that protects the kidney from pathogenic Wnt/β-catenin signaling and from fibrosis following UUO and UIRI. (Am J Pathol 2023, 1–20; https://doi.org/10.1016/j.ajpath.2023.09.008)

With a prevalence reaching 10.5% to 13.1%, the incidence of chronic kidney disease (CKD) is increasing.1 Kidney fibrosis is the inevitable end point of various progressive CKDs, and it eventually leads to end-stage kidney failure.2–4 Despite the high prevalence and poor prognosis of CKD, there is no effective treatment to completely stop or reverse the formation of kidney fibrosis and the progression of CKD.4 Therefore, it is crucial to explore new and effective targets for the therapeutic intervention of kidney fibrosis based on more insight into its pathogenesis. In recent years, significant efforts have been made to identify the unique Wnt/β-catenin target genes associated with kidney injury and fibrosis,5 including fibronectin (an extracellular matrix component),6 fibroblast-specific protein 1 (a marker for fibroblasts and myofibroblasts),7 Snail1,7 matrix metalloproteinase-7 (MMP-7),8 plasminogen activator inhibitor-1 (PAI-1),9 and components of the renin-angiotensin system.10 Moreover, delivery of the Wnt antagonist Dickkopf (DKK) gene reduces β-catenin accumulation and attenuates renal interstitial fibrosis in a mouse model of obstructive nephropathy.9 Similarly, Klotho has been demonstrated to bind multiple Wnts in kidney injury, resulting in a reduction of β-catenin activity and amelioration of interstitial fibrosis.11

The Wnt/β-catenin pathway and its related components therefore represent promising therapeutic targets to attenuate kidney fibrosis and potentially halt CKD progression.12 Wnt ligands induce a spatial interaction between the receptors Frizzled proteins (FZD) and lipoprotein receptor-related

Supported by the National Science Foundation of China grant 82000655 (S.L.); “Yiluqihang and Shenmingyuanyang” Medical Development and Scientific Research Fund project on kidney diseases grant SYY20200301001 (S.L.); and Shanxi Province Health Commission Science Fund grant 2022002 (S.L.). Funding sources did not influence experimental design and analysis/interpretation of results or impact the decision to publish.

X.S. and S.L. contributed equally to this work.

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https://doi.org/10.1016/j.ajpath.2023.09.008
proteins 5 and 6 to initiate β-catenin—dependent signaling. Wnt signal inhibitors can be divided into two categories. One is the confirmed DKK protein family, which has a binding site similar to the ligand Wnt and binds competitively to LPR5/6 in the receptor complex, preventing the activation of the Wnt/β-catenin pathway and reducing kidney interstitial fibrosis. Another group of the regulatory proteins has a homologous ligand binding site and similar amino acid sequence to FZD in the receptor complex, which shares common characteristics of containing the Frizzled—cysteine-rich domain (Fz-CRD) in the molecular structure.

Fz-CRD was found in eight kinds of proteins: FZD, secreted Frizzled-related protein (sFRP), muscle skeletal receptor tyrosine kinase, receptor tyrosine kinase-like orphan receptors 1 and 2, carboxypeptidase Z, collagen type XVIII α 1, membrane Frizzled-related protein, and corin (serine peptidase). With the exception of corin, seven other proteins containing the Fz-CRD structure have all been reported to bind Wnt and affect Wnt signal transduction. Of them, sFRPs 1 to 5 were widely reported to bind to Wnt through the Fz-CRD, thereby blocking the Wnt signaling pathway. Furthermore, exogenous administration of sFRP4 inhibited the Wnt/β-catenin pathway in kidney tubular epithelial cells and thereby slowed the process of interstitial fibrosis in mouse kidney.

Corin has mostly been studied in connection with the heart and is a key enzyme in the natriuretic peptide system. As a type II transmembrane serine protease, corin in structure is consisted of a transmembrane domain near the N-terminus and two Fz-CRDS, eight low-density lipoprotein receptor repeats, a scavenger receptor-like domain, and a tryptasin-like protease domain at the C-terminus, of which Fz-CRDS are mostly associated with Wnt signaling proteins. Corin is also expressed in human kidneys, particularly in the proximal convoluted tubules and the inner medullary collecting ducts. Lower baseline serum corin was associated with higher risk of kidney function decline in patients undergoing coronary angiography. In patients with CKD, reduced kidney corin expression and urine soluble corin levels were reported. Low levels of kidney corin expression were also found in rat models of proteinuric kidney diseases. Studies in rat models of kidney disease and corin knockout mice showed that corin is important for maintaining normal kidney function and sodium homeostasis. These results suggest that local corin expression may play a role in the progression of CKD.

Collectively, it was hypothesized that corin might have a suppressive effect on kidney fibrosis by binding the Wnt ligand based on its Fz-CRDS and consequently disturbing Wnt/β-catenin signaling, similar to sFRP4. In this study, the hypothesis will be systematically investigated, including the regulation of corin in kidney fibrogenesis, its interaction with canonical Wnt signaling pathways, and its role in protecting kidneys from fibrotic injury by using in vitro and in vivo models.

Materials and Methods

Used antibodies in experiments are provided in Table 1, and other detailed materials and sources are listed in Table 2.

Animal Model and Infection of Recombinant Adenovirus

The Ethics Committee for Animals of Shanxi Medical University (Taiyuan, China) approved all animal protocols. Male C57BL/6 mice weighing approximately 22 to 25 g were purchased from the Experimental Animal Center of Shanxi Medical University. Unilateral ureteral obstruction (UUO) surgery was performed, as described previously. Briefly, mice were anesthetized, laparotomy was performed, and the left ureter was identified and ligated with 5.0 silk at two separate points at 7 days in the UUO groups. Kidney ureteral ischemia/reperfusion injury (UIRI) was induced by unilateral clamping of the left kidney pedicle with arterial microclamps for 30 minutes, as described previously. During the ischemic period and recovery, body temperature was controlled between approximately 37°C and 38°C using a temperature-controlled heating system.

Recombinant adenovirus vectors were injected into three to four sites at the lower part of the left kidney cortex using 30-gauge needles immediately after ligation of the left ureter in UUO model or after clamping of the left kidney pedicle in UIRI model. Bioluminescence images of mice were acquired using Xenogen IVIS 200 after 48 hours of UUO/ UIRI and adenovirus infection. At 7 days after UUO, mice were euthanized, and left kidneys were removed and fixed. At 10 days after UIRI, the intact right kidney was removed via a right flank incision. Mice were euthanized at 11 days after UIRI. The surgical procedure was previously described in detail. Serum and kidney tissues were collected for various analyses. The selection of time points in two models is determined by preliminary experiments as well as references from relevant literature.

Cell Culture and Treatment

Human embryonic kidney (HEK-293) cell line, widely employed in protein interaction studies, was selected for its high transfection efficiency. Human kidney proximal tubular epithelial (HKC-8) cells are commonly used in research on tubular epithelial cell metabolism, especially in the fields of kidney fibrosis. HEK-293 and HKC-8 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 mg/mL streptomycin at 37°C in an atmosphere of 5% CO2. HEK-293 cells were treated with recombinant human transforming growth factor (TGF)-β1 at 2 ng/mL for different periods of time, except as otherwise indicated in some experiments. HKC-8 cells were pretreated with TGF-β type I receptor inhibitor (Table 2) at 10 mmol/L, and then were treated with TGF-β1, which refers to the
HEK-293 and HKC-8 cells were transiently transfected with various expression vectors as indicated in different experiments. Whole cell lysates were prepared and analyzed by Western blot analyses.

### Plasmid Construction

Corin cDNAs were transferred to pIRE2-EGFP in frame with or without a C-terminal Flag/myc tag. The following deletion mutants were derived from pIRE2-EGFP:

### Table 1: Used Antibodies in Experiments

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<th>Dilution for WB</th>
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**Table 2: Materials and Sources**

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<tr>
<td>HKC-8 (human kidney proximal tubular epithelial cells)</td>
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**GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IF, immunofluorescence; IHC, immunohistochemical; PAI-1, plasminogen activator inhibitor 1; PCSK6, proprotein convertase subtilisin/kexin-6; PDGFRβ, platelet-derived growth factor receptor β; WB, Western blot analysis.**

Previous literature on the method.11 HEK-293 and HKC-8 cells were transiently transfected with various expression vectors as indicated in different experiments. Whole cell lysates were prepared and analyzed by Western blot analyses.

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corin\textsuperscript{Flag/myc, pIRES2-EGFP-corin\textsuperscript{ΔCRD—Flag} was made by deletion in the two CRDs of amino acids 134 to 259 and 450 to 573. pIRES2-EGFP-corin\textsuperscript{ΔPRO—myc} was constructed by deletion of amino acids 579 to 689. Mouse Wnt1 cDNA was cloned into pcDNA3 together with HA epitope tag at its C-terminus (pcDNA-Wnt1\textsuperscript{HA}). pcDNA-corin and pcDNA—\textbeta-catenin (activated \textbeta-catenin expression vector) were also constructed routinely. All cDNAs were checked by automatic sequencing. All plasmids were purchased from Sangon Biotech (Shanghai, China).

**Generation of Recombinant Adenovirus**

The corin-overexpressing adenovirus (Adeno-corin) and blank adenoviruses (Adeno-blank) expressing green fluorescence protein with a titer of $1 \times 10^{11}$ plaque-forming units were constructed by Sangon Biotech. Briefly, a full-length human cDNA of corin was synthesized and integrated into the vector (CMV-MCS-3FLAG-SV40-EGFP), and then transfected into HEK-293 cell line using Lipofectamine 2000. After amplification and purification, viral preparations were collected and desalted, and titers were determined using Adeno-X Virus Purification Kit, according to the manufacturer’s instructions.

**Cytosolic Free \textbeta-Catenin Analysis with GST—E-Cadherin Binding Assay**

Plasmid pGST—E-cadherin was purchased from Sangon Biotech. The GST—E-cadherin binding assay was performed exactly as previously described.\textsuperscript{16,40} GST—E-cadherin proteins were expressed in bacteria and were purified from the bacterial lysates by incubation for 30 minutes at room temperature with glutathione-Sepharose beads, and HEK-293 cells were transiently transfected with vector control. Wnt1 and corin/corin\textsuperscript{ΔCRD—Flag}/corin\textsuperscript{ΔPRO—myc} were lysed in lysis buffer, and extracts were clarified by centrifugation. For a quantitative analysis, two different amounts of cell extracts, 0.1 or 0.0 mg, were incubated with Sepharose beads bound to the GST—E-cadherin. After 1 hour of incubation at 4°C, the Sepharose beads were collected by centrifugation, washed twice with lysis buffer, which consisted of 0.1% SDS and 0.5% bovine serum albumin, and two times with phosphate-buffered saline, and boiled in SDS sample buffer containing \textbeta-mercaptoethanol. Samples were subjected to SDS-PAGE and Western blot analysis with the anti—\textbeta-catenin antibody.

**TOPflash/FOPflash Luciferase Assay**

The effect of corin on \textbeta-catenin—mediated transcription was determined using the TOPflash/FOPflash Luciferase Assay. TOPFlash Reporter plasmid is designed to evaluate \textbeta-catenin—dependent signaling events that drive the expression of T-cell factor (TCF) in the Wnt signaling pathway and contains two sets of three copies of the TCF binding site. As a negative control, the FOPflash construct contained mutated TCF4 binding sites that cannot be activated by \textbeta-catenin. HKC-8 cells were cotransfected with the reporter plasmid along with Wnt1 expression vector (pcDNA-Wnt1\textsuperscript{HA}) or activated \textbeta-catenin expression vector (pcDNA—\textbeta-catenin) in the absence or presence of corin expression vector (pcDNA-corin) at different concentrations as indicated. The pRL-TK vector that provided the constitutive expression of Renilla luciferase was also cotransfected as an internal control for normalizing the transfection efficiency. All transfections were performed using Lipofectamine 2000 reagent. At 24 hours following transfection, the luciferase assay was performed using a Dual Luciferase Reporter Gene Assay Kit, according to the manufacturer’s instructions. The results are represented as normalized TOPFlash/FOPFlash values.

**Western Blot Analysis**

Western blot analysis was performed following the standard procedure, as previously described. Briefly, kidney tissue or cells\textsuperscript{15} were lysed using radioimmunoprecipitation assay buffer (25 mmol/L Tris-HCl, 150 mmol/L NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, and 0.1% SDS). Samples were resolved by SDS-PAGE, transferred onto the polyvinylidene difluoride membrane, and probed with specific antibodies (Table 1). Anti-rabbit horseradish peroxidase—conjugated secondary antibodies were used for 1 hour at room temperature and followed by chemiluminescence detection. Relative band intensities were quantified by the ImageJ software version 1.53a (NIH, Bethesda, MD; https://imagej.nih.gov/ij/). Glyceraldehyde-3-phosphate dehydrogenase was used as a loading control. The data were normalized to the loading controls to calculate the fold change.

**Coimmunoprecipitation**

Coimmunoprecipitation analysis was performed to determine the interaction of corin and Wnt1 in HEK-293 cells. Cells were cotransfected with Wnt1 plasmid (pcDNA-Wnt1\textsuperscript{HA}) and corin (pcDNA-corin) or its mutants vectors (pIRES2-EGFP-corin\textsuperscript{ΔCRDFlag} or pIRES2-EGFP-corin\textsuperscript{ΔPROmyc}) for 24 hours. Cells were solubilized in buffer after transfection, and extracts were clarified by centrifugation for 20 minutes at 4°C. Total cell lysates were immunoprecipitated overnight at 4°C with anti-HA antibody and protein A/G plus agarose. The precipitated complexes were collected by centrifugation and washed three times. The precipitated proteins were separated by SDS-PAGE and followed by detection with each specific antibody, such as anti-corin, anti-Flag, anti-myc, or glyceraldehyde-3-phosphate dehydrogenase.

**Histology and Immunohistochemical Staining**

For the evaluation of fibrosis, kidney tissue was stained with Masson trichrome and Sirius red in sections (4 \textmu m thick) of
Figure 1  Wnt/β-catenin signaling is activated and corin expression is decreased along with the increased expression of β-catenin in mouse kidney after unilateral ureteral obstruction (UUO). A and B: Western blot analyses of kidney expression of several protein, including Wnt1, α-smooth muscle actin (α-SMA), type I collagen, fibronectin, Snail, and plasminogen activator inhibitor-1 (PAI-1), in sham and obstructed kidneys at day 7 after UUO. A and B: Representative
kidney biopsies, fixed with 10% neutral formalin, and embedded in paraffin, according to the kit’s instructions. Tubular damage score evaluation: Twenty randomly selected cortical tubulointerstitial fields were evaluated in each mouse. Tubular damage included tubular dilation, change in shape of tubular cells from cuboid to flat (1 score); brush border injury (1 score) or loss (2 score); formation of casts (2 score); and death and detachment of tubular cells (1 score). The severity of interstitial renal fibrosis was evaluated by the calculation of the positive area of Aniline blue or Sirius red staining of one slide section. The density of positive signals was analyzed using ImageJ software version 1.53a and expressed as the number of percentage positive area (Sirius red staining area percentage and Aniline blue staining area percentage). The observer was unaware of the origin of the slides.

Immunohistochemical staining was performed using routine protocols, as previously described. Antibodies (Table 1) used were as follows: anti-corin, anti-active β-catenin, anti-fibronectin, anti-α-protein convertase subtilisin/kexin-6 (PCSK6), anti-platelet-derived growth factor receptor β, anti-CD31, and anti-z-smooth muscle actin (z-SMA). The sections were incubated overnight at 4°C with primary antibodies. This was followed by biotinylated secondary antibodies and finally by avidin-conjugated horseradish peroxidase. All slides were counterstained with hematoxylin. Nonimmune normal IgG was used to replace primary antibodies as a negative control, and no staining occurred. Slides were viewed under Olympus DP74 microscope equipped with a digital camera. Staining was quantified in 10 randomly captured fields on each slide, and the data were analyzed using ImageJ software version 1.53a.

Immunofluorescence Assay

HKC-8 monolayers were fixed in methanol (20 minutes, −20°C), and blocked with 2% bovine serum albumin in phosphate-buffered saline (1 hour, room temperature). Cells were then incubated with the antibody against fibronectin, platelet-derived growth factor receptor β, anti-CD31, and z-SMA, followed by incubation with the fluorescent secondary antibody. Cellular nuclei were stained using DAPI (Life Technologies, Carlsbad, CA). Images were obtained using Olympus FV1000 Confocal microscope. Ten fields under ×400 microscope were randomly selected. The percentage of staining positive area in the selected field was analyzed by Image Pro plus 6.0.

Estimation of Blood Urea Nitrogen and Serum Creatinine

Serum creatinine and urea nitrogen levels were measured by use of a Creatinine assay kit and Urea assay kit, according to the protocols specified by the manufacturer.

Echocardiography

Transthoracic echocardiographic analysis was performed on mice to access the cardiac function. Briefly, mice were sedated using 1.5% inhaled isoflurane. A commercially available echocardiography system (Mylab X5 Vet; Esaote, Italy) was used. The hemithorax of each mouse was shaved, and two-dimensional and M-mode images of the left ventricle at the long axis were recorded. Left ventricular end-diastolic dimension and left ventricular end-systolic dimension were measured at least three times and averaged for each mouse. The fractional shortening and ejection fraction (percentages) were calculated.

Statistical Analysis

Results were obtained from three independent experiments. Results are presented as means ± SEM. Statistical analyses were performed using IBM SPSS Statistics 19.0. The t-test was used to determine the significance of differences between two groups. Comparison between groups was made using one-way analysis of variance, followed by Student-Newman-Kuels test. P < 0.05 was considered significant.

Results

Wnt/β-Catenin Signaling Is Activated and Expression of Corin Is Decreased in Mouse Kidney after UUO

The expression and regulation of corin during kidney interstitial fibrosis induced by UUO was first examined. The increased Wnt signaling activity after UUO was detected by measuring the Wnt1 and active β-catenin (non-phosphorylated) levels. As shown in Figure 1, A and B, several widely known Wnt/β-catenin target genes were up-
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Figure 2  The Frizzled—cysteine-rich domains (Fz-CRDs) of corin are required for Wnt1 binding and inhibition of Wnt/b-catenin signaling in vitro. A: Schematic representation of corin and its mutant derivatives. Wild-type corin has a transmembrane domain (TM), two Fz-CRDs, eight low-density lipoprotein receptor repeats (LDLRs), a scavenger receptor domain (SR), and a trypsin-like protease domain (PRO). Corin^ΔCRD-Flag represents the mutant with deletion of two Fz-CRDs. Corin^ΔPRO-Myc represents the mutant with deletion of the PRO. B: Coimmunoprecipitation of corin or corin^ΔCRD-Flag with Wnt1HA. HEK-293 cells were transfected with vector, HA-tagged pcDNA-Wnt1 (pcDNA-Wnt1HA) plus pcDNA-corin, or pcDNA-Wnt1HA plus Flag-tagged pIRES2-EGFP-corin^ΔPRO-Myc (pIRES2-ΔPRO-Myc) CRD (pIRES2-ΔCRD-Flag). The lysates were subjected to immunoprecipitation (IP) with anti-HA, followed by immunoblotting (IB) with anti-cerin or anti-Flag. The corin/Wnt1HA complex was detected (lane 11), whereas the corin^ΔCRD-Flag/Wnt1HA complex was not found (lane 12). C: Corin^ΔPRO-Myc coprecipitated with Wnt1HA. HEK-293 cells were transfected with vector, pcDNA-Wnt1HA plus pcDNA-corin, or pcDNA-Wnt1HA plus myc-tagged pIRES2-EGFP-corin^ΔPRO-Myc (pIRES2-ΔPRO-Myc). The lysates were subjected to IP with anti-HA, followed by immunoblotting with anti-cerin or anti-myc. The complexes of corin/Wnt1HA and corin^ΔPRO-Myc/Wnt1HA were detected with anti-cerin or anti-myc antibodies, respectively (lanes 2 and 3). D: HEK 293 cells were transiently transfected with vector control, pcDNA-Wnt1HA, pcDNA-Wnt1ΔN plus pcDNA-corin, pcDNA-Wnt1HA plus pIRES2-EGFP-corin^ΔCRD-Flag, or pcDNA-Wnt1HA plus pIRES2-EGFP-corin^ΔPRO-Myc in the GST—E-cadherin binding assay. E and F: Corin inhibition of the Wnt1-induced increase in active b-catenin levels was performed using either 0.1 mg (lanes 1, 3, 5, 7, and 9) or 1 mg (lanes 2, 4, 6, 8, and 10) of total cell lysates. E and F: Representative Western blot analysis (E) and quantitative data (F) are presented. G and H: Luciferase activity of the TOPFlash/FOPFlash reporter plasmid in HCK-8 cells. HCK-8 cells were cotransfected with TOPFlash or FOPFlash reporter plasmid and different amounts of corin plasmid (pcDNA-corin) as indicated in the absence or presence of pcDNA-Wnt1HA (G) or pcDNA-b-catenin (H). Luciferase assays were performed 24 hours after transfection using the Dual-Luciferase Reporter Assay System. Firefly luciferase activity was standardized to the Renilla luciferase control. Data are given as means ± SEM (F-H). n = 5 independent experiments (E and F); n = 3 independent experiments (G and H). *P < 0.05, **P < 0.01, and ***P < 0.001. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
Figure 3  
Transforming growth factor (TGF)-β1 represses corin expression, and corin inhibits TGF-β1-induced fibrogenic action based on its Frizzled-cysteine-rich domains in vitro. A–D: Western blot analyses of corin expression under TGF-β1 treatment. A–D: HKC-8 cells were incubated with the same concentration of TGF-β1 (2 ng/mL) for various periods of time (A and C) or different doses of TGF-β1 for 24 hours (B and D), as indicated. A–D: Representative
regulated in the obstructed kidney. Wnt/β-catenin signaling activity increased along with markers of fibrosis after UUO, which was confirmed in the current study.

Corin proteins were expressed in normal mouse kidneys. The down-regulation in corin protein expression after UUO compared with the sham controls was found. Notably, loss of corin was accompanied by increased expression of active β-catenin in tubular epithelial cells after UUO (Figure 1, C–E). Further linear regression analysis revealed an inverse correlation between kidney corin protein and β-catenin levels in the obstructed kidneys (Figure 1F).

On the basis of the previous studies indicating that PCSK6 acts as a corin activator, the expression of PCSK6 in UUO mouse kidney was investigated. In line with the down-regulation of corin, the protein expression of PCSK6 was also reduced at days 4 and 7 after UUO, compared with the sham group (Figure 1G–I).

Fz-CRDS of Corin Are Required for Wnt1 Binding and Inhibition of Wnt/β-Catenin Signaling in Vitro

Next, the potential connection between loss of corin and activation of Wnt/β-catenin signaling was investigated. Previous studies have shown that several proteins containing Fz-CRD,15 including FZD (receptor of Wnt), sFRP,16 carboxypeptidase Z,19 and receptor tyrosine kinase-like orphan receptor,18 can coimmunoprecipitate members of the Wnt family and regulate Wnt signaling. The two Fz-CRDs in corin (Figure 2A) and the down-regulation of corin protein expression along with the activation of Wnt signaling after UUO raised the question of whether corin can directly bind to Wnt1 and, if so, which part of the protein may mediate this interaction. Therefore, the immunoprecipitation experiments were performed, in which the lysates of HEK-293 cells were cotransfected with HA-tagged Wnt1 (Wnt1HA) in combination with corin or corinFlag (the Flag-tagged mutant with deletion in the two Fz-CRDS of corin) (Figure 2A).

HEK-293 cells transfected with Wnt1HA in combination with corinFlag expressed similar Wnt1 levels (Figure 2B). Immunoprecipitation with anti-HA antibody (Figure 2B) or IgG (Figure 2B) followed by immunoblotting with anti-corin was performed. Analysis of the coprecipitates demonstrated the presence of the corin/Wnt1 complex (Figure 2B). An antibody directed against the Flag epitope was used to detect corinFlag. When these Fz-CRDSs were absent in the constructs corinFlag (Figure 2B), corinFlag could not be coprecipitated with Wnt1Flag (Figure 2B), although the mutant proteins were present in the lysates (Figure 2B). In contrast, Wnt1Flag coprecipitated with corinPro-myc (Figure 2C), a myc-tagged mutant lacking the trypsin-like protease domain (Figure 2A). These results indicate that the physical association of Wnt1 with corin protein, and the Fz-CRDSs of corin, plays a crucial role in the combination process.

Then, the experiment of investigating the ability of corin to inhibit Wnt1 functions was performed. The β-catenin levels were quantitated in the transient transfection of HEK-293 cells using a GST–E-cadherin binding assay.43 Different plasmids were successfully transfected or cotransfected into HEK-293 cells (Figure 2D). Figure 2E shows that Wnt1 induced a significant increase in the amount of active β-catenin over that of the vector control (Figure 2, E and F). The expression of corin dramatically inhibited this increase (Figure 2, E and F). When these Fz-CRDSs were deleted in the construct corinFlag, the effect of inhibition was significantly reduced (Figure 2, E and F). When the plasmid corinFlag was changed to corinPro-myc, the induction of active β-catenin by Wnt1 was still inhibited (Figure 2, E and F). These findings establish that corin acts to inhibit the Wnt1 signaling functions responsible for increasing active β-catenin levels through the Fz-CRDSs.

TOPflash/FOPflash reporter gene assays in HK-8 cells were next performed to determine the functional effect of corin on Wnt-mediated gene transcription.31,45 The TOPflash reporter plasmid contains three TCF/lymphoid enhancer-binding response elements, whereas these sites are mutated in the FOPflash plasmid. Activation of the Wnt/β-catenin pathway induces binding of β-catenin to TCF/lymphoid enhancer-binding elements and thereby activates transcription of the firefly luciferase reporter gene. The present results demonstrated that Wnt/β-catenin signaling was activated following the transfection of Wnt1HA (pcDNA-Wnt1HA) alone, whereas the cotransfection of corin (pcDNA-corin) induced a dose-dependent reduction in Wnt1-stimulated transcriptional activity, as evidenced by the alterations in luciferase activity (Figure 2G). However, corin did not impair the luciferase activity induced by the cotransfection of activated β-catenin (pcDNA–β-catenin) and corin (pcDNA-corin) (Figure 2H). These observations suggest that inhibition of Wnt/β-catenin signaling by corin is mediated by targeting Wnt1 rather than β-catenin.

Western blot analyses (A and B) and quantitative data (C and D) are presented. E and F: Western blot analyses of corin expression after pretreatment with a TGF-β1 receptor inhibitor. HK-8 cells were pretreated with TGF-β1 type I receptor inhibitor (10 μmol/L) for 1 hour, followed by incubation with TGF-β1. E and F: Representative Western blot analysis (E) and quantitative data (F) are presented. G and H: Western blot analysis was used to detect the expression of the indicated proteins at 24 hours after exposure to TGF-β1. HK-8 cells were transfected with empty vector, pcDNA-corin, or pRES2-EGFP-corin (ΔCRD–Flag) as indicated, followed by incubation with TGF-β1 (2 ng/mL). Total cell lysates were immunoblotted with specific antibodies. G and H: Representative Western blot analysis (G) and quantitative data (H) are presented. I and J: Immunofluorescence staining for fibronectin (red) and DAPI (blue) in HK-8 cells treated with 2 ng/mL TGF-β for 24 hours. I and J: Representative micrographs (I) and quantitative data (J) are presented. Data are the means ± SEM (C, D, F, H, and J). **P < 0.01, ***P < 0.001. Scale bar = 25 μm (I). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PAI-1, plasminogen activator inhibitor-1; α-SMA, α-smooth muscle actin.
TGF-β1 Represses Corin Expression and Corin Inhibits TGF-β1–Induced Fibrogenic Action Based on Fz-CRDs in Vitro

To investigate the mechanism responsible for mediating corin loss in obstructed kidneys, the next experiment was conducted to test whether corin expression is regulated by TGF-β1, a fibrogenic cytokine that is up-regulated in almost all cases of CKD. As shown in Figure 3, A and B, TGF-β1 inhibited corin expression in HKC-8 cells in a time- and dose-dependent manner. Quantitative data of corin protein after TGF-β1 incubation are presented in Figure 3, C and D. The TGF-β1 type I receptor inhibitor largely restored corin expression in HKC-8 cells (Figure 3, E and F). Therefore, TGF-β1 was identified as a crucial factor that triggers the down-regulation of corin protein expression in HKC-8 cells.

Furthermore, whether overexpression of corin inhibits the fibrogenic actions of TGF-β1 in vitro was examined. HKC-8 cells were transfected with vector of empty, corin or corin ΔCRD–Flag, and then were treated with TGF-β1. Western blot analyses revealed that exogenous corin attenuated TGF-β1–mediated induction of fibronectin and α-SMA (Figure 3G). Moreover, expression of corin was able to inhibit β-catenin activation triggered by TGF-β1, as well as PAI-1 and Snail induction (Figure 3G). After the transfection of corin was changed to the corin ΔCRD–Flag, the inhibition of fibrogenic actions, the activation of β-catenin, and the induction of Snail1 and PAI-1 were attenuated (Figure 3G). Quantitative data of Western blot analyses are presented in Figure 3H. Similar results were obtained when fibronectin expression was assessed by immunostaining (Figure 3, I and J). Collectively, these data indicate that corin inhibits the TGF-β1–mediated induction of various fibrogenic genes in tubular epithelial cells, and the inhibitory effect depends on its Fz-CRDs.

Kidney overexpression of corin after intrarenal adenovirus gene delivery is successfully constructed in UUO/UIRI model

For analysis of the possible beneficial effects of corin on kidney fibrosis, 5 × 10⁸ plaque-forming units of Adeno-corin, Adeno-blank, or saline were infused intrarenally into the left kidneys of mice immediately after ligation of the left ureter in the UUO model or clamping of the left kidney pedicle in the UIRI model. Approximately 10% of all mice that underwent transplantation died within 24 hours of the operation. Deaths were equally distributed among the groups. No animals died if they survived the first 24 hours after transplantation. Injected mice were imaged for luciferase activity 48 hours later. Figure 4A shows that intrarenal delivery of Adeno-corin resulted in strong luciferase activity that appeared near the region of the kidney. The injected kidney showed markedly higher levels of luciferase activity than the contralateral kidney. Quantitative flux data of luciferase activity are presented 48 hours after UUO/UIRI in Figure 4B. Immunohistochemical staining showed that diffuse corin overexpression was evident in the proximal and distal tubules of the mice intrarenally injected with the Adeno-corin at 7 days after UUO and 11 days after UIRI (Figure 4C). Moreover, Western blot analyses confirmed that the kidney expression of corin in the Adeno-corin–injected mice significantly increased in the two models, compared with that of the Adeno-blank–injected mice (Figure 4, D and E). Quantitative data of Western blot analyses are presented in Figure 4, F and G. Taken together, these data suggest that adenovirus-mediated corin overexpression in mouse kidney was successfully generated in the UUO/UIRI model.

The cardiac function of mice in different groups was assessed using echocardiography at 7 days after UUO and 11 days after UIRI. There were no significant differences in cardiac function between the mice with only UUO and those with UUO along with intrarenal injection of Adeno-corin, as observed in the echocardiography studies (Supplemental Figure S1 and Supplemental Table S1). These findings suggest that adenovirus-mediated corin overexpression of corin had no discernible impact on cardiac function in the UUO/UIRI model.

Adenovirus-Mediated Overexpression of Corin Ameliorates Kidney Fibrosis after UUO

Because corin blocks the fibrogenic action induced by β-catenin or TGF-β1 in vitro, the experiment in vivo was designed and performed to test the efficacy of adenovirus-mediated overexpression of corin in mitigating kidney fibrosis after UUO. An improvement in kidney tubular injury and fibrosis scores was detected after injection of Adeno-corin. At 7 days after UUO and injection of adenovirus, the tissues of the left kidney were analyzed (Figure 5A). As shown by Sirius red staining, used to provide a histologic correlate to total kidney collagen, the percentage of the tubulointerstitial occupied by collagen was significantly reduced in the Adeno-corin–injected mice compared with the Adeno-blank–injected mice and those with UUO alone (Figure 5B). The same results were observed in Masson trichrome staining (Figure 5B). Quantitative determinations of the area of Sirius red and Masson trichrome staining are presented in Figure 5C. Immunohistochemical staining revealed that overexpression of corin in vivo substantially inhibited the expression of α-SMA, the molecular signature protein for myofibroblasts (Figure 5D). In the normal adult mouse kidney, α-SMA expression is restricted to vascular smooth muscle cells. In the 7-day obstructed kidneys, α-SMA was also expressed by the increased number of interstitial myofibroblasts. The increases in fibronectin and the major interstitial matrix proteins after UUO were suppressed in mice intrarenally injected with Adeno-corin (Figure 5D). Similar results were obtained using Western blot analyses of whole kidney lysates (Figure 5E). The mice intrarenally injected with...
Adeno-corin showed significant reductions in α-SMA, fibronectin, and collagen-I protein levels compared with the mice with Adeno-blank in quantitative determinations (Figure 5F). Therefore, it was observed that adenovirus-mediated overexpression of corin ameliorates kidney fibrosis after UUO.

Pericytes have been identified as the primary source of interstitial myofibroblasts in the fibrotic kidney.46 Platelet-
Figure 5  Adenovirus-mediated overexpression of corin ameliorates kidney fibrosis after unilateral ureteral obstruction (UUO). A: Experimental design. The green arrow indicates the time point of UUO and injection of corin-overexpressing adenovirus (Adeno-corin) or blank adenovirus (Adeno-blank). Detection of fluorescence activity was performed 48 hours after UUO and adenovirus infection to confirm adenovirus gene delivery. B: Representative micrographs of kidney
derived growth factor receptor β was selected as a pericyte marker for labeling purposes, given its established verification in this role.46 CD31 is a marker found in mature endothelial cells. After UUO, the number of pericytes significantly increased with a higher density expression of platelet-derived growth factor receptor β (Figure 5, G and H), whereas CD31-positive endothelial cells showed a significant decrease (Figure 5, I and J) compared with the sham group. Overexpression of corin resulted in reduced pericyte presence (Figure 5, G and H) and increased CD31-positive endothelial cells (Figure 5, I and J) in the UUO + Adeno-corin group compared with the UUO + Adeno-blank group, suggesting that corin alleviated capillary rarefaction induced by UUO.

Adenovirus-Mediated Overexpression of Corin Blocks Wnt/β-Catenin Signaling after UUO

To establish the relevance of corin in regulation of Wnt/β-catenin signaling, the next step in the experiment examined whether adenovirus-mediated overexpression of corin affects the activation of β-catenin in the kidney after UUO. As shown in Figure 6A by immunohistochemical staining, adenovirus-mediated overexpression of corin inhibited the expression of β-catenin in both tubular epithelial and interstitial cells in the Adeno-corin— injected mice after obstructive injury. After the administration of Adeno-corin, tubular epithelial β-catenin was restricted primarily to the basolateral membrane, with only a small amount present in the cytoplasm and nucleus in the 7-day obstructed kidneys. Western blot analysis also showed that kidney β-catenin abundance was reduced in the Adeno-corin— injected mice after obstructive injury (Figure 6, B and C). Furthermore, overexpression of corin inhibited expression of Snail and PAI-1 (Figure 6, D and E). Hence, adenovirus-mediated overexpression of corin effectively impedes the canonical Wnt/β-catenin signaling pathway after UUO.

Adenovirus-Mediated Overexpression of Corin Ameliorates Kidney Injury in the UIRI Model

To further confirm the relevance of corin in the regulation of Wnt/β-catenin signaling in vivo, the next experiment examined whether forced expression of corin affects kidney activation of β-catenin in another model induced by UIRI, which is characterized by sustained Wnt/β-catenin activation and progressive fibrotic lesions.39 As shown in Figure 7A, Adeno-corin, Adeno-blank, or saline was infused intrarenally into the left kidneys of mice immediately after clamping the kidney pedicle. Overexpression of corin in the UIRI model was confirmed as described above (Figure 4). Western blot analyses of whole kidney homogenates revealed that Wnt1, β-catenin, and its target gene expression were significantly induced at day 11 after UIRI compared with those of the sham group (Figure 7, B and C), which indicates that Wnt/β-catenin was activated in this UIRI model. Adenovirus-mediated overexpression of corin appeared to largely restore kidney expression of corin proteins and inhibited β-catenin and its target gene expression at day 11 after UIRI. Similar data were obtained when kidney sections were immunostained for corin (Figure 4C) and active β-catenin (Figure 7D). These results indicate that adenovirus-mediated overexpression of corin effectively blocks Wnt/β-catenin signaling in the UIRI model as well.

The fibrotic lesions in the kidney were analyzed. Kidney histology, as shown by Masson staining and Sirus red staining, revealed significant lesions, characterized by dilated tubules and interstitial inflammation and expansion, at day 11 after UIRI (Figure 7, E and F). Adenovirus-mediated overexpression of corin largely abolished these morphologic lesions. Fewer fibrotic lesions and reduced collagen deposition were observed in the kidneys injected with Adeno-corin, suggesting that corin attenuates the development of kidney fibrosis. Similarly, expression of corin in vivo significantly reduced serum creatinine and blood urea nitrogen (P values are both 0.01; n = 10) (Figure 7G). Furthermore, profound induction of α-SMA—positive myofibroblasts and fibronectin protein were observed by immunohistochemical staining predominantly in the kidney interstitial space after UIRI (Figure 7H); however, overexpression of corin inhibited these effects (Figure 7H). The results were similar when α-SMA, fibronectin, and collagen-I protein were quantitatively analyzed by Western blot analysis (Figure 7, I and J). The overexpression of corin in the UIRI + Adeno-corin group, consistent with the findings in the UUO model, led to a decrease in pericyte presence and an increase in CD31-positive endothelial cells compared with the UIRI + Adeno-blank group (Figure 7, K—N). Taken together, these results indicate that adenovirus-mediated overexpression of corin can ameliorate kidney injury in both UUO and UIRI models.

tissues in different groups at day 7 after UUO as indicated. Kidney sections were subjected to Sirius red and Masson trichrome staining. C: Graphic presentation showing the area of Sirius trichrome and Sirus red staining in kidney tissues among the indicated groups. D: Representative immunohistochemical micrographs of α-smooth muscle actin (α-SMA) and fibronectin at day 7 after UUO. Paraaffin-embedded kidney sections were stained with α-SMA and fibronectin antibodies. Arrows indicate positive staining. E and F: Western blot analysis of α-SMA, fibronectin, and collagen I in kidney tissues at day 7 after UUO. E and F: Representative gel images (E) and quantitative data (F) are shown. G and H: Immunohistochemical and immunofluorescence staining of platelet-derived growth factor receptor β (PDGFR-β) in kidney tissues of different groups as indicated at day 7 after UUO. PDGFR-β serves as a pericyte marker. G and H: Representative micrographs (G) and quantitative data (H) are presented. I and J: Immunohistochemical and immunofluorescence staining of CD31 in kidney tissues of different groups as indicated at day 7 after UUO. CD31 serves as the marker of mature endothelial cells. I and J: Representative micrographs (I) and quantitative data (J) are presented. Data are the means ± SEM (C, F, H, and J). n = 5 animals per group (C, F, H, and J). *P < 0.05, **P < 0.01, and ***P < 0.001. Scale bars = 50 μm (B, D, G, and I). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
Discussion

The results presented in this study demonstrate that the suppression of corin is a pathologic feature along with the increased activity of Wnt/β-catenin signaling in mouse kidneys after UUO. This process leads to the activation of the fibrinogen signaling pathway and consequently triggers the expression of a series of fibronectin genes. In vitro, it was found that corin can physically bind to Wnt1 ligands through its Fz-CRDs and thereby inhibit Wnt1 signaling, which is responsible for increasing active β-catenin levels. TGF-β1 represses corin expression, and corin inhibits the TGF-β1-mediated induction of various fibrogenic genes in vitro. Furthermore, adenovirus-mediated overexpression of corin can alter the progression of fibrosis by inhibiting Wnt/β-catenin signaling and the expression of its target genes in two models of CKD induced by UUO and UIRI. These results illustrate that corin acts as an endogenous antagonist that protects the kidney from the pathogenic Wnt/β-catenin signaling pathway and from fibrosis following UUO and UIRI (Figure 8).
Figure 7  Adenovirus-mediated overexpression of corin ameliorates kidney injury after ureteral ischemia/reperfusion injury (UIRI). A: Experimental design. The **green arrow** indicates the time point of UIRI and injection of corin-overexpressing adenovirus (Adeno-corin) or blank adenovirus (Adeno-blank). The **black arrowhead** indicates the time point of unilateral nephrectomy (UNx). Detection of fluorescence activity was performed 48 hours after UIRI and...
Corin, a type II transmembrane serine protease responsible for converting pro-atrial natriuretic peptide into atrial natriuretic peptide, was identified to be markedly reduced in puromycin aminonucleoside–induced nephrotic syndrome and anti-Thy1 glomerulonephritis. Reduced corin expression in the kidney and urinary levels of soluble corin have also been reported in patients with CKD. Increasing cardiac corin expression in mice with dilated cardiomyopathy reduces cardiac fibrosis, improves cardiac function, and prolongs survival. In terms of protein structure, corin harbors two Fz-CRDs at the C-terminus (Figure 2A), and Fz-CRD is mostly associated with Wnt signaling proteins.

As mentioned in Introduction, the eight proteins containing the Fz-CRD structure are Fzd, sFRP, receptor tyrosine kinase-like orphan receptor, muscle skeletal receptor tyrosine kinase, membrane Frizzled-related protein, carboxypeptidase Z, collagen type XVIII α 1, and corin. With the exception of corin, seven other proteins have been reported to bind Wnt and affect Wnt signal transduction. The current study bridges this gap. The results presented that corin binds to Wnt1 ligands, which are up-regulated in injured kidneys. Although not proven, corin might be able to bind other members of the Wnt family. More important, the corin/Wnt interaction is apparently mediated by Fz-CRDs. By using different immunological tags, it was observed that the Fz-CRDs of corin possessed the ability to form complexes with Wnt1. Furthermore, corin inhibited Wnt-induced alterations in β-catenin regulation and TCF transcription. Similar conclusions were also derived from the other proteins containing Fz-CRD, such as sFRP, receptor tyrosine kinase-like orphan receptor 2, and collagen type XVIII α 1. Certain Fz-CRD deletion mutants lost the ability to bind Wnt1 and to block the Wnt-induced axis, which should help to evaluate the proposed model for corin inhibition of Wnt signaling.

These findings are further reinforced by the fact that the corin mutants with deletion of the PRO domain retained the ability to bind Wnt1. Therefore, the current results conclude that corin physically binds to Wnt through its Fz-CRD structural domain and thus functionally isolates antagonism of Wnt/β-catenin signaling.

TGF-β1, a putative key regulator in inflammation, tissue repair, and fibrosis of the kidney, activates β-catenin and triggers the expression of its target genes in HKC-8 cells (Figure 3). It was also observed that TGF-β1 does not significantly affect Wnt1 expression, consistent with a previous study. TGF-β1 markedly suppresses corin expression. Conversely, transfection of corin in turn functionally blocks TGF-β1–mediated β-catenin activation and the subsequent induction of profibrotic genes, such as Snail1, PAI-1, fibronectin, and α-SMA, underlining that corin is an important player in mediating profibrotic TGF-β1 signaling. Such antagonistic interaction between TGF-β1 and corin suggests a possible vicious cycle between corin deficiency and TGF-β1/β-catenin activation in the diseased kidney. When the Fz-CRDs were knocked out, the blocking effect on TGF-β1/β-catenin activation disappeared, which further confirmed that the inhibitory effect of corin on TGF-β1–induced fibrogenic action is dependent on its Fz-CRD.

Several inhibitors of Wnt-dependent signaling cascades have been identified, such as sFRP, Wnt inhibitory factor, Klotho, and a family of DKK proteins. DKK family proteins and sFRP are up-regulated in the obstructed kidneys after UUO. Similar to Klotho, the down-regulation of corin expression in fibrotic kidneys indicates that corin expression is not under the positive control of the Wnt/β-catenin pathway, suggesting that overexpression of corin may be the preferred strategy to inhibit Wnt/β-catenin and its resulting fibrosis.

Adenovirus are widely used for transgene delivery because of the high convertibility, ability to infect non-dividing cells, and high titers yields. In the current study, the UUO and UIRI models of the kidney with the delivery of Adeno-corin were successfully constructed. In this model, kidney overexpression of corin using gene transfer with Adeno-corin was effective in preventing fibrotic kidney injury after UUO/UIRI. In most molecular therapies, the i.v. route is the preferred method for delivery because of the ease of administration. However, the selectivity of the glomeruli will hinder rather than facilitate delivery of these vectors from the blood. In the preliminary studies, the method of corin gene transfer in vivo using standard tail vein
injection or percutaneous injections failed to result in the enhanced expression of corin in the kidney. Parenchymal injection was used herein according to previous studies, which successfully resulted in overexpression of corin in the proximal and distal tubules of mice. Therefore, adenovirus gene delivery by parenchymal injection allowed us to directly test the hypothesis that restoration of corin expression in mouse kidneys ameliorates fibrosis after UUO/UIRI injury.

Sustained activation of Wnt/β-catenin signaling is detrimental and could aggravate kidney fibrosis in CKD progression. In the canonical Wnt cascade, the Wnt protein interacts with target cells by binding the heterodimer receptor complex, which consists of FZDs and lipoprotein receptor-related proteins 5/6. The primary platform for Wnt binding is FZDs, which are seven-pass transmembrane receptors with an extracellular N-terminal CRD domain. Wnt/β-catenin signaling is regulated at many levels, such as by proteins that function as antagonists of ligands. Among them are sFRPs and Wnt inhibitory factor, both of which bind to Wnts and thus inhibit the interaction between Wnt and Wnt receptors. Administration of sFRP4 inhibited the progression of kidney fibrosis, possibly because of recombinant sFRP4 preventing Wnt proteins from binding to FZDs

Figure 8 Schematic diagram showing the hypothesized mechanisms by which corin ameliorates kidney interstitial fibrosis. Right panel: After Wnt binds the Frizzled (FZD)/lipoprotein receptor-related protein (LRP) coreceptor complex, the canonical Wnt/β-catenin (β-Cat) signaling pathway is activated. Wnt can induce the phosphorylation of LRP by GSK3β and casein kinase 1-γ (CK1γ), thus regulating the docking of Axin. The recruitment of Axin leads to the stabilization of β-catenin. In the nucleus, the binding of β-catenin to T-cell factor (TCF) promotes the transcription of Wnt target genes associated with fibrosis.

Left panel: Corin can bind to Wnt ligands through its Frizzled cysteine-rich domains (CRDs), which results in Wnts that are not bound by receptor complexes. β-Catenin is phosphorylated and then is targeted for rapid destruction by the proteasome. In the nucleus, the binding of Groucho to TCF inhibits the transcription of Wnt target genes and ameliorates kidney fibrosis.
receptors during UUO. Other Wnt inhibitors include DKK proteins, which antagonize signaling by binding lipoprotein receptor-related proteins 5/6. Delivery of DKK1 also significantly reduced the expression of Wnt/β-catenin-dependent genes and matrix accumulation after UUO. In various animal or cell models of CKD, several strategies have been reported to attenuate kidney fibrosis by inhibiting the activity of the Wnt/β-catenin signaling pathway, in which certain components of the pathway were examined to explore the underlying mechanisms of this strategy.

The present study offers novel insights into the ability of corin to mitigate kidney fibrosis damage in vivo, which may be closely associated with the Wnt/β-catenin signaling pathway. The kidney protection elicited by corin is associated with its inhibition of β-catenin activation and suppression of Snail1, PAI-1, α-SMA, and fibronectin. Similar to sFRP4 with the Fz-CRD, corin has two Fz-CRDs in its structure, and the inhibition of fibrotic action at the cellular level and in animal models was confirmed. Collectively, the hypothesis was formulated and eventually verified that corin can inhibit kidney fibrosis. Specifically, corin binds to Wnt1 via Fz-CRDs and thus interferes with the binding of Wnt1 to its receptor, followed by inhibition of Wnt/β-catenin signaling and reduction of fibrosis. The combination between Wnt1 and the Fz-CRDs of corin is apparently given priority to the FZD proteins of the receptor complex. The exact molecular mechanisms of competitive protein binding action remain unclear.

There are many questions that remain to be answered. First, the identity of the protease that activates corin in the kidney remains elusive. It has been shown that PCSK6 acts as a primary corin activator in the heart, and that PCSK6 deficiency causes salt-sensitive hypertension in mice. It was found that expression of PCSK6 was attenuated in the kidney tissues after UUO. It is presumed that impaired corin in UUO may be at least partly attributable to decreased PCSK6 expression. However, the consequent modification of the corin expression in kidney after knocking PCSK6, and whether the overexpression of PCSK6 affects the corin/Wnt signaling pathway and the kidney fibrosis, needs to be confirmed by further studies. Second, it cannot be ruled out the possibility that corin may exert its beneficial action by other mechanisms as well (eg, the involvement of the natriuretic peptide system, in which corin is a key protease of activating the natriuretic pro-atrial natriuretic peptide and probran natriuretic peptide). Third, it was only speculated, but not confirmed, that all 19 members of the Wnt family can bind corin. Finally, there is a lack of human relevance as no patient samples were used. In this regard, future studies on corin in patients with CKD are warranted.

In summary, the present study has provided novel insights into a protective role of corin in fibrotic kidney. Corin exerts inhibitory effects on Wnt signaling and subsequent kidney fibrosis, which is potentially associated by the synergistic interaction between Wnt and corin via Fz-CRDs. Down-regulation of corin protein is a common pathologic feature and is associated with activation of β-catenin during progression of kidney fibrosis. In this case, adenovirus-mediated overexpression of corin can restore the inhibition of Wnt/β-catenin signaling and may be a reasonable strategy for the treatment of fibrotic kidney injury, although more research is needed.

Disclosure Statement

None declared.

Author Contributions

X.S. designed the experiments and performed part of the experiments; L.W. and X.Q. provided technical and material support; S.L., Y.Z., R.F., and X.G. performed experiments; S.L. and X.S. analyzed the results; S.L., X.S., and Y.Z. wrote the original draft; and X.S. and L.W. reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Supplemental Data

Supplemental material for this article can be found at http://doi.org/10.1016/j.ajpath.2023.09.008

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