Mechanism of Fibrosis in HNF1B-Related Autosomal Dominant Tubulointerstitial Kidney Disease

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ABSTRACT

Background Mutation of HNF1B, the gene encoding transcription factor HNF-1β, is one cause of autosomal dominant tubulointerstitial kidney disease, a syndrome characterized by tubular cysts, renal fibrosis, and progressive decline in renal function. HNF-1β has also been implicated in epithelial–mesenchymal transition (EMT) pathways, and sustained EMT is associated with tissue fibrosis. The mechanism whereby mutated HNF1B leads to tubulointerstitial fibrosis is not known.

Methods To explore the mechanism of fibrosis, we created HNF-1β-deficient mIMCD3 renal epithelial cells, used RNA-sequencing analysis to reveal differentially expressed genes in wild-type and HNF-1β-deficient mIMCD3 cells, and performed cell lineage analysis in HNF-1β mutant mice.

Results The HNF-1β-deficient cells exhibited properties characteristic of mesenchymal cells such as fibroblasts, including spindle-shaped morphology, loss of contact inhibition, and increased cell migration. These cells also showed upregulation of fibrosis and EMT pathways, including upregulation of Twist2, Snail1, Snail2, and Zeb2, which are key EMT transcription factors. Mechanistically, HNF-1β directly represses Twist2, and ablation of Twist2 partially rescued the fibroblastic phenotype of HNF-1β mutant cells. Kidneys from HNF-1β mutant mice showed increased expression of Twist2 and its downstream target Snail2. Cell lineage analysis indicated that HNF-1β mutant epithelial cells do not transdifferentiate into kidney myofibroblasts. Rather, HNF-1β mutant epithelial cells secrete high levels of TGF-β ligands that activate downstream Smad transcription factors in renal interstitial cells.

Conclusions Ablation of HNF-1β in renal epithelial cells leads to the activation of a Twist2-dependent transcriptional network that induces EMT and aberrant TGF-β signaling, resulting in renal fibrosis through a cell-nonautonomous mechanism.
tubular atrophy, thickening and lamellation of tubular basement membranes, kidney microcysts or macrocysts, and absence of immune complex deposits. ADTKD encompasses disorders previously known as medullary cystic kidney disease and familial juvenile hyperuricemic nephropathy. ADTKD is genetically heterogeneous and can arise from mutations in at least five genes: UMOD, which encodes uromodulin/Tamm–Horsfall protein, the major urinary protein produced in the loop of Henle; MUC1, which encodes the apical membrane protein mucin-1; REN, which encodes the enzyme renin that catalyzes the conversion of angiotensinogen to angiotensin-1; SEC61A1, which is involved in protein translocation in the endoplasmic reticulum; and HNF1B, which encodes the transcription factor hepatocyte NF-1β (HNF-1β). Causative genes have not yet been identified in a significant portion of affected individuals.

HNF-1β is a homeodomain-containing transcription factor that regulates tissue-specific gene expression in the kidney, liver, pancreas, and other epithelial organs. HNF-1β is essential for normal kidney development; ablation of HNF-1β in nephron progenitors leads to disturbances in Notch-dependent nephron patterning, and ablation in the ureteric bud inhibits branching morphogenesis and Wnt9b-dependent nephron induction and GDNF/Ret signaling. In humans, mutations of HNF1B have been linked to congenital abnormalities of the kidney and urinary tract, including renal agenesis/hypoplasia, multicystic dysplastic kidneys, horseshoe kidneys, and glomerulocystic kidney disease. Mutations of HNF1B can also produce ADTKD, often associated with hyperuricemia, hypomagnesemia, hypokalemia, diabetes mellitus, and Müllerian duct abnormalities. Previous studies suggested that HNF-1β regulates the transcription of UMOD, raising the possibility that disturbances in a common transcriptional network may underlie the pathogenesis of ADTKD. Abnormalities in unfolded protein response (UPR) and mitochondrial metabolism have also been implicated. In addition to inherited kidney diseases, mutations of HNF1B have been detected in sporadic cases of renal hypoplasia/dysplasia. Expression of HNF1B is also downregulated in polycystic kidney disease.

Several genetically modified mouse models have been developed to unravel the pathogenesis of human HNF1B-related kidney disease. Common features of the HNF-1β mutant mouse models are enlarged kidneys with fluid-filled cysts, multilayered cyst epithelium, and hydropnephrosis. Molecular characterization of HNF-1β mutant mice has shown that HNF-1β plays a significant role in cystic kidney diseases through the regulation of cystogenes such as Pkhd1, Pkd2, Umod, and Kif12. Recent chromatin immunoprecipitation sequencing (ChIP-seq) experiments have shown that HNF-1β regulates cholesterol metabolism through transcriptional activation of Srebp2 and Pckα. Urinary concentration through activation of Fxr and potassium and magnesium homeostasis through activation of Kir5.1. However, the complete gene network governed by HNF-1β in the kidney and the mechanisms whereby human mutations lead to a broad spectrum of clinical phenotypes remain to be fully elucidated. Constitutive ablation of Hnf1b in mice results in embryonic lethality due to failure of endoderm development, and kidney-specific deletion of Hnf1b using the Ksp/Cre deleter strain results in postnatal kidney failure. We recently used Pkd1/Cre to generate Ksp/Cre;Hnf1b mice to specifically in renal collecting ducts. Collecting duct-specific deletion of Hnf1b results in longer survival and slower progression of cystic disease, renal fibrosis, and hydropnephrosis. Mutant mice also exhibit polyuria, polydipsia, and impaired urinary concentration recapitulating clinical features of ADTKD in humans with mutations in HNF1B.

To investigate the pathogenesis of renal interstitial fibrosis in ADTKD, three ADTKD–UMOD mouse models had been generated. Characterization of the mutant mice suggests that renal fibrosis arises from endoplasmic reticulum (ER) stress and secondary mitochondrial dysfunction. However, the mechanism whereby mutations of HNF1B produce tubulointerstitial fibrosis has not been explored. Using HNF-1β mutant cell lines and mouse models, we found that loss of HNF-1β induces epithelial–mesenchymal transition (EMT) via derepression of the transcription factor Twist2. As a consequence, the expression of TGF-β ligands is upregulated in renal tubules, which leads to renal fibrosis via a cell-nonautonomous process.

**METHODS**

**Transgenic Mice**

Ksp/Cre mice that express Cre recombinase under the control of the Ksp-cadherin (Cdhl6) promoter and Hnf1b floxed mice containing loxP sites flanking exon 1 of Hnf1b have been described previously. R26R-EYFP mice that express EYFP after Cre/loxP recombination were provided by Dr. Frank Costantini (Columbia University). Ksp/Cre mice were crossed with Hnf1b floxed mice, and the bitransgenic progeny were intercrossed to generate Ksp/Cre;Hnf1b floxed mice (HNF-1β mutant mice). Cre-negative or Ksp/Cre;Hnf1b floxed littermates were used as negative controls. Mice of both sexes were used for experiments.
Cell Culture

mIMCD3 cells were obtained from American Type Culture Collection and maintained in DMEM supplemented with 10% FBS and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin). Cells were cultured at 37°C under 5% CO₂.

Gene Editing

Cells were seeded on six-well plates (Corning) at a density of 200,000 cells per well. After 24 hours, when 60%–70% confluent, the cells were transfected with 2 μg Cas9 and U6-sgRNA vector (PX459, #48139; Addgene) using Lipofectamine 3000 (Life Technologies) according to the manufacturer’s directions. The single-guide RNA (sgRNA) sequences are listed in Supplemental Table 1. After incubation for 72 hours, an aliquot of the transfected cells was lysed using QuickExtract DNA extraction kits (Epicentre), following the manufacturer’s directions. Genomic DNA was amplified using PCR kits (Quanta Biosciences). The sequences of PCR primers are listed in Supplemental Table 2. PCR products were cloned using CloneJET PCR cloning kits (Thermo Fisher Scientific), and plasmids were sequenced using the Sanger method, with a universal T7 primer.

Quantitative Real-Time RT-PCR

Total RNA was extracted from cells or kidneys using TRIzol (Invitrogen) or RNeasy Mini Kits (Qiagen) according to the manufacturers’ directions. Complementary DNA was synthesized using cDNA Synthesis Kit (Roche), and quantitative real-time PCR was performed with PerfeCTa SYBR Green FastMix (Quanta Biosciences) using a CFX Connect Real-Time System (Bio-Rad). Gene expression levels in cell lines or whole kidneys were normalized to 18S ribosomal RNA and β2 microglobulin, respectively. Fold changes in messenger RNA (mRNA) expression levels were calculated using the comparative Ct method, as described.29 Primers used for quantitative real-time PCR are listed in Supplemental Table 3.

RNA-sequencing

TruSeq stranded mRNA libraries were generated from each sample and sequenced in a 100 bp paired-end run on a HiSeq 2500 instrument using v4 chemistry by the University of Minnesota Genomics Center. Raw paired-end RNA-sequencing (RNA-seq) reads in fastq format were analyzed using gopher-pipeline software (Rnaseq-Pipeline version 1.4; https://bitbucket.org/jgarbe/gopher-pipelines), which (1) assesses base call quality, cycle uniformity, and contamination using fastQC, (2) maps reads to the reference mouse genome (ensembl GRCm38) via HiSAT2,30 using ensembl mouse annotation (Mus_musculus.GRCm38.83), (3) cleans the alignments using picard tools (https://broadinstitute.github.io/picard/), and (4) quantifies the reads mapped per gene using subread.31 The resultant count table was imported into DESeq2 (v1.10.1)31 to test for differential expression using default parameters. Genes that showed at least a two-fold change in expression with a raw P value < 0.05 were considered differentially expressed. R package biomaRt (v2.26.1)32 and heatmap (v1.0.8) were used for the downstream data exploration and visualization. Raw and processed data have deposited with the National Center for Biotechnology Information Gene Expression Omnibus under accession number GSE97770.

Lentiviral Transduction

A lentiviral vector expressing HA-tagged GFP (pLV-HA-GFP) has been described previously.29 To generate a vector expressing HA-tagged Twist2 (pLV-HA-Twist2), the Twist2 coding sequence was amplified with primers containing EcoRV and SalI restriction sites at the 5’ and 3’ ends, respectively. The PCR product was cloned in-frame into compatible restriction sites of pLV-HA-GFP. mIMCD3 cells were infected with lentivirus and selected by growth in hygromycin (200 μg/ml) for 10 days. Surviving cells were pooled for subsequent experiments.

Chromatin Immunoprecipitation (ChIP)

Quantitative ChIP was performed using an antibody specific for anti–HNF-1β (sc-22840; Santa Cruz Biotechnology) as described previously.29 Isotype-specific IgG (sc-2027; Santa Cruz Biotechnology) was used as a negative control. DNA (ChIP-enriched or input) was quantified by real-time PCR using PerfeCTa SYBR Green FastMix. Sequences of gene-specific primers are listed in Supplemental Table 4.

Immunoblot Analysis

Cells were lysed in Laemmli buffer and subjected to immunoblot analysis as described previously.33 Immunoblots were incubated with primary antibodies overnight at 4°C then incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 hour. Immunoblots were developed by incubation with chemiluminescence reagent (Amersham) and exposed to x-ray film. Antibodies are listed in Supplemental Table 5.

Wound Healing Assays

Cells (5 × 10⁵) were seeded in 24-well Culture-Insert plates (#80241; iBidi) containing silicon inserts with a 500 μm cellfree gap. After incubation for 36 hours to 100% confluence, the inserts were removed and culture medium was replaced. Phase-contrast photomicrographs were obtained at time 0 hours and after 12 hours incubation at 37°C. The area of the cellfree gap was measured using Photoshop, and the percentage of gap closure was calculated.

Cell Invasion Assays

Cells (5 × 10⁵) were seeded in Transwell cell culture inserts containing permeable membranes with 3 μm pore size (#353096; Falcon), and placed in 24-well plates with 0.5 ml culture medium. After 48 hours, the inserts were fixed and stained with Giemsa. Phase-contrast photomicrographs were obtained using a Nikon microscope.
Cell Cycle Analysis
Trypsinized cells were centrifuged at 524×g for 5 minutes. The cell pellets were washed with ice-cold PBS and fixed in 70% ethanol overnight at 4°C. Fixed cells were centrifuged at 524×g for 5 minutes and stained with 500 μl propidium iodide master mix (0.1% Triton X-100, 1 mM EDTA, 0.05 mg/ml RNase A [50 U/mg], 50 μg/ml propidium iodide, and PBS; pH 7.4) for 1 hour. Stained cells were analyzed with a LSRII (Becton Dickinson) using the 20-mW blue laser (488 nm) and a 610/20 band pass filter with a 600LP filter to register the fluorescence signal. Doublet discrimination was used to analyze the cellular expression of propidium iodide via histogram and to collect a statistically relevant percentage of single cells to analyze for the cell cycle profile. To quantify apoptosis, the percentage of cells with hypodiploid DNA content was measured by flow cytometry.

Antibody Staining
Mice were euthanized, and kidneys were fixed by perfusion and incubation with 4% paraformaldehyde for 48 hours. Tissues were incubated overnight in 70% ethanol, embedded in paraffin, and sectioned at 4 μm. For immunohistochemistry, slides were incubated in heat-induced antigen retrieval 1× Reveal Decloaker (RV100M; Biocare Medica) for 30 minutes, treated with 3% hydrogen peroxide, and blocked in 100% Rodent Block M (RBM961; Biocare Medical) for 1 hour. Primary antibodies were added in 10% Rodent Block and incubated overnight at 4°C. The following primary antibodies were used: Twist (#15393; 1:200; Santa Cruz Biotechnology), Snail (#ab85936; 1:10,000; Abcam), E-Cadherin (#3195, 1: 2000; Cell Signaling Technology), Vimentin (#5741; 1:2000; Cell Signaling Technology), and α-SMA (#5694, 10,000; Abcam). Slides were incubated in Rabbit-on-Rodent HRP-Polymer (RMR622; Biocare Medical) for 30 minutes and then treated with 3′-3′′-diaminobenzidine (926603-buffer A, 926503-buffer B; BioLegend) for 5 minutes. Sections were counterstained with hematoxylin. For immunofluorescence, the following antibodies were used: HNF-1β (#sc22840; 1:500; Santa Cruz Biotechnology), GFP (#600–901–2155, 1:400; Rockland), Entactin (#ab14511, 1:200; Abcam), Smad3 (#ab40854; 1:2000; Abcam), phospho-Smad3 (#S40450253, 1:50; Sigma), SMA-Cy3 (#C6198; 1:500; Sigma), and Vimentin-Alexa Fluor 647 (#9855; 1:2000; Cell Signaling Technology). Secondary antibodies were conjugated to Cy3 or Alexa Fluor 647 (1:2000; Jackson ImmunoResearch). Nuclei were counterstained with DAPI (4′,6-diamidino-2-phenylindol). Images were captured using a Leica DM5500 B upright microscope with DFC7000 T camera. Confocal laser scanning microscopy was performed by the Imaging Core of the University of Minnesota Center for Immunology with an Olympus FV1000 Confocal Microscope.

In Situ Hybridization
RNA in situ hybridization was performed using RNAscope 2.0 HD-Assay-Brown Kit (cat#210020) per the manufacturer’s instructions (Advanced Cell Diagnostics). Then, 4-μm-thick kidney sections were prepared and RNAscope was performed, using commercial probes for TGF-β2 (cat#406181, accession #NM_009367.3) and TGF-β3 (cat#406211, accession #NM_009368.3). Hybridization signals were detected after kit amplification and incubated with 3′-3′″-diaminobenzidine. Nuclei were counterstained with hematoxylin, and slides were mounted with Permount (Sigma). Images were captured using a Leica DM5500 B upright microscope with DFC7000 T camera.

Collagen Level Determination
The Masson trichrome method was used to stain wild-type and HNF-1β knockout p28 kidney paraffin sections. All sections were stained at the same time, and blue collagen stain was quantified using ImageJ. The blue channel was separated using the IHC Toolbox plugin (https://imagej.nih.gov/ij/plugins/ihc-toolbox/index). Area fraction of blue collagen staining in total non-cystic area was calculated from wild-type and HNF-1β knockout kidneys. A two-tailed t test was used to compare the relative collagen deposition between wild-type and HNF-1β knockout littermates.

ELISA
Two confluent T175 flasks of cells were rinsed with serum-free Advanced/F12 medium and replenished with 20 ml Advanced/F12 medium, then incubated at 37°C for 24 hours. Then, 40 ml of the conditioned medium was centrifuged at 3724×g for 10 minutes, and the supernatant was concentrated by centrifugation (Centricon Plus 70 with NMWL:10K) at 3500×g for 30 minutes at 4°C. TGF-β2 was measured using TGF-β2 Quantikine ELISA Kits according to the manufacturer’s directions (cat#MB200; R&D Systems).

Fibroblast Activation
NRK-49F normal rat kidney fibroblasts (American Type Culture Collection) were seeded in six-well plates at 2×105 cells per well and incubated for 48 hours in DMEM containing 5% FBS, then switched to serum-free Advanced/F12 medium for 4 hours. NFK-49F cells were then incubated with an equal volume of conditioned medium and fresh Advanced/F12 medium containing 0.5% BSA at 37°C for 48 hours. Cells were lysed in 0.5 ml Laemmli buffer and subjected to immunoblot analysis.

Statistical Analyses
A Student two-tailed unpaired t test was used for pairwise comparisons. One-way ANOVA followed by Tukey test was used for multiple comparisons. P<0.05 was considered significant.

Study Approval
All experiments involving animals were performed under the auspices of the Institutional Animal Care and Use Committee at the University of Minnesota.
RESULTS

Ablation of HNF-1β in mIMCD3 Renal Epithelial Cells Alters Cell Morphology

We used CRISPR-based gene-editing to delete exon 1 of Hnf1b in the collecting duct-derived cell line mIMCD3. Previous studies using homologous recombination in mice showed that deletion of exon 1 produces a null allele.14 We synthesized sgRNAs complementary to sequences flanking exon 1 and cotransfected cells with Cas9 to delete the intervening sequence by nonhomologous end joining (Figure 1A). Deletion-spanning PCR confirmed the complete removal of exon 1 from the Hnf1b locus. Three independent clones lacking exon 1 were generated and used to establish permanent lines (Figure 1B). Sanger sequencing of the PCR fragments confirmed deletion of the first exon and end-joining at the predicted cut sites (Figure 1C). Immunoblot analysis showing undetectable HNF-1β protein in three knockout cell lines compared with wild-type cell lines. β-actin was used as a loading control. (E and F) Quantitative RT-PCR showing greatly reduced expression of Hnf1b and Pkhd1 in three HNF-1β knockout cell lines compared with wild-type mIMCD3 cells. Error bars indicate SD. (G) Phase-contrast photomicrographs showing polygonal morphology of wild-type mIMCD3 cell lines and spindle-shaped morphology of HNF-1β knockout cell lines. Scale bars, 100 μm. KO, knockout; WT, wild-type.

HNF-1β Null mIMCD3 Cells Exhibit EMT Characteristics and Form Multilayered Epithelia In Vitro

To determine the whole-genome transcriptome that is regulated by HNF-1β, we performed RNA-seq on three wild-type mIMCD3 cell lines and three HNF-1β–deficient cell lines. HNF-1β–deficient cells showed upregulation of 1135 genes and repression of 759 genes compared with control cells (Figure 2A). All differentially expressed genes are listed in Supplemental Table 6. As a positive control, we confirmed changes in expression of previously identified HNF-1β–dependent genes, such as Pkhd1, Pcsk9, Cdh16, Kif12, and Socs3.14,20 To identify the differentially expressed genes that are directly regulated by

Figure 1. Ablation of HNF-1β alters epithelial cell morphology. (A) Diagram showing the Hnf1b locus before (above) and after (below) CRISPR-based nonhomologous end joining (NHEJ). Blue arrows indicate sgRNA binding sites. F1 and R1 indicate PCR primers used for genotyping. (B) PCR of genomic DNA showing deletion of exon of Hnf1b in three independent mIMCD3 cell lines. (C) Sanger sequencing of the NHEJ junction from an HNF-1β knockout cell line. (D) Immunoblot analysis showing undetectable HNF-1β protein in three knockout cell lines compared with wild-type cell lines. β-actin was used as a loading control. (E and F) Quantitative RT-PCR showing greatly reduced expression of Hnf1b and Pkhd1 in three HNF-1β knockout cell lines compared with wild-type mIMCD3 cells. Error bars indicate SD. (G) Phase-contrast photomicrographs showing polygonal morphology of wild-type mIMCD3 cell lines and spindle-shaped morphology of HNF-1β knockout cell lines. Scale bars, 100 μm. KO, knockout; WT, wild-type.
HNF-1β, we compared the RNA-seq results with our previous ChIP-seq analysis of genome-wide binding of HNF-1β in native chromatin from mIMCD3 cells. This analysis revealed that 75% of the differentially expressed genes contained nearby HNF-1β binding sites and were therefore likely to be directly regulated by HNF-1β.

Ingenuity Pathway Analysis (IPA) revealed that EMT and fibrosis were the two most dysregulated pathways in HNF-1β–deficient mIMCD3 cells (Supplemental Figure 1A). Examination of the EMT gene set in HNF-1β–deficient cells showed upregulation of key EMT transcription factors Snail1, Snail2, Zeb2, and Twist2 and increased expression of TGF-β ligands TGF-β2, and TGF-β3. Conversely, the expression of E-cadherin (Cdh1) was reduced (Figure 2B). Examination of 44 genes within the fibrosis gene set showed that genes involved in collagen synthesis were significantly
upregulated in HNF-1β-deficient cells (Supplemental Figure 1B). Expression of other known ADTKD genes was either undetectable (Umod, Ren1) or unchanged (Sec61a1, Muc1) (Supplemental Figure 2).

Next, we determined whether the molecular signature of EMT was associated with corresponding changes in cell phenotype. First, we performed wound healing assays to measure cell migration. Compared with wild-type mIMCD3 cells, HNF-1β-deficient cells exhibited faster rates of wound healing (Figure 2C). The rates of cell growth and cell-cycle progression were similar, whereas apoptosis was decreased in HNF-1β-deficient cells (Supplemental Figure 3). To confirm the increased cell motility, we performed cell invasion assays using Transwell filters. HNF-1β-deficient mIMCD3 cells could invade through the 3-μm pores of a permeable filter, whereas wild-type cells exhibited no invasion (Figure 2D). When grown to confluence on Lumox discs, wild-type mIMCD3 cells exhibited contact inhibition and formed an epithelial monolayer. In contrast, HNF-1β-deficient cells produced a multilayered epithelium reminiscent of the multilayered cyst epithelium seen in HNF-1β mutant mice and humans (Figure 2E). These cellular and molecular phenotypes indicate that ablation of HNF-1β in renal epithelial cells is sufficient to induce EMT.

**Figure 3.** Ablation of HNF-1β activates an EMT transcriptional network in vivo. (A) Immunoblot showing increased levels of Twist, Snail1, Snail2, Zeb1, and Zeb2 and unchanged levels of E-cadherin in protein lysates from wild-type (WT) and HNF-1β mutant (KO) kidneys (n=5). GAPDH was used as a loading control. (B–G) Protein bands were quantified by Image Studio Lite software (LI-COR). Relative abundance was normalized to GAPDH. Error bars indicate SD. *P<0.05. (H and I) Immunohistochemical staining using an (H) anti-Twist antibody or (I) anti-Snail antibody on P28 kidney sections from control mice (left) and HNF-1β mutant mice (right) (n=3). Positive staining is indicated in brown. Strong staining is seen in nuclei of epithelial cells (arrowheads) lining the cysts (cy). Scale bars, 10 μm. KO, knockout; WT, wild-type.

Ablation of HNF-1β Induces Partial EMT In Vivo

To determine whether ablation of HNF-1β also induces EMT in vivo, we measured the expression of master EMT transcription factors in kidneys from HNF-1β mutant mice. For these experiments, we utilized Ksp/Cre;Hnf1bF/F mice in which the first exon of Hnf1b is deleted by Cre/loxP recombination specifically in renal tubules. Previous studies have shown that kidney-specific deletion of Hnf1b results in the formation of kidney cysts and rapid decline in kidney function. Kidneys were harvested from wild-type and mutant mice at P28, and protein lysates were analyzed by immunoblotting and quantified by band densitometry. Like the findings in HNF-1β-deficient mIMCD3 cells, the expression of EMT transcription factors Twist, Snail1, Snail2, Zeb1, and Zeb2 was significantly higher in kidneys from HNF-1β mutant mice compared with wild-type kidneys (Figure 3, A–G). However, in contrast to HNF-1β mutant cells, the expression of E-cadherin was not significantly altered in mutant kidneys. To confirm the presence of EMT in epithelial cells, we performed immunohistochemistry on kidney sections using antibodies against Twist and Snail. As shown in Figure 3, H and I, the nuclei of cyst epithelial cells in HNF-1β mutant kidneys showed more intense staining for Twist and Snail proteins compared with wild-type tubular epithelial cells. Taken
together, these results suggest that ablation of HNF-1β in vivo results in partial EMT, as evidenced by induction of EMT transcription factors but preserved expression of E-cadherin.

Twist2 Is a Novel EMT Transcription Factor Regulated by HNF-1β

Knowledge-based IPA suggested that the induction of EMT genes in HNF-1β–deficient mIMCD3 cells could be explained by upstream activation of the transcription factor Twist1 (Supplemental Figure 4). Twist1 and its homolog Twist2 constitute a family of basic-helix–loop-helix transcription factors that are highly expressed in mesenchymal cells, bind to an E-box sequence in DNA, are essential for the development of mesodermal tissues, and promote EMT and cancer metastasis.34

Analysis of our previous ChIP-seq data20 indicated that Twist1 was unlikely to be regulated by HNF-1β (data not shown). In contrast, Twist2, which encodes a protein with similar DNA-binding properties, contained two potential binding sites for HNF-1β. One site (HNF-1β BSI) was located in the first intron 16 kb downstream from the transcription start site and a second site (HNF-1β BSII) was located in the 3' flanking region 4 kb downstream from the gene body (Figure 4A). Using quantitative ChIP, we validated the binding of HNF-1β to the Twist2 locus in wild-type mIMCD3 cells
Compared with control IgG, immunoprecipitation with an HNF-1β antibody resulted in enrichment of chromatin containing BSI and BSII but no enrichment of an irrelevant genomic region. No significant binding was detected in HNF-1β-deficient mIMCD3 cells, further indicating that the binding was specific. To determine whether the binding of HNF-1β was functionally significant, we measured Twist2 mRNA transcripts by quantitative RT-PCR (Figure 4E). The level of Twist2 transcripts was significantly increased in HNF-1β mutant mIMCD3 cells compared with wild-type cells, suggesting that HNF-1β normally represses Twist2 transcription (Figure 4E).

Figure 5. Ablation of Twist2 rescues the EMT phenotype in HNF1β-deficient cells. (A) Representative phase-contrast images of wild-type mIMCD3 (WT), HNF-1β knockout cells (KO), and HNF-1β and Twist2 double knockout cells (DKO). Scale bar, 100 μm. (B–G) Quantitative RT-PCR showing normalized expression of E-Cadherin, TGF-β2, TGF-β3, Snail2, Zeb1, and Zeb2 in HNF-1β and Twist2 double knockout cells (DKO) compared with HNF-1β single knockout cells (KO). WT indicates expression in wild-type mIMCD3 cells. (H) Cell migration measured by wound healing assays in wild-type (WT), HNF-1β mutant (KO), and HNF-1β and Twist2 double knockout (DKO) cells. (I) Cell invasiveness measured by Transwell assays in wild-type (WT), HNF-1β mutant (KO), and HNF-1β and Twist2 double knockout (DKO) cells. Data shown represent mean±SEM from three independent experiments. *P<0.05.
Previous studies showed that Twist1 regulates the expression of Snai2 (Slug), a zinc-finger transcription factor that is upregulated during EMT. Because the expression of Snai2 is elevated in HNF-1β mutant cells, and Twist1 and Twist2 share similar DNA-binding properties, we tested whether Twist2 can also regulate Snai2 transcription. We used lentiviruses to generate mIMCD3 cell lines stably expressing HA-tagged Twist2, or HA-tagged GFP as a negative control. Quantitative ChIP with an anti-HA antibody demonstrated that Twist2 (but not GFP) was bound to the Snai2 promoter (Figure 4F). Using CRISPR-based gene editing, we deleted Twist2 in mIMCD3 cells. Quantitative RT-PCR showed that the expression of Snai2 was reduced in Twist2-deficient mIMCD3 cells compared with wild-type cells. (Figure 4G). Taken together, these studies identify Snai2 as a novel transcriptional target of Twist2. Upregulation of Twist2 and subsequent activation of Snai2 may underlie the induction of EMT in HNF-1β-deficient renal epithelial cells.

Ablation of Twist2 Rescues the Fibroblastic Phenotype of Hnf1b Null Cells

To determine whether Twist2 is required for the induction of EMT, we generated HNF-1β and Twist2 double-knockout cells using CRISPR-based gene editing. Ablation of Twist2 in HNF-1β-deficient cells converted the cells to a more epithelial morphology (Figure 5A). Quantitative RT-PCR showed that ablation of Twist2 restored the normal expression of E-cadherin and reduced the overexpression of EMT transcription factors Snai2, Zeb1, and Zeb2 (Figure 5, B and E–G). Importantly, the expression of TGF-β ligands TGF-β2 and TGF-β3 was also increased in HNF-1β-deficient cells and was reduced in double-knockout cells (Figure 5, C and D). Functionally, HNF-1β and Twist2 double-knockout cells migrated more slowly and were less invasive compared with HNF-1β single-knockout cells (Figure 5, H and I). These findings indicate that the induction of EMT in HNF-1β-deficient mIMCD3 cells is Twist2-dependent.

HNF-1β Mutant Kidneys Develop Interstitial Fibrosis through a Cell-Nonautonomous Mechanism

Previous studies have shown that collecting duct-specific deletion of HNF-1β using the Pkhdl1/Cre deleter strain produces renal fibrosis. To ablate HNF-1β in additional nephron segments, we generated kidney HNF-1β mutant mice using the Ksp/Cre deleter strain, which expresses Cre recombinase in all nephron segments and collecting ducts. Trichrome staining showed that significant interstitial fibrosis was present in kidneys from Ksp/Cre;Hnf1bfl/fl mice at age 28 days (Supplemental Figure 5A). Quantitative RT-PCR showed that mRNA transcripts encoding the mesenchymal marker vimentin and the myofibroblast marker α-smooth muscle actin (α-SMA) were upregulated in HNF-1β mutant kidneys, whereas the expression of E-cadherin, a canonical marker of epithelial cell polarity, was unchanged (Supplemental Figure 5, B–D). Consistent with these results, immunohistochemistry showed accumulation of vimentin and α-SMA in the interstitium and maintenance of E-cadherin in the epithelium (Supplemental Figure 5, B–D).

Earlier studies suggested that EMT is a source of myofibroblasts that are responsible for renal interstitial fibrosis. However, more recent studies suggest that myofibroblasts originate from resident interstitial cells, which may include fibroblasts, pericytes, endothelial cells, and macrophages. To test whether myofibroblasts in HNF-1β mutant kidneys originate by EMT, we performed genetic cell fate mapping. First, we used Cre/loxP recombination to label renal tubular epithelial cells with an enhanced yellow fluorescent protein (EYFP) reporter gene. Kidney sections were costained with antibodies against HNF-1β and GFP (which also recognizes EYFP). In wild-type mice, EYFP was expressed exclusively in renal tubules that also expressed HNF-1β (Supplemental Figure 6A). Next, we generated Ksp/Cre;Hnf1bfl/fl;R26R-EYFP mice in which HNF-1β mutant renal epithelial cells were labeled with EYFP. Antibody staining showed that EYFP was expressed in cyst epithelial cells that lacked HNF-1β and was not expressed in surrounding non-cystic tubules in which the expression of HNF-1β was maintained (Supplemental Figure 6B).

To assess whether HNF-1β mutant cells migrate into the interstitium, we costained kidney sections with antibodies against EYFP and the basement membrane marker entactin. EYFP-positive cells were located exclusively on the luminal side of the basement membrane and were not detected in the renal interstitium, indicating that the mutant cells did not migrate out of the tubules (Supplemental Figure 6C). To determine whether HNF-1β-deficient epithelial cells can transdifferentiate into myofibroblasts, we costained kidney sections with antibodies against EYFP and α-SMA. As shown in (Supplemental Figure 6D), α-SMA–positive cells were present in the interstitium but did not express EYFP, indicating that they were not derived from HNF-1β mutant epithelial cells. Taken together, these findings indicate that HNF-1β mutant epithelial cells undergo EMT but do not transdifferentiate into myofibroblasts in vivo.

Aberrant TGFβ Signaling Underlies Interstitial Fibrosis in HNF-1β Mutant Kidneys

The preceding studies suggested that renal myofibroblasts originate via a cell-nonautonomous mechanism in which HNF-1β mutant kidney tubules produce signals that activate interstitial cells. RNA-seq analysis of HNF-1β-deficient mIMCD3 cells showed increased expression of profibrotic signaling molecules TGF-β2 and TGF-β3 (Figure 5, C and D). Quantitative RT-PCR analysis confirmed upregulation of Tgfβ2 and Tgfβ3 mRNA transcripts in HNF-1β mutant kidneys (Figure 6, A and B). In contrast, the expression of TGF-β1 was only slightly increased in mutant cells and kidneys, and the increase was not statistically significant (Supplemental Figure 7). To confirm that TGF-β2 and TGF-β3 were upregulated in epithelial cells, we performed in situ hybridization (RNAscope).
Transcripts encoding TGF-β2 and TGF-β3 were strongly expressed in the cyst epithelium of HNF-1β mutant kidneys compared with wild-type tubules, consistent with the cell line model results and further supporting the EMT phenotype of HNF-1β mutant cells (Figure 6, C and D).

In the canonical TGF-β signaling pathway, active TGF-β ligands bind to the TGF-β receptor type II (TβRII), resulting in activation of the TGF-β receptor type I (TβRI) kinase and phosphorylation of downstream effectors Smad2 and Smad3. Phosphorylated Smad2 and Smad3 bind to Smad4 forming a heteromeric complex that translocates to the nucleus and activates transcription of ECM genes. Immunoblot analysis with phosphospecific antibodies showed increased phosphorylation of Smad2 and Smad3 in protein lysates from HNF-1β mutant kidneys (Figure 7A). The levels of total Smad2 and Smad3 were also increased. Antibody staining showed increased phospho-Smad3 within the nuclei of both cyst epithelial cells and interstitial cells in HNF-1β mutant kidneys compared with wild-type tubules (Figure 7B). Costaining with antibodies against vimentin and α-SMA showed that phospho-Smad3 accumulated in the nuclei of interstitial fibroblasts and myofibroblasts, respectively (Supplemental Figure 8). Consistent with activation of downstream TGF-β signaling, staining with an antibody that recognizes both the phosphorylated and dephosphorylated forms of the protein showed that Smad3 was primarily cytoplasmic in wild-type kidneys, whereas it was present in the nucleus in mutant kidneys (Figure 7C). These results indicate hyperactivation of canonical TGF-β signaling in HNF-1β mutant kidneys.

To confirm the epithelial–mesenchymal crosstalk, we measured TGF-β2 levels in conditioned medium using ELISA. The levels of immunoreactive TGF-β2 were elevated in conditioned medium from HNF-1β-deficient mIMCD3 cells compared with wild-type cells (Figure 7D). To confirm that the secreted TGF-β2 was biologically active, we incubated the conditioned medium with normal rat kidney fibroblasts (NRK-49F) and measured Smad3 phosphorylation. Incubation with conditioned medium from HNF-1β-deficient mIMCD3 cells produced greater Smad3 phosphorylation than wild-type cells (Figure 7E). Total Smad3 was unchanged, and treatment with the TβRI (ALK5) inhibitor SB431542 reduced Smad3 phosphorylation. Taken together, these findings indicate that ablation of HNF-1β activates a transcriptional network that results in increased production of TGF-β2 and TGF-β3, which signal in a paracrine manner to interstitial cells and thereby stimulate renal fibrosis (Figure 8).

**DISCUSSION**

Germline mutations of the transcription factor HNF-1β produce a spectrum of inherited human diseases. First
Figure 7. TGFβ signaling is aberrant in HNF-1β mutant kidneys. (A) Immunoblot analysis showing increased phosphorylation of Smad2 and Smad3 and increased total levels of Smad2 and Smad3 in kidneys from Ksp/Cre;Hnf1b<sup>−/−</sup> mice (KO) and wild-type littermates (WT) at P28. β-actin was used as a loading control; n=5. (B) Indirect immunofluorescence of kidney sections from Ksp/Cre;Hnf1b<sup>−/−</sup> mice (KO) and wild-type littermates (WT) at P28. β-actin was used as a loading control; n=5.
identified as a cause of maturity-onset diabetes of the young type 5,39 mutations of HNF1B are strongly associated with congenital cystic kidney diseases, including multicystic dysplastic kidneys and glomerulocystic kidney disease. The association of maturity-onset diabetes of the young type 5 with cystic kidney abnormalities has been coined renal cysts and diabetes.40 A link between HNF1B mutations and cyst formation was strengthened by studies showing that HNF-1β directly controls the transcription of the autosomal dominant polycystic kidney disease gene PKD2 and the autosomal recessive polycystic kidney disease gene PKHD1.14 Transgenic expression of dominant-negative mutant HNF-1β in mice confirmed the causal association between mutations of HNF1B and cystic kidney disease.18 ChIP-seq experiments have revealed that HNF-1β plays a central role in a transcriptional network that regulates the expression of multiple cystogenes. Besides PKD2 and PKHD1, HNF-1β regulates the transcription of UMOD, which is mutated in medullary cystic kidney disease; CYS1, which is mutated in the cpk mouse model of ARPKD; and KIF12, a cpk modifier gene.14 The development of kidney cysts in individuals with HNF1B mutations can be attributed to global inhibition of this transcriptional network.

In addition to the formation of kidney cysts, HNF-1β mutant kidneys are characterized by increased interstitial fibrosis.21 A similar phenotype is seen in humans with heterozygous mutations of UMOD, MUC1, REN, and SEC61A1, which has led to the recent nosology of ADTKD.1 The resulting clinical manifestations include small kidneys, absence of significant hematuria or proteinuria, slowly progressive decline in GFR, and tubular abnormalities such as hyperuricemia, hypomagnesemia, and reduced urinary concentrating ability. The mechanism of renal fibrosis in ADTKD is poorly understood. Previous studies of UMOD-related ADTKD have shown that impaired trafficking of mutant uromodulin produces ER stress, activation of the UPR, and secondary mitochondrial dysfunction.15,26,27 Although the transcription of UMOD is regulated by HNF-1β, it is unlikely that this mechanism is responsible for the phenotype of HNF1B-related ADTKD because most patients with UMOD-related ADTKD harbor missense mutations, which produce misfolded proteins that accumulate in the ER,27 whereas patients with HNF1B-related ADTKD have wild-type UMOD sequence.

Figure 8. Tubular-interstitial crosstalk mediates renal fibrosis in HNF1B-related ADTKD. Normally, HNF-1β binds to the Twist2 locus and inhibits Twist2 expression. Loss of HNF-1β results in depression of Twist2 and activation of its downstream target Snail2. Snail2 is known to increase TGF-β3 production through activation of β-catenin/TCF4 and may also regulate TGF-β2 expression. Excess TGF-β2 and TGF-β3 produced by mutant epithelial cells signals to interstitial cells (green) where activation of TGF-β receptors leads to Smad2/3 phosphorylation and increased fibrogenesis.

Here, we used HNF-1β mutant cells and mice to identify a novel mechanism of renal fibrosis in HNF1B-related ADTKD (Figure 8). We show that ablation of HNF-1β in renal epithelial cells results in derepression of its target gene, Twist2, which in turn causes upregulation of Snail2 and its downstream...
targets, TGF-β3 and TGF-β2. Increased TGF-β2 and TGF-β3 produced in renal epithelial cell signal to interstitial cells, where activation of Smad proteins is known to stimulate fibrogenesis.41 Consistent with this model, cell-lineage analysis demonstrated that renal fibroblasts in HNF-1β mutant kidneys arise via a cell–nonautonomous mechanism. Although HNF-1β-deficient epithelial cells do not directly generate interstitial myofibroblasts, expression of collagen genes is upregulated and may contribute to the expansion of extracellular matrix (Supplemental Figure 1).

EMT refers to a process in which epithelial cells undergo phenotypic changes, including loss of cell–cell adhesion and apical–basal polarity, and acquire mesenchymal characteristics, such as spindle-shaped morphology, increased motility, and loss of contact inhibition.42 EMT is essential for normal ontogeny, wound healing, and tissue regeneration, but sustained EMT is associated with tissue fibrosis.43 Cancer cells that undergo EMT produce distant metastasis. The converse process, mesenchymal–epithelial transition, is responsible for the conversion of metanephric mesenchyme into renal tubules during nephrogenesis. EMT is regulated by transcription factors such as Twist1, Twist2, Snail1, Snail2, Zeb1, Zeb2, and signaling pathways such as TGF-β, Wnt/β-catenin, Hedgehog, Notch, and receptor tyrosine kinases.44 HNF-1β has previously been implicated in EMT in renal fibrosis and cancer.45,46 A dominant negative form of HNF-1β has previously been implicated in EMT in renal tubules during nephrogenesis. EMT is regulated by transcription factors such as Twist1, Twist2, Snail1, Snail2, Zeb1, and Zeb2, and signaling pathways such as TGF-β, Wnt/β-catenin, Hedgehog, Notch, and receptor tyrosine kinases.44 HNF-1β has previously been implicated in EMT in renal fibrosis and cancer.45,46 A dominant negative form of HNF-1β induces EMT when expressed in an ovarian carcinoma cell line SKOV3.47 HNF-1β cooperates with ERBB2 to induce EMT in NMuMG cells.46 However, the precise mechanism whereby loss of HNF-1β induces EMT is poorly understood. Here, we show that loss of HNF-1β by itself is sufficient to induce EMT in cultured renal epithelial cells, as evidenced by changes in cell morphology, increased motility, increased invasiveness, decreased expression of E-cadherin, and induction of an EMT gene signature. Importantly, HNF-1β mutant epithelial cells also exhibit EMT in vivo, as evidenced by increased expression of EMT transcription factors and TGF-β ligands. However, the expression of E-cadherin was unchanged, which contrasts with the decreased expression in HNF-1β mutant cells. The in vivo phenotype suggests partial EMT, in which epithelial cells dedifferentiate and express both epithelial and mesenchymal characteristics.38,48 Similar findings of complete EMT in vitro and partial EMT in vivo have been made previously. For example, overexpression of Twist1 or Snail1 induces complete EMT in cultured epithelial cells but partial EMT with preserved E-cadherin expression in tissues and organoids.49,50 The absence of complete EMT explains why HNF-1β mutant epithelial cells fail to differentiate into myofibroblasts and migrate into the renal interstitium.

RNA-seq analysis of HNF-1β-deficient mIMCD3 cells showed increased expression of EMT transcription factors Twist2, Snail1, Snail2, and Zeb2. Integration of this RNA-seq dataset with our published ChIP-seq dataset20 identified Twist2 as a novel HNF-1β–regulated gene. We found that HNF-1β binds to two sites in the Twist2 gene and inhibits Twist2 expression in mouse tubular epithelial cells. Importantly, ablation of Twist2 in HNF-1β–deficient cells reduced cell migration and invasiveness and normalized the expression of EMT transcription factors and TGF-β ligands, indicating that EMT is Twist2-dependent. Twist2 has previously been identified as a “master” transcription factor in EMT. Twist2 is upregulated during EMT, and ectopic expression of Twist2 in ovarian cancer cells is sufficient to induce EMT.51 Twist2 is homologous to Twist1, which is well studied for its roles in EMT and cancer progression. Although Twist1 and Twist2 are highly conserved and contain similar protein motifs encoded by a single exon,52 they have distinct functions and different knockout phenotypes.53 By IHC, we detected increased nuclear Twist and Snail proteins in HNF-1β mutant kidneys, indicating activation of an EMT transcriptional network in cyst lining cells. In Drosophila, Twist has been shown to activate Snail,54 and human Twist1 activates Snail2 in carcinoma cells.55 Here, we demonstrated that the homologous protein Twist2 binds to the Snai2 promoter and regulates its expression in kidney epithelial cells. Collectively, these studies demonstrate that HNF-1β regulates EMT expression in mouse tubular epithelial cells. Importantly, ablation of Twist2 in HNF-1β–deficient cells reduced cell migration and invasiveness and normalized the expression of EMT transcription factors and TGF-β ligands, indicating that EMT is Twist2-dependent. Twist2 has previously been identified as a “master” transcription factor in EMT. Twist2 is upregulated during EMT, and ectopic expression of Twist2 in ovarian cancer cells is sufficient to induce EMT.51 Twist2 is homologous to Twist1, which is well studied for its roles in EMT and cancer progression. Although Twist1 and Twist2 are highly conserved and contain similar protein motifs encoded by a single exon,52 they have distinct functions and different knockout phenotypes.53 By IHC, we detected increased nuclear Twist and Snail proteins in HNF-1β mutant kidneys, indicating activation of an EMT transcriptional network in cyst lining cells. In Drosophila, Twist has been shown to activate Snail,54 and human Twist1 activates Snail2 in carcinoma cells.55 Here, we demonstrated that the homologous protein Twist2 binds to the Snai2 promoter and regulates its expression in kidney epithelial cells. Collectively, these studies demonstrate that HNF-1β regulates EMT by repressing transcription of Twist2, thereby inhibiting its downstream target Snai2 (Figure 8).

HNF-1β was first implicated in renal fibrosis by studies showing that the HNF1B gene promoter is repressed by Snail1.45 Fibrosis induced by Snail1 was attributed to decreased expression of HNF-1β, which leads to downregulation of Ksp-cadherin (Cadherin-16), a putative cell adhesion protein that we previously identified as an HNF-1β–dependent gene. However, a Cdh16 knockout phenotype has not been reported, so the significance of this mechanism remains unclear. Recently, we reported the identification of the members of the epithelial-specific miR-200 family of microRNAs as novel transcriptional targets of HNF-1β.55 HNF-1β binds to two evolutionarily conserved sites located 28 kb upstream to miR-200b and regulates the transcription of a 28-kb long noncoding RNA encoding the miR-200b/200a/429 cluster. The expression of the miR-200 cluster was decreased in kidney epithelial cells expressing dominant-negative mutant HNF-1β and in HNF-1β mutant kidneys, resulting in the upregulation of Zeb2. These findings suggest that HNF-1β may regulate EMT through both transcriptional and post-transcriptional mechanisms.

Interstitial fibrosis is a common feature of ADTKD and other CKDs. Sustained activation of fibroblast and myofibroblast cell populations within the interstitium results in laying down of excessive extracellular matrix causing renal failure.56 The origin of activated myofibroblasts is controversial.58 Cell lineage analysis in mice subjected to unilateral ureteral obstruction suggested that myofibroblasts were derived from kidney tubular epithelial cells through a process of EMT.37 Other studies have suggested that myofibroblasts originate from fibrocytes, pericytes, macrophages, or endothelial cells.57,58 The disparate findings could reflect differences in the nephron segments or fibrosis models that were studied. The results presented here in a mouse model of HNF1B-
related ADTKD indicate that interstitial myofibroblasts do not originate from tubular epithelial cells. Although kidney epithelial transdifferentiation does not generate myofibroblasts in HNF1B-related ADTKD, partial EMT may promote renal fibrosis, as proposed by two recent studies.49,59

Consistent with a cell-nonautonomous mechanism of fibrosis, HNF-1β mutant epithelial cells showed marked upregulation of profibrotic signaling molecules TGF-β2 and TGF-β3 both in vitro and in vivo. Expression of TGF-β1 was slightly increased in mutant cells and kidneys compared with controls, but the difference was not statistically significant. These results suggest that TGF-β2 and TGF-β3 are the major TGF-β ligands that are dysregulated in HNF-1β mutant kidneys. TGF-β signaling is a central mechanism for renal fibrosis through the activation of genes required for fibrogenesis41,60 and the initiation and maintenance of EMT.43 In situ hybridization confirmed that Tgfβ2 and Tgfβ3 mRNA transcripts were upregulated in HNF-1β mutant epithelial cells in the kidney. Increased mRNA expression is likely due to upregulation of Snai2, which is known to stimulate Tgfβ3 transcription through induction of β-catenin/TCF4 complexes.62

Both TGF-β2 and TGF-β3 have been implicated in renal fibrosis, although antifibrotic activity has also been described.63 Consistent with profibrotic activity, we observed increased phosphorylation and nuclear translocation of Smads3 in interstitial cells in HNF-1β mutant kidneys. We further showed that conditioned medium from HNF-1β knockout cells could stimulate TβRII-dependent phosphorylation of Smad3 in cultured fibroblasts. These results support epithelial/myofibroblast crosstalk as an important mechanism of renal fibrosis in HNF1B-related ADTKD (Figure 8).64 Previous studies have shown that transgenic overexpression of TGF-β1 in renal tubules increases renal fibrosis65 and knock-out of TβRII in SMA-positive myofibroblasts, but not fibroblasts, reduces fibrosis.58,66 Besides TGF-β, epithelial cells undergoing EMT can produce other cytokines and signaling molecules that promote renal fibrosis.38 In this regard, PDGF ligands and receptors were also upregulated in HNF-1β null cells (data not shown). Phosphorylation of Smads was also observed in the HNF-1β mutant epithelium itself, suggesting that TGF-β may signal in an autocrine manner. Because TGF-β can induce Snail proteins, such signaling would provide a feed-forward mechanism for potentiating EMT and further stimulating renal fibrosis.61 Alternatively, autocrine TGF-β signaling may be protective, as evidenced by increased fibrosis after collecting duct-specific ablation of TβRII.67

In summary, we have identified a novel EMT transcriptional network in renal epithelial cells. Mutation of HNF-1β upregulates this network through depression of Twist2 and its transcriptional target Snail2. Renal interstitial fibrosis arises through a cell-nonautonomous mechanism that may involve aberrant TGF-β signaling. Targeting this new axis may provide opportunities for treating HNF1B-related ADTKD and other kidney diseases associated with decreased expression of HNF-1β.

ACKNOWLEDGMENTS

We thank Sophia Vrba, Shayan Farahani, Alan Mickelson, and Steffan Nye for expert technical assistance; Colleen Forster for her excellent histology support and consultation (Bionet, Clinical and Translational Science Institute, University of Minnesota); and Dr. Brian Fife (University of Minnesota) for assistance with laser scanning confocal microscopy.

S.C., A.S., S.A., J.H., and K.A. conducted experiments. Y.Z. conducted bioinformatics analysis. M.P. provided the HNF-1β floxed mice. S.C.C. and P.I. designed studies, analyzed data, and wrote the manuscript.

This work was supported by National Institutes of Health grant R37DK042921 (to P.I.).

DISCLOSURES

None.

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