Effects Therefore, it is imperative to explore the molecular and immune responses; thus, directly targeting TGF-β process, such as cell apoptosis, proliferation, differentiation and In addition to renal fibrosis, which is characterized by the excessive deposition of extracellular matrix in the kidney, is the common final pathologic pathway of nearly all chronic kidney diseases and leads to end-stage renal failure [2]. Although great efforts have been put into finding the molecular and cellular regulators of kidney fibrosis in recent years, there are currently no effective therapies to prevent the onset or progression of renal fibrosis [1]. Many studies have established TGF-β as the master regulator of renal fibrosis [50]. In addition to renal fibrosis, TGF-β regulates many other biological process, such as cell apoptosis, proliferation, differentiation and immune responses; thus, directly targeting TGF-β may have adverse effects [50]. Therefore, it is imperative to explore the molecular and cellular mechanisms underlying TGF-β-induced renal fibrosis, which might provide new treatment strategies. TGF-β is the most important inducer of epithelial-to-mesenchymal transition (EMT) in embryogenesis, fibrosis, and cancer [3], and EMT is the key mechanism underlying TGF-β-driven renal fibrosis [50]. Renal EMT is a process in which renal tubular epithelial cells (RTECs) lose epithelial cell markers, such as E-cadherin or Ksp-cadherin, and gain mesenchymal cell markers, such as N-cadherin, fibronectin, vimentin. RTECs are the major constituents of the renal parenchyma and are often the target in kidney injury [4]. Many studies have observed the phenomenon of EMT and its role in renal fibrosis [5], Genetic ablation of key TGF-β-induced EMT targets Tgfb2 [6], Smad4 [7], Snai1 [8] and Twist1 [9], specifically in RTECs, can prevent the progression of renal fibrosis. Consistently, overexpressing Snai1 in tubular epithelial cells induces fibrosis [10]. Partial EMT, a status that RTECs do not transdifferentiate into interstitial fibroblasts but remain integrated in the tubules, could induce RTECs dysregulation of absorption, secretion, cell cycle and repair [11]. Partial EMT is one of the important mechanisms for renal fibrosis progression [8,9,11]. TGF-β–induced renal fibrosis and EMT includes both a Smad-dependent pathway, which involves the activation of Smad2/3/4, and Smad-independent pathways, including the activation of JNK, p38, JNK, and p38, and other signaling pathways such as PI3K/Akt and Smad3, which are involved in the regulation of EMT and renal fibrosis [12]. Therefore, understanding the molecular mechanisms underlying TGF-β-induced renal fibrosis and EMT is crucial for developing new therapeutic strategies.

1. Introduction

Chronic kidney disease is one of the leading cause of death worldwide, is highly prevalent in many countries, and is becoming a major public health problem [1]. Renal fibrosis, which is characterized by the excessive deposition of extracellular matrix in the kidney, is the common final pathologic pathway of nearly all chronic kidney diseases and leads to end-stage renal failure [2]. Although great efforts have been put into finding the molecular and cellular regulators of kidney fibrosis in recent years, there are currently no effective therapies to prevent the onset or progression of renal fibrosis [1]. Many studies have established TGF-β as the master regulator of renal fibrosis [50]. In addition to renal fibrosis, TGF-β regulates many other biological process, such as cell apoptosis, proliferation, differentiation and immune responses; thus, directly targeting TGF-β may have adverse effects [50]. Therefore, it is imperative to explore the molecular and cellular mechanisms underlying TGF-β-induced renal fibrosis, which might provide new treatment strategies.

Renal fibrosis is widely considered a common mechanism leading to end-stage renal failure. Epithelial-to-mesenchymal transition (EMT) plays important roles in the pathogenesis of renal fibrosis. Run-related transcription factor 1 (RUNX1) promotes TGF-β-induced renal tubular epithelial-to-mesenchymal transition (EMT) and renal fibrosis through the PI3K subunit p110δ.

**Research Paper**

**Runt-Related Transcription Factor 1 (RUNX1) Promotes TGF-β-Induced Renal Tubular Epithelial-to-Mesenchymal Transition (EMT) and Renal Fibrosis through the PI3K Subunit p110δ**

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**A B S T R A C T**

Renal fibrosis is widely considered a common mechanism leading to end-stage renal failure. Epithelial-to-mesenchymal transition (EMT) plays important roles in the pathogenesis of renal fibrosis. Run-related transcription factor 1 (RUNX1) promotes TGF-β-induced renal tubular epithelial-to-mesenchymal transition (EMT) and renal fibrosis through the PI3K subunit p110δ.

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**1. Introduction**

Chronic kidney disease is one of the leading cause of death worldwide, is highly prevalent in many countries, and is becoming a major public health problem [1]. Renal fibrosis, which is characterized by the excessive deposition of extracellular matrix in the kidney, is the common final pathologic pathway of nearly all chronic kidney diseases and leads to end-stage renal failure [2]. Although great efforts have been put into finding the molecular and cellular regulators of kidney fibrosis in recent years, there are currently no effective therapies to prevent the onset or progression of renal fibrosis [1]. Many studies have established TGF-β as the master regulator of renal fibrosis [50]. In addition to renal fibrosis, TGF-β regulates many other biological process, such as cell apoptosis, proliferation, differentiation and immune responses; thus, directly targeting TGF-β may have adverse effects [50]. Therefore, it is imperative to explore the molecular and cellular mechanisms underlying TGF-β-induced renal fibrosis, which might provide new treatment strategies. TGF-β is the most important inducer of epithelial-to-mesenchymal transition (EMT) in embryogenesis, fibrosis, and cancer [3], and EMT is the key mechanism underlying TGF-β–driven renal fibrosis [50]. Renal EMT is a process in which renal tubular epithelial cells (RTECs) lose epithelial cell markers, such as E-cadherin or Ksp-cadherin, and gain mesenchymal cell markers, such as N-cadherin, fibronectin, vimentin. RTECs are the major constituents of the renal parenchyma and are often the target in kidney injury [4]. Many studies have observed the phenomenon of EMT and its role in renal fibrosis [5], Genetic ablation of key TGF-β–induced EMT targets Tgfb2 [6], Smad4 [7], Snai1 [8] and Twist1 [9], specifically in RTECs, can prevent the progression of renal fibrosis. Consistently, overexpressing Snai1 in tubular epithelial cells induces fibrosis [10]. Partial EMT, a status that RTECs do not transdifferentiate into interstitial fibroblasts but remain integrated in the tubules, could induce RTECs dysregulation of absorption, secretion, cell cycle and repair [11]. Partial EMT is one of the important mechanisms for renal fibrosis progression [8,9,11]. TGF-β–induced renal fibrosis and EMT includes both a Smad-dependent pathway, which involves the activation of Smad2/3/4, and Smad-independent pathways, including the activation of JNK, p38, JNK, and p38, and other signaling pathways such as PI3K/Akt and Smad3, which are involved in the regulation of EMT and renal fibrosis [12]. Therefore, understanding the molecular mechanisms underlying TGF-β-induced renal fibrosis and EMT is crucial for developing new therapeutic strategies.
ERK, and PI3K/Akt [12]. Many co-activators or co-repressors are known to interact with Smads, including the Runx family of transcription factors Runx1, Runx2 and Runx3 [13]. Previous studies have shown that Runx2 mediates the antipapoptotic effects of parathyroid hormone in proximal tubule cells [14] and that Runx3 is involved in regulating the expression of AT1 receptor-associated proteins in renal distal convoluted tubule cells [15]. Runx1 is critical for generating definitive hematopoietic stem cells via the Endothelial-to-Hematopoietic Transition (EHT) [16], which is conceptually similar to EMT. In addition, the role of Runx1 in non-immune cells has recently received great attention, such as lung epithelial cells [17], gastric epithelial cells [18], colon epithelial cells [19], hepatocytes [20], and mesenchymal cells [21]. However, the roles of Runx1 in TGF-β-induced EMT and renal fibrosis are still unclear.

In this study, we used a conditional knockout mouse model that specifically deleted Runx1 in proximal tubular epithelial cells and investigated whether and how Runx1 mediated renal fibrosis and EMT. Our results show that Runx1 expression was enhanced both in response to TGF-β-treatment and in renal fibrosis. Runx1 promoted TGF-β-induced partial EMT by increasing transcription of the PI3K subunit p110α. Deletion of Runx1 in RTECs protected the host against renal fibrosis induced by unilateral ureteral obstruction (UUO) or treatment with folic acid (FA).

2. Materials and Methods

2.1. Reagents

Antibodies against Runx1, Slug and N-cadherin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against Runx1 for IHC were from Abcam (Cambridge, MA, USA). Antibodies against GAPDH, and secondary HRP-conjugated goat anti-mouse and anti-rabbit IgG were purchased from Beyotime Biological Technology (Shanghai, China). Electrochemiluminescent (ECL) reagents were purchased from Thermo Fisher Scientific (San Jose, CA, USA). Recombinant human TGF-β was purchased from PeproTech (Rocky Hill, NJ, USA). P110α inhibitor CAL-101, PI3K inhibitor LY294002 and SMAD3 inhibitor SIS3 were purchased from Selleck Chemicals (Houston, TX, USA). Folic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). The siGENOME SMARTpool human Runx1 siRNA was obtained from Dharmacon (Lafayette, CO, USA). SMAD3, PTEN, ATP1B1, and PIK3CD siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Lipofectamine RNAiMAX and Lipofectamine 2000 were purchased from Invitrogen (Carlsbad, CA, USA). The Dual-Glo Luciferase Assay System was purchased from Promega (Madison, WI, USA). The RNAiso reagent was obtained from TaKaRa Ltd. (Kyoto, Japan).

2.2. Cell Culture

HEK 293T cells (kind gifts from Dr. J. F. Chen, SIBCB) and NRK-52E cells (Cell Bank, Chinese Academy of Sciences) were maintained in DMEM containing 10% FBS, penicillin (100 units/ml), streptomycin (100 μg/ml) and 1% L-glutamine. HK-2 cells (Cell Bank, Chinese Academy of Sciences) and RPTEC/TERT1 cells (Keli Biological Technology Co., Ltd) were maintained in DMEM/F12 containing 10% FBS, penicillin (100 units/ml), streptomycin (100 μg/ml) and 1% L-glutamine. HK-2 cells (5 × 10⁴/well) or RPTEC/TERT1 cells (5 × 10⁴/well) were seeded in 12-well plates and then stimulated with 5 ng/ml TGF-β for 24 h in the presence of SIS3 (5 μM), LY294002 (10 μM), or CAL-101 (1 μM). NRK-52E cells (1 × 10⁵/well) were seeded in 12-well plates and then stimulated with 20 ng/ml for 48 h.

2.3. Animal Models

Runx1loxP/loxP mice and γGT-Cre mice were obtained from the Jackson Laboratory. Runx1loxP/loxP mice are on C57BL/6 background and γGT-Cre mice are on the mixed C57BL/6 and BALB/c background. γGT-Cre mice were bred with Runx1loxP/loxP mice to generate a specific disruption of Runx1 in proximal tubular cells, and these mice were crossed for 5 generations to the C57BL/6 background. The littermates on the same genetic background including the WT control mice or Runx1−/− mice were used in the study. Tail DNA samples were genotyped with the following primer pairs:

γGT-Cre-F: GCTTCTTTGACTTCCAGAGTTC
γGT-Cre-R: CAGGGTGTATAGACGATCC;
Runx1loxP/loxP-F: GCTTCCAACTCGTGGTAAGCC
Runx1loxP/loxP-R: CTGATTGCCCCCTGGTGGACG.

Renal fibrosis was induced in a UUO model by cutting between two ligated points of the left ureter in mice as previously described [22], and sham operated mice underwent the same procedure except for the obstruction. Mice were injected intraperitoneally with folic acid (250 mg/kg body weight dissolved in 300 mM NaHCO₃) for the first 7 days (UUO model) or 30 days (FA model), and blood and kidneys were collected for various analyses. All animal procedures were conducted in strict accordance with institutional guidelines and were approved by the Institutional Animal Care and Use Committee of Shanghai Institute of Biochemistry and Cell Biology (IBCB0057).

2.4. Retroviral Transduction

To generate retroviral particles, 6 μg MigR1-RUNX1 (a gift from Dr. Shi Jingyi, Shanghai Institute of Hematology) was co-transfected with 6 μg pCL-10A1 into 293T cells by the calcium phosphate method. Then, retroviral supernatants were collected to infect HK-2 or NRK-52E cells to overexpress RUNX1, followed by FACS sorting of GFP+ cells. Cells that stably overexpressed RUNX1 and control cells were seeded in 12-well plates, and the cells were stimulated with 5 ng/ml or 20 ng/ml TGF-β1. Cells were collected for RT-qPCR or western blot analysis after 24 h.

2.5. siRNA Transfection

HK-2 or RPTEC/TERT1 cells (5 × 10⁴/well) were seeded in 12-well plates. After 24 h, they were transfected with 20 nM RUNX1, SIS3, PTEN, ATP1B1, PTEN + RUNX1 or ATP1B1 + RUNX1 siRNA and a non-specific siRNA using the Lipofectamine RNAiMAX kit according to the manufacturer’s instructions. After 36 h, HK-2 cells were stimulated with 5 ng/ml TGF-β1 for an additional 24 h. Cells were then prepared for RT-qPCR or western blot analysis.

2.6. RT-qPCR

Total RNA was extracted with RNAiso reagent, and cDNA was generated from 1 μg RNA using M-MLV reverse transcriptase (Promega, Madison, WI, USA) and then analyzed using RT-qPCR with SYBR Green Master Mix on a CFX-96 machine (Bio-Rad, CA, USA). Primer sequences [23,24,51] are listed in the Supplemental information.

2.7. Coimmunoprecipitation and Western Blot Analysis

HK-2 cells were stimulated with 5 ng/ml TGF-β1 for 1.5 h, and then the cell lysates were collected. Endogenous interactions between RUNX1 and SNAI1, Slug or Twist1 were detected by coimmunoprecipitation as previously described [24]. HK-2 cells were seeded in 12-well plates and stimulated with 5 ng/ml TGF-β1 for 6, 12, 24, 48 and 72 h, and then whole cell lysates were subjected to immunoblotting with anti-RUNX1, SNAI1, Slug, and N-cadherin antibodies.

2.8. Luciferase Assay

293 T (5 × 10³) cells were co-transfected with 0.5 μg of pcDNA3.1-SMAD3/A plasmid and 0.2 μg of CAGA luciferase reporter plasmid...
containing 1/20 Renilla luciferase plasmid with 0.5 μg of pcDNA3.1-RUNX1 or pcDNA3.1-GFP plasmid, using the Lipofectamine 2000 reagent. The cells were allowed to recover for 24 h and were then examined with a dual luciferase reporter assay system (Promega, USA).

2.9. Immunohistochemistry (IHC) and Masson Trichrome Staining (MTS)

Paraffin-embedded kidney sections were prepared as previously described [49] and then analyzed by immunohistochemistry staining using anti-RUNX1 antibodies according to the 2-step plus® Poly-HP HR Anti-Mouse/Rabbit IgG Detection System (ZSGB-BIO, Beijing, China). Tissue sections from kidneys were subjected to MTS (Shanghai Bogoo Biotechnology. Co., Ltd, Shanghai, China) according to the standard protocol.

2.10. Renal Function Evaluation

Serum was obtained from mice and analyzed for levels of blood urea nitrogen (BUN) by a AU5800 series Automatic Biochemical Analyzer (Beckman Kurt, USA).

2.11. Statistical Analysis

Statistical analysis was performed with GraphPad Prism 5, and statistically significant differences were determined by two-tailed Student’s t-tests or one way of analysis of variance.

3. Results

3.1. RUNX1 Expression Levels Are Increased in TGF-β-induced EMT and Renal Fibrosis

To better understand the effects of RUNX1 in TGF-β-induced EMT and renal fibrosis, we first examined the expression of RUNX1 in human renal tubular epithelial cell line HK-2 after stimulation with TGF-β. Compared to the unchanged expression of Runx2 and Runx3, only the RUNX1 mRNA levels were significantly increased by TGF-β treatment (Fig. 1a). In addition, we observed enhanced expression of RUNX1 and other EMT marker genes at the mRNA level, including CDH-2, SNAI1 and SLUG, which were induced by TGF-β in a time-dependent manner (Fig. 1b). We also observed the enhanced mRNA levels of profibrogenic PAI-1 by TGF-β treatment (Fig. 1b, right panel). In addition, the enhanced expression levels of RUNX1, SNAI1, SLUG and N-cadherin were confirmed at the protein level by western blotting assays (Fig. 1c).

Next, we explored the molecular mechanisms regulating RUNX1 expression in RTECs. Because SMAD3 is the key effector in TGF-β signaling [25], we used specific siRNA or inhibitors to target SMAD3. As expected, TGF-β treatment increased RUNX1 expression at the protein level in HK-2 and another human renal proximal tubule cell line RPTEC/TERT1 cells; in contrast, knockdown and inhibition of SMAD3 prevented the upregulation of RUNX1 expression induced by TGF-β, despite a minimal increase in RUNX1 in tubular cells (Fig. 1d).

Fig. 1. RUNX1 expression levels are increased in TGF-β-induced EMT and renal fibrosis. (a) HK-2 cells were stimulated with 5 ng/ml TGF-β for 24 h. RUNX1, RUNX2 and RUNX3 mRNA levels were detected by RT-PCR. Data are shown as the means ± SEM of three independent experiments. (b) HK-2 cells were stimulated with 5 ng/ml TGF-β for the indicated durations. Data are shown as the means ± SD of three independent experiments. (c) HK-2 cells were stimulated with 5 ng/ml TGF-β for the indicated durations to detect RUNX1, N-cadherin, SNAI1 and SLUG protein levels by immunoblotting. (d) HK-2 cells or RPTEC/TERT1 cells were transfected with SMAD3 siRNA (named sSD3) and nonspecific siRNA (named NC) or pretreated with the SMAD3 inhibitor SIS3 and control DMSO, followed by TGF-β stimulation to detect RUNX1 expression by immunoblotting. (e-g) Immunohistochemical and immunoblotting analysis of RUNX1 expression on day 7 after UUO, and Runx1, Tgfβ, Sna1 and Col1a1 mRNA levels by RT-qPCR in kidneys of UUO-induced and sham-control mice (n = 4). Data are shown as the mean ± SD of a representative of three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001.
epithelial cells without TGF-β stimulation (Fig. 1d). These findings indicate that RUNX1 expression is induced in renal tubular epithelial cells upon TGF-β treatment via a SMAD3-dependent mechanism.

Because TGF-β-induced EMT plays vital roles in regulating renal fibrosis [12], we further investigated the in vivo expression of RUNX1 in kidneys from a mouse model of renal fibrosis induced by unilateral ureteral obstruction (UUO) as previously described [22]. Compared to normal kidney structure in non-obstructed contralateral kidneys of UUO mice (hereafter designated contralat. in Fig. 1e), UUO treatment obstructed kidneys via the formation of an enlarged tubular lumen. Immunohistological analysis confirmed that RUNX1 expression was enhanced in renal tubular epithelial cells, rather than in the glomerulus, in UUO-treated mice (black arrows in Fig. 1e). In agreement with these in vitro findings, Runx1 expression at both the protein (Fig. 1e-f) and mRNA levels (Fig. 1g, left panel) was significantly increased in obstructed kidneys. In addition, we observed an upregulation of several genes related to EMT or fibrogenesis, including TGF-β, Snai1 and Col1a1, at the mRNA level in kidneys from UUO-treated mice (Fig. 1g).

3.2. RUNX1 is Required for TGF-β-Induced Renal Tubular EMT

To investigate the role of RUNX1 in EMT, we used specific siRNA to knock down RUNX1 in HK-2 cells. Light microscopy revealed that TGF-β-induced morphological changes in HK-2 cells from a cobblestone-like appearance to an elongated fibroblast-like shape. In contrast, this effect was abolished by the siRNA-mediated knock-down of RUNX1 expression (termed siRUNX1) in HK-2 cells (Fig. 2a). Western blotting analysis confirmed the knock-down efficiency of RUNX1 expression by siRNA (Fig. 2b, left panel). Furthermore, in response to TGF-β stimulation, siRUNX1-transfected HK-2 cells had profoundly diminished expression of EMT marker genes at the protein level, including N-cadherin, SNAI1 and SLUG, compared with controls (Fig. 2b, left panel). In addition, siRUNX1-transfected HK-2 cells decreased SLUG expression at the mRNA level (Fig. 2b, right panel).

To further confirm that RUNX1 promotes renal tubular EMT, we overexpressed RUNX1 in HK-2 cells. The overexpression levels of RUNX1 in HK-2 cells were evaluated by western blotting analysis (Fig. 2c, left panel). Overexpression of RUNX1 indeed enhanced the expression of N-cadherin and SNAI1 at the protein level (Fig. 2c, left panel).
and increased SLUG expression at the mRNA level, compared with controls (Fig. 2c, right panel).

We next overexpressed RUNX1 in the rat renal tubular epithelial cell line NRK-52E and confirmed that the overexpression of RUNX1 increased the expression of EMT marker genes, including SNAI1 at the protein level (Fig. 2d, left panel) and SLUG at the mRNA level (Fig. 2d, right panel). We also used siRNA to knock down RUNX1 in the human tubular epithelial cell line RPTEC/TERT1, and the results showed that knockdown of RUNX1 reduced N-cadherin expression at the protein level (Fig. 2e, left panel) and SLUG at the mRNA level (Fig. 2e, right panel). Thus, we used overexpression or knockdown strategies in different cells to demonstrate that RUNX1 promotes TGF-β-induced renal tubular EMT.

3.3. RUNX1 Promotes EMT Via p110δ-Mediated Akt Activation

SMAD3 plays a critical role in TGF-β-induced EMT, and previous reports have demonstrated a direct interaction between SMADs and RUNX1 [13]. We therefore examined the effect of RUNX1 on SMAD3 activation using a dual-luciferase reporter driven by Smad-binding CAGA elements. Unexpectedly, the co-expression of RUNX1 with SMAD3 in 293 T cells did not further increase CAGA reporter readings compared with 293 T cells transfected with SMAD3 alone (Fig. 3a, left panel). In addition, we found that overexpression of RUNX1 did not profoundly affect the phosphorylation levels of SMAD3 (Fig. 3a, right panel). We then investigated whether RUNX1 could interact with key effectors for EMT, including SNAI1, SLUG and TWIST1, by immunoprecipitation assays. However, we did not detect any interactions between RUNX1 and these EMT regulators (Fig. 3b).

Recent studies have reported that loss of ATP1B1, a Na/K-ATPase subunit, can promote renal EMT [26]. Since we observed that knockdown of RUNX1 in HK-2 cells promoted expression of ATP1B1 in the absence or presence of TGF-β treatment (Fig. 3c, middle lane), we next asked whether ATP1B1 could functionally cooperate with RUNX1 in EMT. Profound knockdown efficiency of ATP1B1 in HK-2 cells was confirmed by western blotting analysis (Fig. 3c, upper lane). Unexpectedly, knockdown of ATP1B1 did not reverse the reduced N-cadherin expression in siRUNX1 knock-down cells, with or without TGF-β treatment (Fig. 3c, upper lane).

Previous studies have reported that RUNX1 interacts with HIF-1α [27] and that HIF-1α promotes renal fibrosis via EMT [28]. We next asked whether RUNX1 affected HIF-1α expression in response to TGF-

Fig. 3. RUNX1 promotes EMT via p110δ-mediated Akt activation. (a, left panel) Plasmids for expressing GFP or RUNX1, together with plasmids for expressing SMAD3/4, were transfected into 293T cells in the presence of the CAGA luciferase reporter plasmid and the Renilla luciferase plasmid. After 24 h, cell lysates were prepared and analyzed by a dual luciferase reporter assay. Data are shown as the mean ± SD of a representative of three independent experiments. (a, right panel) NRK-52E cells were transfected with plasmids expressing RUNX1 or GFP, then stimulated with 20 ng/ml TGF-β for 30 min. RUNX1 and p-SMAD3 expression levels were detected by immunoblotting. (b) HK-2 cell lysates were immunoprecipitated with anti-SNAI1, SLUG or TWIST1 antibodies to detect endogenous interactions with RUNX1 by immunoblotting using anti-RUNX1 antibodies. (c-f) HK-2 cells were transfected with control siRNA, RUNX1 siRNA, or ATP1B1 siRNA as indicated, followed by stimulation with 5 ng/ml TGF-β for 24 h. Expression levels of N-cadherin and ATP1B1 (c) or HIF-1α and SMAD4 (d) or p110δ and phosphorylated AKT (e) or p110δ and phosphorylated ERK/p38 (f) were detected by immunoblotting. (g) RPTEC/TERT1 cells were transfected with RUNX1 siRNA or control siRNA, then stimulated with 5 ng/ml TGF-β for 24 h, p110δ, RUNX1 and Akt phosphorylation levels were detected by immunoblotting. (h) RUNX1 was knocked down or overexpressed in HK-2 cells to measure PIK3CD mRNA levels by RT-qPCR. Data are shown as the means ± SEM of three independent experiments. (i) HK-2 cells were stimulated with 5 ng/ml TGF-β for 24 h in the absence or presence of the PI3K inhibitor LY294002 (10 μM, named LY) or the p110δ inhibitor CAL-101 (1 μM, named CA). N-cadherin expression was detected by immunoblotting. (j, k) HK-2 or RPTEC/TERT1 cells were transfected with the control siRNA (named NC), RUNX1 siRNA (named siR), PTEN siRNA (named siP), then stimulated by 5 ng/ml TGF-β for 24 h. The mRNA levels of PTEN were detected by RT-qPCR (j). N-cadherin, RUNX1 and SNAI1 protein levels were detected by immunoblotting (k). Data are shown as the mean ± SD from three independent experiments. *P<0.05, **P<0.01.
β treatment. Although knockdown of RUNX1 reduced HIF-1α expression at the protein level in resting HK-2 cells, TGF-β treatment did not affect HIF-1α expression in control HK-2 cells or RUNX1 siRNA-treated HK-2 cells (Fig. 3d). Together, these results show that RUNX1 might not depend on SMAD3, ATP1B1 or HIF-1α to promote TGF-β-induced renal tubular EMT.

TGF-β signaling may also regulate EMT via a non-SMAD dependent pathway [12,50]. We therefore asked whether RUNX1 affected the activation of MAPKs and Akt by TGF-β. The results showed that RUNX1 knockdown decreased the Akt phosphorylation levels (Fig. 3e) but not the phosphorylation levels of ERK (Fig. 3f). Although the phosphorylation levels of p38 were increased in HK-2 cells with RUNX1 knock down, as the role of p38 in promoting EMT, it could not explain the molecular mechanism of RUNX1 promoting TGF-β-induced renal tubular EMT.

Because loss of phosphatase and tensin homolog deleted on chromosome ten (PTEN) may enhance Akt activation and promote EMT [29], we next examined the effect of RUNX1 on the PTEN/Akt pathway. We confirmed the knockdown efficiency of PTEN by the specific siRNA in HK-2 and RPTEC/TERT1 cells (Fig. 3i). As expected, siRNA-mediated knockdown of PTEN (named siP in Fig. 3k) enhanced TGF-β-induced expression levels of N-cadherin and SNAI1. Co-transfection of siRUNX1 and siPTEN (named siP+siR in Fig. 3k) attenuated the enhanced expression of N-cadherin and SNAI1, when compared to HK-2 and RPTEC/TERT1 cells transfected with siPTEN alone (Fig. 3k). These results indicate that RUNX1 promotes EMT by upregulating p110δ expression and Akt activation.

3.4. Tubule-Specific Deletion of Runx1 Ameliorates UUO or FA-Induced Renal Fibrosis

To further explore the role of RUNX1 in vivo, we generated conditional knockout mice with tubular epithelial cell-specific ablation of Runx1. Mice with the γGT-Cre locus, which express Cre recombinase uniformly in proximal tubular epithelial cells [30], were bred with homozygous Runx1-flxed mice. This could generate the mouse model with a specific disruption of Runx1 in proximal tubular cells (i.e. γGT-Cre−Runx1fl/fl or γGT-Cre−Runx1fl/+ mice, hereafter named Runx1fl/+ mice). Runx1fl/+ mice were fertile and showed no phenotypic abnormalities, compared to the control mice (γGT-Cre−Runx1fl/fl, hereafter designated WT mice). The littermates of the control mice or Runx1fl/+ mice were subjected to UUO induction.

After UUO induction, kidneys were enlarged and swollen, and there were no obvious differences in the appearance or morphology of

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**Fig. 4.** Tubule-specific deletion of Runx1 ameliorates UUO-induced renal fibrosis. (a) Morphological changes of kidneys in control or UUO-induced Runx1fl/+ and WT mice. (b-e) mRNA levels of Runx1, Col1a1, Col3a1, Fibronectin and Pai-1 (b) or Runx2 (c) or Slc22a6 (d), or Il-6 (e), or Pik3cd (f) were detected by RT-qPCR in kidneys of control and UUO-induced Runx1fl/+ and WT mice (n = 4). (g) Immunoblotting analysis showed expression levels of SNAI1, SLUG, VIMENTIN and α-SMA in kidneys of control or UUO-induced Runx1fl/+ and WT mice. (h) Representative images of MTS staining of kidneys from the indicated groups. Black bar: 50 μm. Data are shown as mean ± SD of a representative of three independent experiments. *P<0.05, **P<0.01, NS, not significant.
kidneys between Runx1<sup>cKO</sup> and WT mice (Fig. 4a). UUO treatment induced renal destruction in WT mice (Fig. 1e), which significantly enhanced mRNA expression levels of Runx1 and profibrogenic genes including Col1a1, Col3a1, Pai-1 and Fibronectin (Fig. 1e & Fig. 4b). In contrast, kidneys of Runx1<sup>cKO</sup> mice had significantly decreased mRNA expression levels of Runx1, Col1a1, Col3a1, Pai-1 and Fibronectin after UUO induction when compared to those in WT mice (Fig. 4b). Two independent groups previously reported the contradictory findings of Runx2 in renal fibrosis [14,31]. We therefore examined whether loss of RUNX1 could affect RUNX2 expression. Deletion of Runx1 in RTECs did not substantially affect the mRNA level of Runx2 mRNA in the UUO-induced renal fibrosis model (Fig. 4c). In addition, we checked the expression level of SLC22A6, an organic anion transporter that is involved in regulating toxicant metabolism, in kidneys from these UUO mouse models. Indeed, UUO induction significantly reduced SLC22A6 expression at the mRNA level compared to untreated healthy kidneys (Fig. 4d), and the genetic ablation of Runx1 in kidneys partially rescued SLC22A6 expression (Fig. 4d), which indicated that RUNX1 ablation improved tubular health.

In agreement with the improvement in kidney injury and fibrosis of Runx1<sup>cKO</sup> mice after UUO induction, mRNA levels of the inflammatory cytokine IL-6 were also decreased (Fig. 4e). We further found that the p110δ mRNA levels tended to be decreased in kidneys of Runx1<sup>cKO</sup> mice after UUO induction (Fig. 4f), although the difference between WT mice and Runx1<sup>cKO</sup> mice was not statistically significant. Nevertheless, the expression of EMT markers at the protein level, including SNAI1, SLUG, VIMENTIN and α-SMA was significantly decreased in kidneys of UUO-induced Runx1<sup>cKO</sup> mice compared to those in WT mice (Fig. 4g). Consistently, Masson trichrome staining (MTS) revealed a reduction of interstitial fibrosis in kidneys of Runx1<sup>cKO</sup> mice compared to WT mice after UUO induction (Fig. 4h).

To further determine the role of RUNX1 in renal fibrosis, we utilized another renal fibrosis model induced by folic acid (FA). As shown in Fig. 5a, sizes of kidneys in WT mice were profoundly reduced after FA induction, whereas genetic ablation of Runx1 in kidneys almost completely reversed the FA-induced changes in renal morphology. The expression levels of profibrogenic genes, including Col1a1, Col3a1, and Pai-1, were significantly reduced in FA-treated kidneys of Runx1<sup>cKO</sup> mice compared to those in WT mice (Fig. 5b). The mRNA levels of IL-6 and p110δ were also decreased in FA-treated kidneys of Runx1<sup>cKO</sup> mice (Fig. 5b). Like in the UUO-induced kidney fibrosis model, ablation of Runx1 in kidneys partially rescued the loss of SLC22A6 expression in FA-treated kidneys (Fig. 5c). After the treatment with FA, Runx1<sup>cKO</sup> mice showed better renal function compared to that of WT mice (Fig. 5d). Masson trichrome staining (MTS) also confirmed that renal fibrosis was inhibited in kidneys of FA-treated Runx1<sup>cKO</sup> mice compared to that of FA-treated WT mice (Fig. 5e). These results, taken together, suggest that RUNX1 is required for the progression of renal fibrosis.

4. Discussion

In the current study, we have demonstrated that expression of RUNX1, but not RUNX2 or RUNX3, was induced in the process of TGF-β-induced EMT in a SMAD3-dependent manner and in UUO-induced
renal fibrosis in vivo. RUNX1 is mainly localized in RTECs in UUO-induced renal fibrosis. We used knockdown, overexpression and conditional KO mouse models to further demonstrate that RUNX1 is required for TGF-β1-induced EMT and renal fibrosis in vivo. RUNX1 enhanced the expression of EMT markers including N-cadherin, SNAI1 and SLUG. Previous studies focused on the role of RUNX1 in immune cells [24,32,33], and this study has uncovered the role of RUNX1 in non-immune cells.

A recent study suggests that RUNX1 promotes the EMT process during mesendodermal differentiation via controlling TGF-β2 expression [34]. In addition, there are inconsistent or contradictory findings about whether RUNX1 promotes or inhibits EMT in breast cancer cells and epithelial ovarian carcinoma [35–38]. Our study has identified that RUNX1 promotes renal tubular EMT and renal fibrosis. However, we did not find that RUNX1 could regulate the expression of TGF-β2 in HK-2 cells (data not shown).

Although SMAD3 is critical for TGF-β1-induced EMT [25] and may interact with RUNX1 [52], we did not find that RUNX1 affected the activity of SMAD3 in dual luciferase reporter assays or the phosphorylation levels of SMAD3 in NRK-52E cells. However, our results did show that TGF-β1-induced RUNX1 expression is SMAD3-dependent, which may indicate that SMAD3 promotes EMT, at least partially, via RUNX1. Although RUNX2 has been reported to interact with canonical EMT regulators [39], we excluded the possibility that RUNX1 interacts with SNAI1, SLUG or TWIST1. ATP1B1 and HIF-1α are also important in the processes of EMT and renal fibrosis [9,26,28]. Although RUNX1 may regulate ATP1B1 and HIF-1α expression, we suggest that RUNX1-induced EMT might be independent of ATP1B1 and HIF-1α because the knockdown of ATP1B1 did not reverse the EMT phenotype induced by RUNX1 and TGF-β1 treatment did not affect HIF-1α expression when comparing control and RUNX1 knock-down cells. We also found that RUNX1 did not affect TGF-β1-mediated activation of MAPKs including ERK, and p38. After excluding the above possibility, we ultimately found that RUNX1 promotes EMT by enhancing the TGF-β1-induced expression of the P13K subunit p110α and the phosphorylation of Akt. Our results are consistent with a previous study showing that RUNX1 directly controls the transcription of p110α and activation of Akt in acute megakaryocytic leukemia [40]. Because the P13K-Akt pathway controls the protein stability of SNAI1 [41] and HIF-1α [42], it may explain the observation that loss of RUNX1 only affects expression of EMT markers, including SNAI1 and N-cadherin, at the protein level.

EMT has been suggested as one of the key mechanisms of TGF-β1-induced fibrosis [12,50]. We found that RUNX1 promotes renal tubular EMT and that the deletion of RUNX1 specifically in RTECs significantly attenuated renal fibrosis. RUNX2 is another RUNX family member, and there is some discrepancy about the role of RUNX2 in EMT. One study used full RUNX2 KO mice and demonstrated that RUNX2 prevents renal fibrosis by inhibiting EMT and that RUNX2 expression is decreased in UUO kidneys [31]. This is contradictory to another study which reported that RUNX2 was overexpressed in UUO kidneys [14]. In addition, several studies have demonstrated that RUNX2 promotes EMT in other systems [43–45]. Therefore, a conditional RUNX2 knockout mouse model is needed to resolve this discrepancy. Our results show that only RUNX1, but not RUNX2 or RUNX3, is induced in renal epithelial cells by TGF-β treatment. This finding might indicate that RUNX1 plays a more important role in renal fibrosis. In agreement with previous studies showing that the inhibition of EMT prevents the loss of RTECs transporter and inhibits inflammation [8,9], deletion of RUNX1 promoted SLC22A6 expression and inhibited IL-6 expression in renal fibrosis. Interestingly, several studies have suggested that RUNX1 regulates the expression of another transporter, SLC22A4, in rheumatoid arthritis [46–48]. Consistent with the in vitro results, conditional KO of RUNX1 in renal tubular epithelial cells also reduced p110α expression. This finding suggests that RUNX1 promotes renal EMT and fibrosis at least partially via p110α-mediated Akt activation. In summary, our findings demonstrate the importance of RUNX1 in EMT and renal fibrosis, and we propose that RUNX1 might be used as a new potential therapeutic target for renal fibrosis.

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Conflicts of Interests

The authors declare that they have no conflicts of interests.

Author Contributions

H.W., T.Z., and M.L. designed this study. M.L., W.C., and S.Z. performed the experiments. H.W., M.L., T.Z., and W.C. analyzed the results. S.Z., D.F., and C.X. coordinated the project. T.Z., M.L., and H.W. wrote the paper with input from S.Z., and W.C. All authors approved the final version of manuscript.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.04.023.

References

[1] Klinkhammer BM, Goldschmeding R, Floege J, Boor P. Treatment of renal fibrosis-turning challenges into opportunities. Adv Chronic Kidney Dis 2017;24:117–29.
[2] Duffield JS. Cellular and molecular mechanisms in kidney fibrosis. J Clin Invest 2014;124:2299–306.
[3] Derynck R, Mathusman BP, Saeptemey KV. Signaling pathway cooperation in TGF-β-induced epithelial-mesenchymal transition. Curr Opin Cell Biol 2014;31:56–66.
[4] Liu Y. Epithelial to mesenchymal transition in renal fibrogenesis: pathologic significance, molecular mechanism, and therapeutic intervention. J Am Soc Nephrol 2004;15:1–12.
[5] Zhou D, Liu Y. Renal fibrosis in 2015: understanding the mechanisms of kidney fibrosis. Nat Rev Nephrol 2016;12:68–70.
[6] Meng XM, Huang XR, Xiao J, Chen HY, Zhong X, Chung AC, et al. Diverse roles of TGF-β1 receptor II in renal fibrosis and inflammation in vivo and in vitro. J Pathol 2012;227:175–88.
[7] Meng XM, Huang XR, Xiao J, Chung AC, Qin W, Chen HY, et al. Disruption of Smad4 impairs TGF-β1/Smad3 and Smad7 transcriptional regulation during renal inflammation and fibrosis in vivo and in vitro. Kidney Int 2012;81:266–79.
[8] Grande MT, Sanchez-Laorden B, Lopez-Blau C, De Frutos CA, Boutet A, Arevalo M, et al. Snafl-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. Nat Med 2015;21:989–97.
[9] Lovisa S, LeBreus VS, Tampe B, Sigumoto H, Vadnagara K, Carstens JL, et al. Epithelial-to-mesenchymal transition induces cell cycle arrest and parenchymal damage in renal fibrosis. Nat Med 2015;21:989–1009.
[10] Bouret A, De Frutos CA, Maxwell PH, Mayol MJ, Romero J, Nieto MA. Snafl activation disrupts tissue homeostasis and induces fibrosis in the adult kidney. EMBO J 2006;25:5603–13.
[11] Lovisa S, Zeisberg M, Kalluri R. Partial epithelial-to-mesenchymal transition and other new mechanisms of kidney fibrosis. Trends Endocrinol Metab 2016;27:681–95.
[12] Sutariya B, Jhonsa D, Saraf MN. TGF-β1 controls the transcription of p110α and activation of Akt in acute megakaryocytic leukemia. Curr Opin Genet Dev 2003;13:43
[13] Ito Y, Miyazono K. RUNX transcription factors as key targets of TGF-β. Biochim Biophys Acta 2004;1675:1–12.
[14] 2015;12:266–79.
[15] Grande MT, Sanchez-Laorden B, Lopez-Blau C, De Frutos CA, Boutet A, Arevalo M, et al. Snafl-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. Nat Med 2015;21:989–97.
[16] Lovisa S, LeBreus VS, Tampe B, Sigumoto H, Vadnagara K, Carstens JL, et al. Epithelial-to-mesenchymal transition induces cell cycle arrest and parenchymal damage in renal fibrosis. Nat Med 2015;21:989–1009.
[17] Bouret A, De Frutos CA, Maxwell PH, Mayol MJ, Romero J, Nieto MA. Snafl activation disrupts tissue homeostasis and induces fibrosis in the adult kidney. EMBO J 2006;25:5603–13.
[18] Lovisa S, Zeisberg M, Kalluri R. Partial epithelial-to-mesenchymal transition and other new mechanisms of kidney fibrosis. Trends Endocrinol Metab 2016;27:681–95.
[19] Sutariya B, Jhonsa D, Saraf MN. TGF-β1: the connecting link between nephropathy and fibrosis. Immunopharmacol Immunotoxicol 2016;38:39–49.
[20] Ito Y, Miyazono K. RUNX transcription factors as key targets of TGF-β1 superfamily signaling. Curr Opin Genet Dev 2003;13:43–7.
[21] Ardura JA, Sanz AB, Ortiz A, Esbrit P. Parathyroid hormone-related protein protects renal tubulointerstitial cells from apoptosis by activating transcription factor Runx2. Kidney Int 2013;83:825–34.
[15] Matsuda M, Tamura K, Wakuhi K, Dejima T, Maeda A, Ohswa M, et al. Involvement of Runx3 in the basal transcriptional activation of the mouse angiogenin II type 1 receptor-associated protein gene. Physiol Genomics 2011;43:894–94.

[16] Tober J, Maijenburg MW, Speck NA. Taking the leap: Runx1 in the formation of blood from endothelium. Curr Top Dev Biol 2016;118:113–62.

[17] Tang X, Sun L, Jin X, Chen Y, Zhu H, Liang Y, et al. Runt-related transcription factor 1 regulates LPS-induced acute lung injury via NF-κB signaling. Am J Respir Cell Mol Biol 2017;57:174–83.

[18] Matsuo J, Kimura S, Yamamura A, Morin C, Plante M, Gregoire J, Renaud MC, et al. The RUNX1 transcription factor is expressed in serous epithelial ovarian carcinoma and contributes to cell proliferation, migration and invasion. Curr Cell Biol 2013;12:972–86.

[19] Wang Y, Feinberg T, Keller ET, Li XY, Weiss SJ. Slug/Nli binding interactions with YAP/TAZ control skeletal stem cell self-renewal and differentiation. Nat Cell Biol 2016;18:917–29.

[20] Edwards H, Xie X, LaFiuira KM, Dombrowski AA, Buck SA, Boerner JL, et al. RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapeutic sensitivity in acute myeloid leukemia. Blood 2009;114:7444–52.

[21] Wang H, Wang HS, Zhou BH, Li G, Zhang F, Wang XF, et al. Epithelial-mesenchymal transition (EMT) induced by TNF-alpha requires AKT/GSK-3β-mediated stabilization of snail in colorectal cancer. PLoS One 2013;8:e56664.

[22] Agani F, Jiang BH. Oxygen-independent regulation of HIF-1: novel involvement of PI3K/AKT/mTOR pathway in cancer. Curr Cancer Drug Targets 2013;13:245–51.

[23] Chakraborty PK, Scharner B, Jurasovic J, Messner B, Bernhard D, Thévenod F. Chronic cadmium exposure induces transcriptional activation of the Wnt pathway and up-regulation of epithelial-to-mesenchymal transition markers in mouse kidney. Toxicol Lett 2010;198:69–76.

[24] Luo MC, Zhou SY, Peng DY, Xiao J, Li WY, Xu CD, et al. Runt-related transcription factor 1 (RUNX1) binds to p50 in macrophages and enhances TRLR4-triggered inflammation and septic shock. J Biol Chem 2016;291:22011–20.

[25] Kim W, Barron DA, San Martin R, Chan KS, Tran LT, Yang F, et al. RUNX1 is essential for mesenchymal stem cell proliferation and myofibroblast differentiation. Proc Natl Acad Sci 2014;111(46):16389–94.

[26] Wu MJ, Wen MC, Chiou YT, Chiou YY, Shu KH, Tang MJ, Rapamycin attenuates ureteral obstruction-induced kidney fibrosis. Kidney Int 2010;78:20227–39.

[27] Lan R, Geng H, Polichnowski AJ, Singha PK, Saikumar P, McEwen DG, et al. PTEN loss regulates LPS-induced acute lung injury via NF-κB signaling. Am J Respir Cell Mol Biol 2013;50:3733–40.

[28] Edwards H, Xie X, LaFiuira KM, Dombrowski AA, Buck SA, Boerner JL, et al. RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapeutic sensitivity in acute myeloid leukemia. Blood 2009;114:7444–52.

[29] Edwards H, Xie X, LaFiuira KM, Dombrowski AA, Buck SA, Boerner JL, et al. RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapeutic sensitivity in acute myeloid leukemia. Blood 2009;114:7444–52.

[30] Edwards H, Xie X, LaFiuira KM, Dombrowski AA, Buck SA, Boerner JL, et al. RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapeutic sensitivity in acute myeloid leukemia. Blood 2009;114:7444–52.

[31] Edwards H, Xie X, LaFiuira KM, Dombrowski AA, Buck SA, Boerner JL, et al. RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapeutic sensitivity in acute myeloid leukemia. Blood 2009;114:7444–52.

[32] Edwards H, Xie X, LaFiuira KM, Dombrowski AA, Buck SA, Boerner JL, et al. RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapeutic sensitivity in acute myeloid leukemia. Blood 2009;114:7444–52.

[33] Edwards H, Xie X, LaFiuira KM, Dombrowski AA, Buck SA, Boerner JL, et al. RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapeutic sensitivity in acute myeloid leukemia. Blood 2009;114:7444–52.

[34] Edwards H, Xie X, LaFiuira KM, Dombrowski AA, Buck SA, Boerner JL, et al. RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapeutic sensitivity in acute myeloid leukemia. Blood 2009;114:7444–52.

[35] Edwards H, Xie X, LaFiuira KM, Dombrowski AA, Buck SA, Boerner JL, et al. RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapeutic sensitivity in acute myeloid leukemia. Blood 2009;114:7444–52.

[36] Edwards H, Xie X, LaFiuira KM, Dombrowski AA, Buck SA, Boerner JL, et al. RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapeutic sensitivity in acute myeloid leukemia. Blood 2009;114:7444–52.