Twist1: A Double-Edged Sword in Kidney Diseases

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Abstract
Background: Twist1 is a basic helix-loop-helix domain containing transcription factor that regulates cell differentiation, migration, proliferation, survival, and inflammatory responses by transcriptionally regulating a wide range of downstream target genes. Its homologous protein, Twist2, shares many structural and functional similarities with Twist1. Summary: Accumulating evidence from both preclinical and clinical studies suggests that Twist1 is a pivotal regulator of several forms of renal disease. Twist1 is persistently activated following renal insults, particularly in chronic kidney diseases, and contributes to the renal inflammatory responses, tubular cell transformation programs, and possibly fibroblast activation, all of which are involved in the initiation and progression of kidney diseases. Key Message: This review will specifically focus on Twist1 and outline our understanding of its functions in kidney disorders along with the introduction of Twist2 where pertinent. The thorough knowledge of Twist1’s actions in the pathogenesis of kidney diseases should facilitate the development of novel therapeutics for kidney injury.

Introduction
The kidney is a highly specialized organ that is vulnerable to various insults, such as hypoxic, toxic, and immunological insults. Intrinsic kidney parenchymal cells and infiltrating mononuclear cells from the circulation following injury undergo a range of adaptive responses, including the release of chemokines, cytokines and growth factors, transdifferentiation, death or senescence, proliferation and repair depending on the type, severity, and duration of injury. These cellular adaptive responses following kidney injury involve Twist-mediated signaling pathways.

Twist1 and Twist2 are highly conserved members of the Twist subfamily of basic helix-loop-helix transcriptional factors. Twist1 serves as a sensor and integrator of multiple stimuli and factors from local microenvironment. Normally, Twist1 in the adult kidney is relatively silenced; however, it is reexpressed in various chronic kidney diseases (CKDs) [1–3]. This signaling activated by a variety of upstream signals governs multiple cellular events and adaptive responses, such as transdifferentiation, inflammatory signaling cascades, and energy metabolism via the targeting of downstream molecules leading to profound effects on the severity and progression of kidney disorders (Fig. 1) [2–4].
Twist1 signaling directs a variety of functions in embryonic development and disease pathology [5–9]. More detailed information regarding the molecular structure of the Twist1 gene and other biological functions unrelated to kidney disease can be found in other excellent reviews [10, 11]. Thus, the scope of this review is limited to the actions of Twist1 in regulation of cellular behaviors impacting kidney diseases, particularly CKD. We propose that the functions of Twist1 signaling are extremely complex, cell- and disease-context dependent, and dramatically influence kidney disease outcomes.

**Upstream of Twist1**

Twist1 expression is regulated by several key upstream signals. The most fully characterized of these upstream pathways is triggered by transforming growth factor (TGF)-β. Three potent downstream signaling cascades: TGF-β/Smad [12], TGF-β/signal transducer and activator of transcription 3 (STAT3)/hypoxia-inducible factor (HIF) [13–15], and TGF-β/AMP-activated protein kinase [16], induced by TGF-β are critical for Twist1 upregulation. Knockdown of either Smad3 or 4 or inhibition of TGF-β receptor I kinase activity completely abrogates the stimulatory effects of TGF-β on Twist1 expression. Overexpression of a constitutively active TGF-β receptor I not only elevates Twist1 levels but also increases the formation of Twist1 homodimers by induction of inhibitor of DNA-binding proteins, which have high affinity for E12/E47 and compete against Twist1 for E12/E47 binding [12]. Besides canonical TGF/Smad signaling, TGF-β/STAT3/HIF signaling also contributes to Twist1 expression. Constitutive expression of dominant negative mutant of STAT3

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Fig. 1. Twist1 signaling is activated by a variety of upstream signals that govern multiple cellular events and adaptive responses, such as EMT, cell death and senescence, inflammatory signaling cascades, and energy metabolism via the targeting of downstream molecules. PGC1α, peroxisome proliferator-activated receptor γ coactivator 1α; EMT, epithelial–mesenchymal transition; NOD, nucleotide-binding oligomerization domain containing; HIF, hypoxia-inducible factor; MMPs, matrix metallopeptidases; E2F1, E2F Transcription Factor 1; Bmi1, B cell-specific Moloney murine leukemia virus integration site 1; CPT1, carnitine palmitoyl transferase 1; YB-1, Y box-binding protein-1; STAT, signal transducer and activator of transcription; NF-κB, nuclear factor kappa B; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; TGF, transforming growth factor.
represses TGF-β1-induced Twist1 expression. Moreover, STAT3 also stabilizes HIF-1α protein and accumulates in the nucleus, which in turn promotes TGF-β1-mediated Twist1 expression [15]. STAT3 induces Twist1 expression directly by binding to the promoter region and indirectly by stabilizing HIF-1α protein, providing evidence that both STAT3 and HIF-1α are required for Twist1 expression and that STAT3 functions as a transcription factor and HIF-1α stabilizer for Twist1 expression after TGF-β1 exposure.

Hypoxia is a potent driver for Twist1 expression via direct induction of HIF. HIF-1α promotes Twist1 expression by binding to the hypoxia-response element in the Twist1 proximal promoter region [2]. Moreover, HIF-1α cooperates with other transcriptional regulators such as Snail and Slug to contribute to epithelial mesenchymal transition (EMT) under hypoxic conditions [17, 18]. Twist1 expression in human cancer cells is enhanced by hypoxia in a HIF-2α-dependent manner, but does not depend on HIF-1α [14]. Together, these findings highlight HIF either HIF-1α or HIF-2α, as a key regulator of Twist1 expression. Additionally, AMP-activated protein kinase activation induced by upstream physiological cues such as TGF-β and hypoxia mediates upregulation of Twist1 gene expression and its increased nuclear localization, thus leading to EMT [16].

Other potent signals modulating profibrotic or inflammatory responses such as Wnt/β-catenin, Notch, nuclear factor kappa B (NF-κB), interleukin (IL) 6/STAT3, epidermal growth factor (EGF) receptor/STAT3 are all thought to transcriptionally regulate Twist1 expression [19]. Our data also suggest that activation of the renin–angiotensin–aldosterone system in immune cells can influence Twist1 expression.

**Downstream of Twist1**

The best characterized downstream target of Twist1 during EMT is E-cadherin, the most crucial protein in regulating cell-to-cell adhesion. In this process, Twist1 directly binds to the B cell-specific Moloney murine leukemia virus integration site 1 (Bmi1) promotor region and triggers its expression. Bmi1 in turn cooperates with Twist1 to be identified on the proximal regulatory regions of the E-cadherin promotor, resulting in decreased E-cadherin expression. This cooperation requires polycomb repressive complex 2 components such as enhancer of zeste homolog 2 and then enhances histone H2 trimethylation at Lys 27 through chromatin remodeling [20]. Twist1 can also recruit the nucleosome remodeling deacetylase complex to repress E-cadherin, leading to EMT, cell invasion, and metastasis. Moreover, Twist1 drives expression of EMT-related genes including type I collagen [12] and N-cadherin [21], via directly binding to their promoters, enabling epithelial cells to adopt a mesenchymal phenotype. Moreover, Twist1 induces the EMT-promoting transcription factor Snail2 by binding to an evolutionarily conserved E-box on its promotor [22]. Additionally, it is believed that Twist1 upregulates the expression of matrix metallopeptidase (MMP) family proteins, Akt2, Y box binding protein-1 (YB-1), Wnt5a, Jagged1, Slug, PDGFRα by directly binding to E-box elements to promote cell survival, drug resistance, EMT, proliferation, and invadopodia formation [11]. Among of them, the MMP protein family is one of the most potent targets of Twist1. For example, Twist1 drives MMP1 expression through binding to the MMP1 promotor to augment tumor cell migration and invasion via ERK1/2 signaling [23]. More broadly, Twist1 increases mRNA for MMP2, 3, 8, 9, 10, 11, 12, and 13 in human chondrocytes [24]. In nonimmune cell lineages MMP13 expression is governed by T-box transcription factor 20, which is another target gene of Twist1 [4].

In response to an inflammatory stimulus, Twist1 can bind to E-boxes in the promotor region of several cytokines, including tumor necrosis factor (TNF)-α, IL-1β, and IL-6, to blunt their expression levels [8, 25]. Furthermore, Twist1 inhibits T-bet, STAT4, and Runx3 transcription factors to decrease interferon (IFN)-γ production in Th1 cells [26].

Besides targeting downstream genes, Twist1 also directly interacts with several proteins to modulate cellular events. For example, cooperation between Twist1 and AP-1 represents a novel mechanism for EMT and tumor invasion and they cooperatively upregulate integrin α5 expression to induce invasion and EMT [27]. Twist1 interacts with NF-κB to repress cytokine production and inflammatory responses [28]. Twist1 binding to YB-1 results in the promotion of EMT, proliferation, and cell cycle progression [29]. Twist1 may also transcriptionally regulate growth factors and chemokines including C-C motif chemokine ligand 2, vascular endothelial growth factor, connective tissue growth factor, fibroblast growth factor 2, and platelet-derived growth factor, but the underlying mechanisms remain largely unknown [30–33].

Epigenetic modulation represents another mechanism through which Twist1 regulates target gene expression. Twist1 either directly or indirectly interacts with several components of the Mi2/nucleosome remodeling deacetyl-
lase protein complex to remodel chromatin in order to modulate target gene expression [34, 35]. For example, Twist1 promotes histone deacetylase 1 and 3-dependent epigenetic modifications that influence the effects of nucleotide binding oligomerization domain containing 2 (NOD2) on macrophage cytokine production [36]. Another study suggests that Twist1-mediated chromatin remodeling silences gene expression through recruitment of DNA methyltransferase 3B [37].

Twist1 also impacts microRNA expression, adding a regulatory layer to participate in cellular events as summarized in other reviews. Conversely, Twist1 is a downstream target of microRNAs, indicating a close interplay between microRNAs and Twist1 [38].

Therefore, Twist1 can either activate or silence genes to regulate cell behaviors through diverse mechanisms including direct or indirect binding to the promoter of target genes, cooperation with deacetylase- or methyltransferase-mediated epigenetic modification.

**Posttranscriptional Modification of Twist1**

Similar to other signaling proteins, transcription factors undergo posttranscriptional modifications by phosphorylation, ubiquitination, or acetylation in response to various cellular signals. Posttranscriptional regulation is important for Twist1 abundance and function. The regulatory mechanisms underpinning posttranslational modification of Twist1 have been investigated in depth recently.

Twist1 degradation is mainly regulated by 2 degradation systems: the p62-mediated autophagy-proteasome system and ubiquitin-proteasome system composed of the ubiquitin ligase including F-box protein partner-of-paired and p53-induced RING-H2 [39, 40], as well as the b-transducin repeat-containing protein [41].

First, autophagy dysfunction may impact Twist1 degradation. In cells with normal autophagy, Twist1 can be degraded by autophagy with subsequent proteasomal degradation. In cells with defective autophagy, however, p62 as a selective autophagy substrate accumulates, inhibiting Twist1 binding with RAD23 homolog B (Rad23B). Rad23B generally works as a delivery protein for targeting Twist1 to the proteasome for degradation [42]. Accumulated p62 binding to ubiquitinated Twist1 enhances Twist1 protein stabilization and then dampens Twist1 degradation by the proteasome. Thus, Twist1 is a critical downstream target of p62 and targeting p62-induced Twist1 stabilization may be a potential therapeutic strategy for the prevention or treatment of disease [43]. In addition to p62-mediated Twist1 stabilization following autophagy inhibition, p62 is responsible for the up-regulation of Twist1 protein induced by EGF and TGF-β [43].

Second, the ubiquitin-proteasome system influences Twist1 stability and degradation through Twist1 phosphorylation and dephosphorylation mediated by phosphokinase and phosphatase, respectively. It is believed that Twist1 protein can be phosphorylated at multiple sites, including Ser-68, Ser-42, Thr-121, Ser-123, Ser-144, Ser-18, and Ser-20 [44–48]. Phosphorylation at these sites has been demonstrated to impact Twist1 dimerization, transcriptional activity, and stability. Phosphorylation on Ser-68 of Twist1 is the best studied regulatory modification by the mitogen-activated protein kinases (MAPK) p38 and c-Jun N-terminal kinase. This phosphorylation results in enhanced stability of the Twist1 protein as it inhibits Twist1 ubiquitination and degradation by the proteasome. A point mutation of serine to alanine at site 68 of Twist1 significantly accelerates Twist1 ubiquitination and degradation. Moreover, activation of MAPK by an active Ras protein or TGF-β treatment dramatically elevates Ser-68 phosphorylation and Twist1 protein abundance without changes in mRNA levels. Blocking MAPK activation by either specific inhibitors or dominant negative inhibitory mutants is sufficient to decrease the abundance of Ser-68 phosphorylation and Twist1 protein [46]. MAPKs not only stabilize Twist1 in a phosphorylation-dependent manner but also transcriptionally upregulate Twist1 expression as mentioned above.

Small C-terminal domain phosphatase 1 specifically dephosphorylates Ser(P)68-Twist1 to trigger Twist1 degradation [49]. Thus, Twist1 phosphorylation status is tightly regulated by Small C-terminal domain phosphatase 1 and MAPK, which together reciprocally influence Twist1 stability and function. Furthermore, Twist1 dimerization and its DNA binding capacity are enhanced by protein kinase A (PKA)-mediated phosphorylation at Thr-125 and Ser-127 but inhibited by protein phosphatase 2A (PP2A)-mediated dephosphorylation of these residues. The counter regulation between PKA-mediated phosphorylation and PP2A-mediated dephosphorylation of Twist1 on Thr-125 and Ser-127 plays a crucial role in Saethre-Chotzen syndrome and limb morphogenesis [44, 50].

Other known sites of Twist1 phosphorylation are at Ser-144 by protein kinase Ca [48], Thr-121 and Ser-123 by PKA [45] and Ser-42 by Akt1 [45], each with different
effects on cellular activities. In yet another posttranslational modification, Twist1 is acetylated at Lys-63 and Lys-67 by Tat interactive protein 60, which enables Twist1 to interact with bromodomain-containing protein 4 for transcriptional activation of Wnt5a [51].

**Kidney Disease-Related Cellular Events Regulated by Twist1**

A variety of cellular events governed by Twist1, including EMT, inflammatory responses, energy metabolism, proliferation, survival and anti-senescence, impact the pathogenesis of kidney disease as addressed in the following discussion.

**Twist1 with EMT**

Pathological EMT is characterized by the epithelial cell loss of E-cadherin-mediated cell-cell adhesion and apical-basal polarity, gain of mesenchymal markers and cell motility, and induction of cell dissociation and migration. Twist1 expression is limited under normal physiologic conditions, whereas in the setting of pathological EMT, Twist1 reexpression is triggered. As an essential molecule for maintaining epithelial integrity, E-cadherin is a critical and the best characterized downstream target of Twist1. Another critical component of EMT development regulated by Twist1 is N-cadherin, which mediates epithelial adhesion to interstitial matrix proteins. Outside-in signaling from integrin-mediated adhesion to interstitial matrix proteins induces the nuclear cytoplasmic translocation and DNA binding of Twist1, activating N-cadherin transcription [21]. Moreover, Twist1 cooperates with other transcriptional factors such as Snail and AP-1 to enhance EMT [27, 52].

Cytoskeletal changes and motility are other characteristics of EMT. Twist1 facilitates cell motility via activating Rac family small GTPase 1. It cooperatively acts with Bmi1 to blunt microRNA let-7i expression, which contributes to upregulation of dedicator of cytokinesis 3 and neural precursor cell expressed developmentally downregulated protein 9, eventually culminating in Rac family small GTPase 1 activation and enabling mesenchymal-motility [53]. Twist1-induced relocation of activated focal adhesion kinase is also important for cellular cytoskeletal organization and movement [54–56]. Twist1-silenced forkhead box protein A1 is also responsible for Twist1-induced motility, but less involved in Twist1-induced mesenchymal morphogenesis and expression of certain EMT markers [35].

Endothelial to mesenchymal transition is also regulated by Twist1. Under hypoxic conditions, endothelial cells of pulmonary arterioles express a-SMA, a mesenchymal cell marker, whereas Twist1 deficiency in these cells attenuates a-SMA positive cell accumulation in vivo. In vitro studies demonstrate that Twist1 Ser-42 phosphorylation is responsible for endothelial to mesenchymal transition through TGFβ-Smad2 signaling [57]. Thus, Twist1 not only in epithelial but also in endothelial cells governs mesenchymal phenotypes in response to pathogenic stimuli.

**Twist1 in Inflammatory Responses**

Twist1 is a common repressor of cell-mediated and humoral adaptive immunity and limits immunopathology in chronic inflammatory diseases. Mice homozygous for a twist-2 null allele or doubly heterozygous for twist-1 and -2 alleles develop severe systemic inflammation, indicating a central role for Twist in regulating inflammation [28]. Twist1’s functions in shaping inflammatory responses have been extensively studied in T cells and macrophages.

**In T Cells**

Twist1 is upregulated in chronically activated Th1, Th17, and T follicular helper cells and acts as an essential component of a cytokine-induced feedback loop [58, 59]. Niesner et al. [58] found that Twist1 expression in Th1 cells follows TCR stimulation with initially transient induction followed by persistent upregulation after repeated stimulation. Twist1 negatively regulates proinflammatory gene expression and cytokine production in T cell subsets through either diminishing NF-κB and Runx3 activation, resulting in limiting IFN-γ, TNF-α, or IL-2 expression in Th1 cells [26], or repressing IL-6/STAT3 activation, leading to restricted IL-17 and IFN-γ production and Th17 and T follicular helper cells development [59]. Thus, Twist1 acts as a balancing factor to regulate signal integration and a master switch to control chronicity in cellular immunity.

**In Macrophages**

In macrophages, Twist1 can be upregulated by type I IFNs and NOD2 stimulation [25]. Twist1 limits inflammatory responses mainly by suppressing TLR or NOD ligand-induced NF-κB activation and cytokine secretion including TNF-α, IL-1β, and IL-6, which are required for proinflammatory macrophage actions [25, 28, 60]. Twist1 may either blunt the phosphorylation and degradation of IκB or physically bind to p65 to inhibit p65-mediated
transcription [28]. On the other hand, Twist1 binding to E-boxes in the cytokines promoter region reduces their expression levels [28, 60]. Twist1 and 2 upregulated by chronic NOD2 stimulation cooperate to suppress proinflammatory cytokine expression through induction of the inflammation inhibitor c-Maf and transcriptional repressor Bmi1 and suppression of cytokine promoter binding of transcriptional activators, including activating transcription factor 4, CCAAT-enhancer-binding protein a, Runx1, and Runx2 [25]. Therefore, Twist1 and 2 coordinately regulate both transcriptional activators and repressors after chronic NOD2 stimulation, thereby downregulating cytokine expression and function in macrophages.

Accordingly, modulating Twist1 in macrophage may hold promise for therapy for inflammatory disorders. Twist1 levels are elevated in macrophages from patients with colitis and rheumatic diseases. A fluorinated triazole derivative (TT-TFM) can elevate Twist1 levels in colon tissue and attenuate colitis symptoms in colitis models through suppressing NF-κB activation and subsequently inhibiting cytokine production. Thus, TT-TFM may represent a promising therapeutic agent for Crohn’s disease treatment by enhancing localized Twist1 signaling [61].

Apart from limiting cytokine expression, Twist1 diminishes expression of proinflammatory chemokines and chemokine receptors and blunts cytokine receptor signaling by upregulating suppressor of cytokine signaling-1, -2 and the IL-1 decoy receptor [58].

Twist1 with Energy Metabolism

Twist1 regulates energy metabolism in multiple cell lineages and tissues including kidney tubular cells, T cells, white and brown adipocytes, and skeletal muscle. Twist1 deficiency in tubular cells restores gene expression associated with fatty acid metabolism and β-oxidation, which are downregulated and associated with cell death and de-differentiation in tubular cells of fibrotic kidneys [3]. In brown adipose tissue, Twist1 drives a negative feedback regulatory loop to finely tune peroxisome proliferator-activated receptor γ coactivator 1 alpha/peroxisome proliferator-activated receptor δ-controlled brown fat metabolism in response to nutrient status, and to therefore ensure energy homeostasis [62]. However, different from brown adipocytes, Twist1 in white adipocytes positively regulates peroxisome proliferator-activated receptor γ coactivator 1α and carnitine palmitoyl transferase 1 expression along with increased fatty acid oxidation (FAO) and no changes in genes governing basal lipolysis [63]. These results suggest that Twist1 is a promoter of FAO in white adipocytes. Moreover, the studies in skeletal muscle reveal that Twist1 also negatively regulates glucose metabolism through pyruvate dehydrogenase kinase 4, which is the master regulator of pyruvate dehydrogenase. Overexpression of either Twist1 or 2 decreases pyruvate dehydrogenase kinase 4 expression and subsequently enhances pyruvate dehydrogenase activity and glucose flux in the Krebs cycle, rather than glycogen synthesis, suggesting that Twist promotes glucose utilization [64]. Twist1 in T cells inhibits glycolysis and promotes FAO, protecting Th1 cells from reactive oxygen species and allowing Th1s cell to limit that duration of inflammation [65]. Collectively, these results indicate that Twist-regulated energy metabolism is complex and may be cell- or local microenvironment-context dependent.

Twist1 in Cell Proliferation

Twist1 is thought to positively regulate proliferation-related proteins including E2F Transcription Factor 1, Cyclin E1, and c-Myc, which are key proteins regulating cell cycle progression via binding to the canonical E-box of their promoter regions. Twist1 knockdown dampens cell cycle progression at the G1/S transition, reduces levels of the cell cycle proteins E2F Transcription Factor 1, Cyclin E1, and c-Myc, and increases levels of p21 and p53. In vivo, Twist1 deficiency in basal keratinocytes of the epidermis significantly reduces cell number by inhibiting cell cycle progression and repressing proliferation. Twist1 knockout results in an increase in p53 protein levels via its stabilization and nuclear localization. Silencing p53 is sufficient to abolish the effect of Twist1 on cell proliferation [66]. Emerging evidence directly links Twist1 regulation of forkhead box protein M1 transcription to control of the cell cycle [67]. Twist1 also positively regulates downstream target gene YB-1, which induces cell proliferation via transactivation of several genes such as proliferation cell nuclear antigen, EGF receptor, DNA topoisomerase II, thymidine kinase, and DNA polymerase [29, 68]. In addition, Twist1 transcriptionally upregulates Akt2, which coordinates Twist1/YB-1 signaling [10]. Based on these reports, Twist1 drives cell proliferation by targeting several cell cycle-related genes and proteins.

Twist1 with Cell Survival and Antisenescence

Twist1 is an inhibitor of apoptosis. Twist1 suppresses p53 function and expression in response to DNA damage through the N-Myc oncogene [69–71]. This suppression attenuates the activation of p53 target genes such as p21Waf1 and B-cell lymphoma-2-associated X protein with consequent inhibition of apoptosis. The blunted induction of these p53 effector molecules is likely mediated
by Akt-dependent phosphorylation of Twist1 at Ser-42 [45]. Maestro et al. [69] have reported that Twist1 inhibits apoptosis and bypasses p53-induced growth arrest by direct and indirect modulation of the alternative reading frame/mouse double minute 2 homolog/p53 pathway in mouse embryonic fibroblasts.

Twist1 may also increase cell numbers by suppressing senescence. In this regard, overexpression of Twist can override premature senescence by abrogating key regulators of the p53 and Rb-dependent pathways [72].

**Twist1 in CKD**

As discussed above, Twist1 is minimally expressed in the adult kidney. However, it is dramatically reexpressed both at the protein and mRNA level following various renal insults, including unilateral ureteral obstruction (UUO), nephrotoxic nephritis, folic acid- and aristolochic acid (AA)-nephropathy, and subtotal nephrectomy [1–4, 73]. Reactivation of Twist1 underscores that this signaling pathway may play important roles in various kidney diseases. There is no effective agent targeting Twist1 signaling for treatment of kidney diseases until now. A comprehensive understanding of the roles for Twist1 plays in kidney injury or repair may provide opportunities to target important components of Twist1 signaling for designing future therapeutic strategies.

**Twist1 in Tubular Cells**

Twist1 participates in physiologic EMT during embryonic development, as well as pathological EMT during disease development such as cancer metastasis and organ fibrosis. In 2015, 2 studies published in *Nature Medicine* showed that EMT is integral to the pathogenesis of renal fibrotic diseases [3, 74]. In these experiments, a partial EMT program triggered signals to promote myofibroblast differentiation and inflammatory responses but did not directly produce interstitial myofibroblasts (Fig. 2). Elegant studies by Kalluri’s group established that Twist1-induced partial EMT during fibrogenesis causes G2-phase cell cycle arrest and loss of several solute and solvent transporters in tubular cells. In vitro studies revealed that epithelial cells exposed to TGF-β1 can upregulate Twist1 mRNA levels. Knockdown and ectopic overexpression experiments also suggest that an EMT program induced by TGF-β1 is associated with a p21-mediated arrest in G2 phase of cell cycle that requires, at least in part, Twist1 and Snail1 [3].

![Diagram](https://example.com/diagram.png)

**Fig. 2.** A variety of cellular events governed by Twist1 impact the pathogenesis of CKD. In epithelial cells, Twist1 induces a partial EMT program to promote myofibroblast differentiation and inflammatory responses. Recruited infiltrating macrophages under the regulation of Twist1 produce MMP13 to degrade extracellular matrix. RTCs, renal tubular cells; EMT, epithelial-mesenchymal transition; ECM, extracellular matrix; MMP13, matrix metalloproteinase 13.
Distal tubular epithelia express Twist1 more abundantly than the proximal nephron [1]. Based on this observation, our group probed Twist1 actions in the distal nephron during chronic AA-induced nephropathy. We found that in AA-induced chronic nephropathy, Twist1 deletion in the distal tubule attenuates interstitial matrix deposition and kidney injury. In vivo and coculture studies revealed that Twist1 in epithelial cells augments the recruitment and activation of proinflammatory CD64+ macrophages in a fibronectin-dependent manner [75]. Lovisa et al. [3] also established that Twist1-dependent EMT promotes inflammatory cell infiltration and cytokine expression in several chronic nephropathy models. Collectively, these results suggest that Twist1 in both the proximal and distal tubule propagates partial EMT and provokes immune cell infiltration, leading to worsened kidney histopathology.

Chronic hypoxia is a potent driver of renal fibrosis, eventually leading to end-stage renal failure. Sun et al. [73] have reported that hypoxia-induced Twist expression mediated by HIF-1α activation may contribute to the pathogenesis of progressive renal fibrosis. In vitro exposure to hypoxia induces human epithelial cells to upregulate HIF-1α and Twist1 expression along with decreased E-cadherin and zona occludens-1 expression. Inversely, silencing Twist1 by siRNA interference restores E-cadherin expression. Moreover, overexpression of Twist promotes transformation from epithelial to mesenchymal phenotypes, characterized by gain of vimentin expression and loss of E-cadherin expression.

**Twist1 in Fibroblasts**

Twist1 expression is enhanced not only in tubular epithelia of dilated tubules but also in the expanded interstitial areas of UUO kidneys. Interstitial cells in UUO-induced fibrotic kidneys show Twist1-positive staining, including α-SMA-positive interstitial myofibroblasts [1]. Marked upregulation of Twist1 expression can be observed in fibroblasts from lung, skin, and tumors [12, 76, 77]. As mentioned above, Twist1 as a transcription factor regulates multiple processes involved in matrix production and deposition as well as MMP-dependent matrix degradation. However, there have been no reports on the effect of Twist1 on fibroblasts phenotype or activation in vivo during development of kidney fibrotic disorders to date.

**Twist1 in Macrophages**

Among the interstitial cells in fibrotic kidney, infiltrating macrophages in the fibrotic interstitial areas make a critical contribution to UUO-induced renal fibrosis. Our group therefore explored Twist1’s actions in macrophages during the development of UUO nephropathy. We found that Twist1 in inflammatory but not resident macrophages exaggerates extracellular matrix degradation and thereby limits kidney fibrosis after UUO. Specifically, infiltrating CD11b+Ly6C<hi> myeloid cells with Twist1 deficiency exhibit blunted MMP13 expression, which is responsible for matrix degradation. Thus, Twist1 in infiltrating myeloid cells attenuates extracellular matrix accumulation in the fibrotic kidney by enhancing MMP13 expression (Fig. 2) [4]. This observation is congruent with studies showing that loss of Twist1 in the macrophage enhances bleomycin-induced lung fibrosis [78]. However, the underlying mechanism through which Twist1 in macrophages attenuates lung fibrosis has not been elucidated.

The protective roles for Twist1 in myeloid cells are surprising given the known profibrotic actions of Twist1 in intrinsic renal parenchymal cells. These data highlight the complex cell-specific actions of Twist1 in the pathogenesis of kidney fibrosis and can guide the design of pharmacological interventions with minimal off-target side effects.

**Twist1 in Human Kidney Diseases**

Similar to the studies in rodent models, Twist is observed in tubular epithelial cell nuclei from the renal biopsies of patients with a variety of CKD including diabetic kidney disease, focal segmental glomerulosclerosis, IgA nephropathy, hypertensive nephrosclerosis, and tubulointerstitial nephritis. By contrast, little positive staining for Twist is found in the renal tubules of normal kidneys [3, 79, 80]. Levels of Twist1 protein in the tubulointerstitium are inversely correlated with estimated glomerular filtration rate and positively correlated with serum creatinine and the severity of tubulointerstitial fibrosis. Moreover, a high levels of Twist correlate with HIF-1α expression and E-cadherin repression across all disease groups. By multivariate analysis, the levels of Twist expression influence renal survival. Twist may thus become a new surrogate predictor for renal survival in patients with CKD. Furthermore, Twist1 expression, as an EMT marker, is inversely correlated with levels of tubular epithelial cell transporter transcripts and activity and positively associates with tubulointerstitial fibrosis in a cohort of renal fibrotic biopsies and normal kidneys [3].

**Conclusion**

Preclinical evidence indicates that Twist1 plays divergent roles in kidney diseases depending on the cell lineage in which it is generated. Twist1 in renal tubular cells...
Propagates EMT and kidney fibrosis, whereas Twist1 in myeloid cells limits matrix accumulation in the scarred kidney by facilitating MMP13-induced matrix degradation. Based on these findings, understanding the cell-specific actions of Twist1 in other renal and immune cell lineages during the pathogenesis of acute and CKD may provide additional guidance to allowing the safe targeting of the Twist1 pathway while limited unwanted off-target side effects. Twist1 as a transcription factor regulates a variety of secretory proteins, but little is known about multilayered cell–cell communication involving Twist in the kidney following injury. For example, targeting the Snail1 pathway by Snail1-morpholino can dampen partial EMT and even reverse established UUO-induced nephropathy [74]. Thus, strategies for combined targeting of Snail1 and Twist1 may yield benefits in CKD if the putative agents can be targeted to the relevant cell lineages. Nevertheless, further elucidation of Twist1-mediated actions in kidney diseases will be required to facilitate translation of Twist1-related therapies for CKD.

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Twist1 in CKD


