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Nephrol Dial Transplant, 2023, **0**, 1–9
https://doi.org/10.1093/ndt/gfad238
Advance access publication date: 15 November 2023

REVIEW

Endothelial to mesenchymal transition in kidney fibrosis

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ABSTRACT

Fibrotic diseases are characterized by the uncontrolled accumulation of extracellular matrix (ECM) components leading to disruption of tissue homeostasis. Myofibroblasts as the main ECM-producing cells can originate from various differentiated cell types after injury. Particularly, the process of endothelial-to-mesenchymal transition (endMT), describing phenotypic shifts of endothelial cells to adopt a fully mesenchymal identity, may contribute to the pool of myofibroblasts in fibrosis, while leading to capillary rarefaction and exacerbation of tissue hypoxia. In renal disease, incomplete recovery from acute kidney injury (AKI) and the ensuing fibrotic reaction stand out as major contributors to chronic kidney disease (CKD) development. While the focus has largely been on impaired tubular epithelial repair as a potential fibrosis-driving mechanism, alterations in the renal microcirculation post-AKI, and in particular endMT as a maladaptive response, could hold equal significance. Dysfunctional interplays among various cell types in the kidney microenvironment can instigate endMT. Transforming growth factor beta (TGF-β) signaling, with its downstream activation of canonical/Smad-mediated and non-canonical pathways, has been identified as primary driver of this process. However, non-TGF-β-mediated pathways involving inflammatory agents and metabolic shifts in intercellular communication within the tissue microenvironment can also trigger endMT. These harmful, maladaptive cell–cell interactions and signaling pathways offer potential targets for therapeutic intervention to impede endMT and decelerate fibrogenesis such as in AKI–CKD progression. Presently, partial reduction of TGF-β signaling using anti-diabetic drugs or statins may hold therapeutic potential in renal context. Nevertheless, further investigation is warranted to validate underlying mechanisms and assess positive effects within a clinical framework.

Keywords: acute kidney injury, chronic kidney disease, endothelial cells, endothelial to mesenchymal transition, fibrogenesis

INTRODUCTION

Fibrosis development is characterized by the accumulation of macromolecules in the extracellular matrix (ECM) compartment [1]. Although fibrogenesis occurs in numerous organs as part of physiological tissue-healing processes after injury, pathological, excessive accumulation of ECM components compromises organ structure and function in fibrotic disease [1]. Myofibroblasts represent the cell type mainly responsible for this pathological ECM deposition. The exact origin of the increased myofibroblast population in fibrotic disease varies between organs but involves transformation of differentiated cell types such as pericytes and endothelial cells (ECs) into a mesenchymal cell type [1, 2].

Chronic kidney disease (CKD) includes one of the fibrotic diseases with a rapidly increasing global disease burden. Incomplete recovery of kidney function after a single or several episodes of acute kidney injury (AKI) represents one of the most important causes of CKD. During the transition from AKI to CKD, renal epithelial structures undergo incomplete repair processes which results in a sustained local pro-inflammatory state, ultimately enhancing renal fibrogenesis [3–5]. In searching to discover repair mechanisms and possible therapeutic targets for kidney fibrosis, most research focuses on proximal tubule cells (PTCs) as the primary site of injury. However, renal endothelial cell (REC) dysfunction also plays a critical role in the AKI–CKD transition [6, 7]. Endothelial-to-mesenchymal transition (endMT), involving transdifferentiation of ECs to a mesenchymal phenotype, is one of the maladaptive processes that occurs in renal capillaries upon injury further enhancing local inflammation, ECM deposition and capillary rarefaction [2, 7, 8].

This review aims to summarize current knowledge on the molecular mechanisms of endMT in fibrogenesis and more specifically AKI–CKD transition, with particular emphasis on the defective cellular and secretory interactions in the pro-inflammatory kidney micro-environment (KME). Ultimately, this may provide possible therapeutic targets to halt endMT and ECM accumulation in the context of fibrotic diseases.

EndMT IN FIBROTIC DISEASE

EndMT occurs under physiological circumstances during embryonic development of atrioventricular cushions in the heart, later forming the septa and heart valves, and vascular wall formation [2, 9, 10]. The first inflammation-mediated, pathological process of endMT in adult tissue was demonstrated in cardiac tissue contributing to the accumulation of fibroblasts and development of cardiac fibrosis [10]. Nowadays, pathological endMT has been described in many fibrotic disorders. While underlying molecular mechanisms show overlap between different organs, some of the induction processes are more restricted to certain fibrotic

Received: September 4, 2023; Editorial decision: November 5, 2023

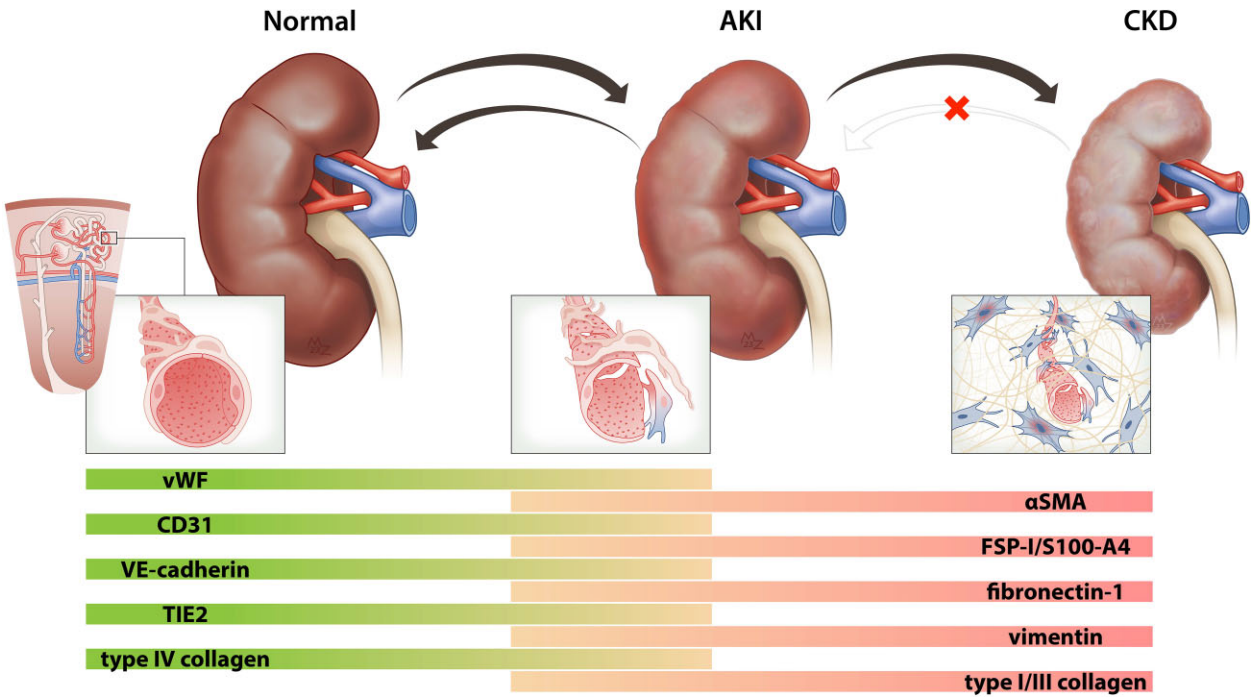

Figure 1: Induction of endMT contributes to the emerging pool of myofibroblasts after injury and causes capillary rarefaction and tissue hypoxia thereby contributing to renal fibrogenesis and disease progression. During endMT, RECs undergo morphological and phenotypic changes with decreasing expression of endothelial protein markers, such as CD31, and increasing expression of mesenchymal protein markers, such as α-SMA.

diseases. In this first section, we will elaborate on general mechanisms of endMT while highlighting its relevance in renal disease.

Molecular mechanisms of endMT

The molecular mechanisms underlying endothelial transdifferentiation in endMT have been partly described. During endMT, ECs lose their adhesive phenotype and apical–basal polarity to transition into a reversible intermediate phenotype characterized by the co-expression of both, EC-specific and mesenchymal-specific markers, and eventually to a fully mesenchymal cell (Fig. 1) [2]. Briefly, these spindle-shaped, invasive and migratory ECs progressively gain expression of several mesenchymal markers such as α-smooth muscle actin (α-SMA), fibroblast-specific protein-1 (FSP-1/S100-A4), collagen type I/III, N-cadherin, fibronectin-I (FN-I) and vimentin while gradually decreasing expression of endothelial protein markers such as von Willebrand Factor (vWF), CD31, vascular endothelial (VE)-cadherin, angiopoietin receptor TIE2 and type IV collagen [2, 8, 9].

Transforming growth factor beta (TGF-β) is widely recognized as the most important inducer of endMT. While all three protein isoforms are capable of enhancing endMT, TGF-β2 has shown most potency to induce endMT *in vitro* [2]. TGF-β is secreted in its inactive precursor form and resides in the ECM as a matricellular ligand. The perivascular ECM contains various matricellular proteins, such as connective tissue growth factor (CTGF) and thrombospondin-1 (TSP-1), with modulatory functions that become upregulated during phases of tissue injury and repair [11]. Release of TGF-β from its latent protein complex by protease cleavage causes binding to its type II transmembrane receptor with subsequent heterodimerization of the type I/II TGF-β receptor complex [2, 8]. Both activin-like kinase 1 (ALK1) and ALK5 belong to the type I TGF-β receptors and play an important role in balancing physiological EC proliferation and quiescence [8, 12]. Box 1 summarizes regulation of TGF-β signaling to maintain EC homeostasis.

Box 1: Regulation of physiological TGF-β signaling in ECs

TGF-β signaling is crucial for maintaining physiological EC function and vascular integrity. As demonstrated from *in vivo* knock-out studies, defects in specific TGF-β ligands or receptors impair early vasculogenesis [12, 13]. TGF-β bioavailability depends on both, the secretion of latent protein forms as well as the release of matricellular TGF-β from its inactivating protein complex in the ECM [14]. The release of TGF-β from this complex can be triggered by various mechanisms, such as traction between cells and ECM components resulting in integrin activation [14]. Binding of TGF-β to ECs induces downstream effects dependent on the specific combination of transmembrane receptors. The main TGF-β receptor complex consists of type I and II receptors; type III includes accessory receptors endoglin and betaglycan [8, 12]. Activation of type I receptors ALK1 and ALK5, respectively, stimulates and inhibits physiological EC proliferation and migration (Fig. 2A) [8, 12]. The EC-specific proliferative effect of TGF-β/ALK1 depends on downstream Smad1/5 activation. Contrarily, TGF-β/ALK5 activation inhibits EC proliferation and migration through Smad2/3 phosphorylation [12]. Accessory receptor betaglycan is important for enhancing binding affinity of TGF-β ligands. The regulatory effects of endoglin are more complex and depend on relative abundance of short and long isoforms of endoglin receptors which promote TGF-β/ALK5 and TGF-β/ALK1 signaling, respectively [12]. The modulation of TGF-β ligand bioavailability and downstream responses represent important regulatory mechanisms that balance TGF-β signaling in ECs.

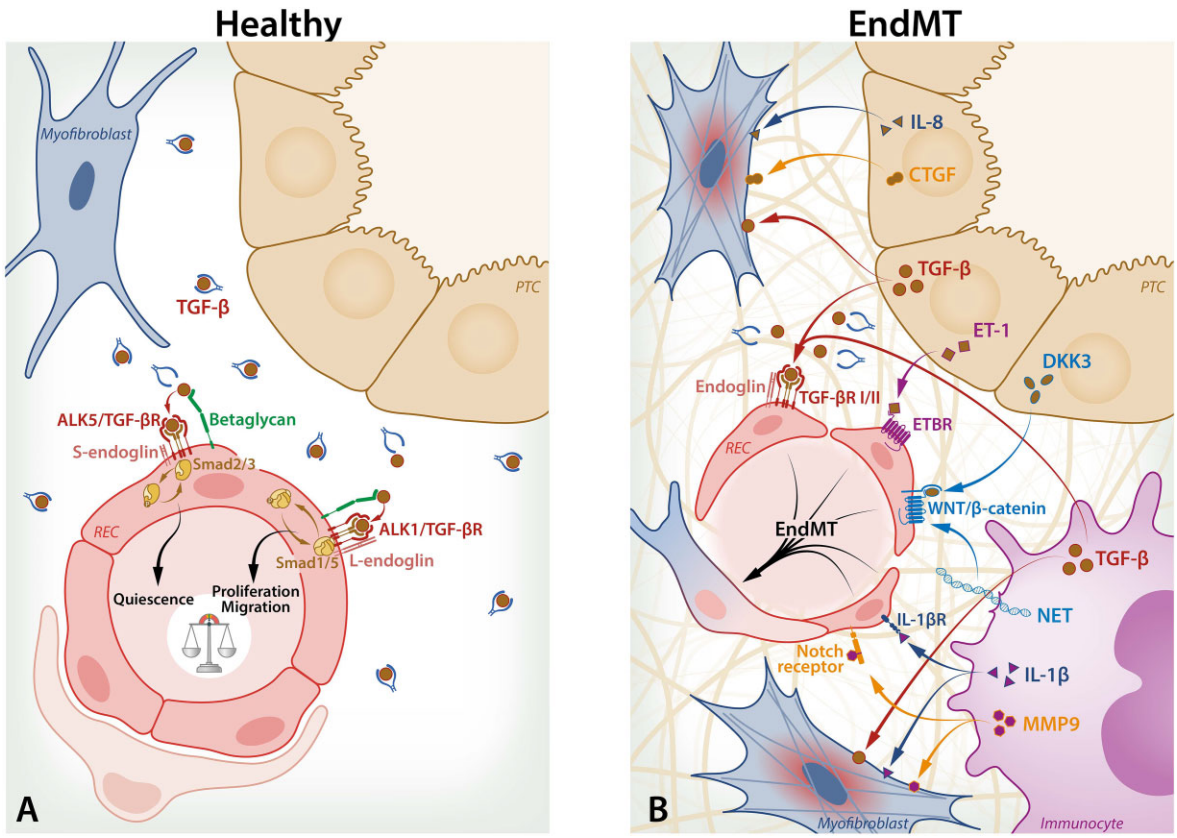

Figure 2: Maladaptive interactions within the KME induce endMT after injury. (**A**) In a physiological context, matricellular TGF-β signaling through ALK1/5 signaling is important for regulating the balance between endothelial proliferation and quiescence. (**B**) After renal injury, endMT can be induced through various mechanisms. Maladaptive PTCs release pro-inflammatory ligands, such as TGF-β, which can directly promote endMT in RECs. Furthermore, increased endothelin and Wnt/β-catenin signaling between PTCs or neutrophils (NETs) and RECs can exert endothelial transdifferentiation. EndMT results in capillary rarefaction and tissue hypoxia thereby enhancing the vicious circle of sustained inflammation in the KME. Within this pro-inflammatory environment, local profibrotic ligands such as TGF-β, IL-1β and MMP9 promote endMT and augment ECM deposition by directly activating myofibroblasts. ETBR: endothelin receptor type B; TGF-βR: transforming growth factor beta receptor.

In the context of endMT, amplification of TGF-β signaling through increased ligand production and matricellular remodeling causes activation of downstream canonical and/or non-canonical pathways [2, 8, 15]. The canonical TGF-β pathway, also known as the Smad-dependent pathway, includes translocation of the phosphorylated Smad2/3/co-Smad4 complex into the nucleus to trigger the expression of specific TGF-β targets such as transcription factors belonging to the Snail and Twist families [2, 13]. Although TGF-β itself represents the most important ligand for Smad-dependent endMT, other ligands from the TGF-β ligand superfamily such as bone morphogenetic proteins (BMPs) can also cause phosphorylation of cytoplasmic Smad1/5/8 with subsequent nuclear translocation of the Smad1/5/8/co-Smad4 complex [2, 8]. TGF-β-induced non-canonical signaling including mitogen activated protein kinases (MAPK) [extracellular signal-regulated kinase, c-Jun N-terminal kinase (JNK), and p38 MAPK] as well as phosphatidylinositol 3-kinase/Akt, c-Abl and protein kinase C also exert Smad-independent and -dependent effects on endMT [2, 8]. Activation of both non-canonical and canonical signaling results in a transcriptional shift from endothelial-specific to mesenchymal-specific gene signature expression.

Besides the TGF-β initiated pathways, endMT can also be induced by many other mechanisms. These mechanisms are often again (in)directly related to TGF-β signaling, such as the caveolar CAV-1 protein acting as a regulator of intracellular TGF-β receptor protein movements and degradation [2]. Notch signaling can synergize with TGF-β signaling to induce *Snai1* expression, while the vasoconstrictor peptide endothelin-1 (ET-1) can promote the expression of various TGF-β ligands as well as drive endMT by itself [2]. Wnt/β-catenin signaling can stimulate both autocrine TGF-β signaling as well as autonomously induce endMT through binding of Wnt ligands such as Dickkopf-1 (DKK-1) and DKK-3. The fact that DKK-1, an apparent inhibitor of Wnt/β-catenin signaling in context of renal fibrosis, can enhance endMT in aortic ECs suggests that its effects via Wnt signaling are highly dependent on the specific cellular context [2, 16]. Furthermore, inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) have shown to stimulate endMT with nuclear factor-κB as an important downstream target in inflammation-mediated transdifferentiation [2].

Other processes frequently occurring under cell stress, such as hypoxia, oxidative stress and reactive oxygen species production, changes in cell metabolism (see Box 2), shear stress and hyperglycemia in diabetic context have been related to endMT induction [2]. More recently, extracellular vesicles (EVs) have gained attention in the context of endMT through their ability to induce endothelial transdifferentiation *in vitro* [17, 18]. EVs are

produced by various cell types and contain biologically active components such as non-coding ribonucleic acids (RNA) that can reflect an inflammatory cell state. These EVs with inflammatory fingerprints seem to carry the ability to induce endMT, likely through microRNA-dependent transcriptional regulation [18]. Additionally, growth factors such as platelet-derived growth factor (PDGF) can induce endMT *in vitro* [2]. The exact involvement of all these (regulatory) mechanisms in endMT is extensively reviewed elsewhere and beyond the scope of this review [2].

Box 2: Metabolic rewiring and endMT induction

Metabolism is a critical component of each and every cell as it provides the necessary energy and building blocks, and eliminates metabolic wastes for the cells to survive and grow. During the past decade, pioneering studies have revealed that metabolism not only supports EC function but also acts as a key determinant of EC fate during vessel growth and specification [19]. For instance, during the developmental transdifferentiation of venous ECs into lymphatic ECs, the upregulation of fatty acid oxidation (FAO)-derived acetyl-CoA production is required to increase histone acetylation at lymphangiogenic genes and their expression [20]. Interestingly, endMT is also directly linked to the cellular levels of acetyl-CoA: while acetate supplementation (a precursor of acetyl-CoA) partially prevents endMT induced by TGF-β and IL-1β, long-term stabilization of TGF-β signaling, on the contrary, relies on the increase in acetate-dependent acetyl-CoA synthesis [21, 22]. Short-term EC co-stimulation with TGF-β and IL-1β lowers FAO, and decreases acetyl-CoA levels, subsequently impairing Smad7 acetylation and signaling, an inhibitor of the TGF-β signaling pathway [21]. Inhibiting FAO by deletion of endothelial CPTs, mitochondrial fatty acid transporters, also promotes endMT [21]. Conversely, prolonged TGF-β stimulation directs glucose metabolism to pyruvate-derived acetate production, which is then converted into acetyl-CoA in the cytosol in an acyl-CoA synthase short chain 2–dependent manner [22]. Acetyl-CoA-dependent acetylation of TGF-β receptor ALK5 and Smads 2 and 4 leads to further activation and long-term stabilization of TGF-β signaling in a positive feedback loop [22]. Further investigation is warranted to clarify the contrasting effects of acetate and resulting changes in acetyl-CoA levels on endoMT [21]. While TGF-β stimulates glucose metabolism by upregulating glucose uptake, hexokinase 1 expression and glycolysis-stimulating 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) enzyme expression and activity in ECs [22, 23], glucose shunt to pentose phosphate pathway is reduced, thereby compromising cytoplasmic nicotinamide adenine dinucleotide phosphate (NADPH) production [24]. The cytoplasmic NADPH pool can be indirectly replenished from the mitochondria but mitochondrial respiration (oxidative phosphorylation) is then subsequently decreased due to the hampering of mitochondrial iron–sulfur cluster biosynthesis [24]. Hence, inhibiting PFKFB3 overexpression, a critical positive regulator of glycolysis, shows the opposite phenotype with preventing endMT, as demonstrated in heart *in vivo* [24]. Overall, EC metabolism is a critical determinant of endMT with FAO, glycolysis and downstream acetate and acetyl-CoA products being involved in and potential metabolic targets of endMT [21–24].

Evidence of renal endMT in kidney disease

Within the kidney, various REC phenotypes co-exist to support compartment-specific processes of glomerular filtration, concentration of urine, tubular reabsorption, and secretion of various ions and metabolites [25]. Upon AKI, RECs get activated and lose their physiological anti-inflammatory and anti-thrombotic properties [26]. After initial activation, injured RECs can entail an even greater phenotypic plasticity resulting in a partial or complete transition to a mesenchymal cell type [2, 25]. This process has been identified in numerous renal diseases such as diabetic nephropathy (DN), lupus nephritis and chronic allograft dysfunction [2, 13, 27]. In this context, endMT contributes to vascular rarefaction and local tissue hypoxia aggravating renal fibrosis and CKD progression (Fig. 1) [26].

The presence of mesenchymal transdifferentiation in mouse RECs was first demonstrated over a decade ago [28]. The actual contribution of RECs to the emerging population of fibroblasts in renal fibrosis was later first confirmed in three different CKD mouse models: unilateral urethral obstructive (UUO) nephropathy, streptozocin (STZ)-induced DN and Alport disease [29]. In these three disease models, co-expression of endothelial marker, CD31, and (myo)fibroblast markers, FSP-1/S100-A4 and α-SMA, was found in 30%–50% of the emerging mesenchymal population [29]. Lineage tracing experiments further proved the existence of endMT 7–10 days after UUO injury with approximately 10% of the (myo)fibroblast population originating from RECs [29, 30]. Similar lineage tracing studies revealed that endMT plays a significant role in the first days after renal ischemic reperfusion injury (IRI), but also during the first few months of the development of DN [31, 32]. EndMT can theoretically occur in different kidney compartments composed of heterogenous REC subtypes [25]. Glomerular mouse ECs have been shown to transdifferentiate in the context of DN in particular, while evidence for this phenomenon in AKI–CKD models is lacking [32, 33]. By contrast, peritubular capillary ECs are prone to transdifferentiate in both DN and AKI–CKD animal models [13, 32].

Importantly, renal capillary endMT results in vascular leakage and tissue hypoxia subsequently affecting proximal tubule function [34]. Reduction of endMT in animal models ameliorates capillary structure and prevents tubulointerstitial fibrosis, highlighting its therapeutic potential [13, 27, 35]. However, despite the evidence on endMT in human renal biopsies and renal fibrosis animal models, controversy remains over to what extent endMT truly contributes to the emerging myofibroblast pool in CKD [36]. Pseudotime trajectory analysis on human biopsies and genetic fate–tracing in the UUO mouse model showed that pericytes and fibroblasts are a main source of ECM-producing myofibroblasts in kidney disease [36]. Whether renal ECs, through the endMT process, contribute to the pool of fibrogenic myofibroblasts, either directly or indirectly via the acquisition of a fibroblast or pericyte intermediate phenotypic state, remains to be further investigated. Nevertheless, the consequences of endMT in terms of enhancing local tissue hypoxia and inflammation through capillary rarefaction seem undisputable.

Mechanisms of endMT in AKI–CKD transition

In the kidney, endMT pathways such as TGF-β signaling are involved during AKI-CKD transition, ultimately promoting renal fibrogenesis [13, 15]. In mice, heterozygous knock-out of TGF-β receptor II (TβRII) in ECs reduced canonical Smad2 phosphorylation upon TGF-β1 and TGF-β2 stimulation [13]. In line with this, these

mice showed decreased renal endMT with inhibition of fibrotic response after UUO- and folate acid (FA)-induced nephropathy [13]. Interestingly, reduction of endothelial TβRII expression in these mice resulted in a decreased expression ratio of the short to long isoforms of endoglin, a well-known TGF-β accessory receptor [12, 13]. Conversely, an increased short to long isoform expression ratio is associated with EC quiescence [12, 13]. *Ex vivo* experiments on aortic vessels obtained from these heterozygous TβRII mice showed improved angiogenic potential with increased pro-angiogenic endoglin-Smad1/5 signaling as the proposed underlying mechanism [13]. Improved angiogenic signaling together with a decrease in endMT may then explain the observed preservation of renal capillaries after injury *in vivo*.

In vitro, TGF-β1 induced human umbilical vein ECs (HUVECs) transdifferentiation and simultaneously triggered upregulation of protein phosphatase 2A (PP2A) activity [27]. Endothelial PP2A affects barrier integrity through dephosphorylation of transmembrane protein occludin [27]. Moreover, abolishment of PP2A activity with selective inhibitor okadaic acid reduced endMT in HUVECs [27]. Consistent with these findings, PP2A activity was increased within 2 weeks after UUO in mouse kidney tissue lysate, and inhibition of PP2A activity reduced renal endMT [27]. Additionally, conditional deletion of downstream pro-mesenchymal transcription factors of the (non-)canonical TGF-β pathway, *Snai1* and *Twist1* genes in ECs, reduced renal fibrosis 10 days after UUO- and FA-induced injury [34]. Endothelial deletion of *Snai1* and *Twist1* suppressed both intermediate (CD31+/α-SMA+) and more advanced (CD31–/α-SMA+) endMT phenotypes resulting in improved renal capillary integrity 10 days after UUO [34].

Wnt/β-catenin signaling, a member of the non-TGF-β-mediated pathways, holds the ability to induce endothelial transdifferentiation [2]. One of its ligands, DKK-3, was identified as an excessively secreted protein by ECs from *Sirt1*-deleted mice exhibiting a permanent dysfunctional endothelial phenotype [16]. Further analysis on DKK3 revealed its autocrine ability to enhance endMT in renal microvascular endothelial cells (RMVECs) *in vitro* [16]. Upon addition of DKK-3, RMVECs showed decreased angiogenic properties with disruption of capillary integrity through increased endothelial transdifferentiation [16]. Another member of the non-TGF-β-mediated pathways, namely Yes-associated protein (YAP) signaling, was previously linked to fibrogenesis in different animal models [37]. Administration of a selective peptide antagonizing F2RL1 G-protein-coupled receptor inhibited endothelial YAP activity thereby decreasing renal endMT and kidney fibrosis after mouse UUO injury [37].

Lastly, inflammatory responses and cellular stress–induced mechanisms are involved in endMT enhancement. Activation of the complement system, a widely recognized contributor to the first phases of IRI, plays a crucial role in renal capillary transdifferentiation [35]. *In vitro*, C3a and C5a endothelial stimulation induce endMT in HUVEC culture [35]. This transdifferentiation could be counteracted by addition of an Akt inhibitor [35]. The Akt pathway plays a role in inflammation and has been linked to inflammatory endMT induction through IL-13 signaling in pulmonary artery ECs [2, 38]. As for renal disease, the downstream role of Akt in complement-induced endMT was confirmed *in vivo* using a porcine IRI model, in which pre-treatment with C1 inhibitors inhibited endMT with simultaneous downregulation of Akt signaling [35]. Furthermore, urinary EVs associated with inflammation and endMT induction were upregulated after 5/6 nephrectomy in a rat model [39]. In transplant patients with renal antibody-mediated rejection, circulating EVs containing a pro-inflammatory microRNA signature were able to induce endMT *in vitro* [17]. Whether EVs carrying pro-inflammatory transcriptional regulators play a broader role in endMT in AKI–CKD transition remains to be elucidated.

CELLULAR CROSSTALK IN KIDNEY MICRO-ENVIRONMENT ENHANCES endMT IN AKI–CKD TRANSITION

Despite the inherent regenerative capacity of the tubular epithelium following injury, AKI patients carry a high risk of developing CKD in later stages. Animal models, in particular IRI mouse models, have provided insights in the subclinical inflammatory processes that hallmark maladaptive repair responses and cause the persistent risk of disease progression [3, 40]. During sustained renal inflammation, RECs, PTCs, pericytes, myofibroblasts and immunocytes communicate in a vicious manner amplifying profibrotic signaling [41–43]. These processes of inflammation and fibrogenesis create focal sites throughout the renal parenchyma with excessive ECM deposition [14]. Within these fibrogenic niches, a specialized ECM network exists in which extracellular vesicles and multiple soluble, profibrotic factors such as TGF-β, Wnt ligands and PDGFs accumulate [14]. Many of these profibrotic factors are known inducers of endMT. Therefore, understanding the maladaptive cellular and secretory crosstalk within the fibrogenic KME represents an important opportunity to halt endMT and preserve vascular integrity.

Cell types involved in the profibrotic kidney micro-environment

Within the kidney, PTCs represent the most susceptible cell population to (ischemic) injury. Tubular reabsorption and secretion are high energy demanding processes and therefore strongly dependent on aerobic respiration [44]. Upon injury, PTCs show great regenerative capacity with induction of epithelial dedifferentiation and proliferation within 24 h. Although a substantial part of damaged epithelial cells is then able to successfully proliferate and redifferentiate to form functional tubular structures, a subpopulation of PTCs undergoes maladaptive repair processes enhancing sustained inflammation and loss of nephron structures [3, 40–42].

Maladaptive PTCs, often referred to as failed repair PTCs (FR-PTCs), acquire different phenotypes after injury. Tubular cell subpopulations can undergo (partial) epithelial-to-mesenchymal transition (EMT) or adopt a senescent, G2/M-arrested state [3, 41, 45]. These latter senescent epithelial cells are far from inactive; activation of JNK signaling induces secretion of profibrotic factors TGF-β, IL-8 and CTGF [3, 45]. *In vivo*, inhibition of this cell cycle arrest in mice through p53 or JNK inhibition reduced development of renal fibrosis after IRI [45]. As senescence of the epithelial compartment is a component of the normal aging process, increasing susceptibility for this G2/M-arrested phenotype may therefore explain worse kidney function outcomes after AKI in the elderly [3]. The initial assumption that Snail- and Twist-driven EMT contributed to the emerging pool of α-SMA+ myofibroblasts in kidney fibrosis was repeatedly disproven by genetic lineage tracing experiments [3, 41, 42, 46]. However, (partially) dedifferentiated tubular cells may also adopt a profibrotic phenotype enhancing inflammation through activation of interstitial myofibroblasts or induction endMT and pericyte-to-mesenchymal transition.

Pericytes are mural cells supporting EC homeostasis and function through direct contact and paracrine signaling.

Tissue-resident pericytes expressing Gli1, a mesenchymal marker for perivascular cells, have been shown to detach, mobilize and transdifferentiate into interstitial α-SMA+ myofibroblasts upon injury [47]. Pericytes, as well as perivascular fibroblasts, represent important subpopulations of renal stromal cells contributing to emerging myofibroblasts in renal fibrosis. Ultimately, these emerging myofibroblasts include the primary cell type accountable for matrix deposition [41]. TGF-β1 signaling can directly induce this matrix secretion of fibrotic components such as collagen (mostly type I), fibronectin and plasminogen activator inhibitor [15]. Numerous other fibrogenic ECM components, such as matricellular proteins tenascin C and TSP-1, contribute to ECM deposition by creating a favorable environment for fibroblast proliferation while carrying the ability to trigger fibroblast activation [14]. Ablation of α-SMA+ or Gli1+ myofibroblasts in an UUO mice model caused a 50%–60% and 55%–60% reduction in interstitial fibrosis, respectively, highlighting the importance of this cell type in renal fibrogenesis [41].

Lastly, immunocytes, such as monocytes and neutrophils, abundantly infiltrate tubulointerstitial spaces during acute inflammation and chronic fibrogenic phases of kidney disease. In the early phase, monocytes are recruited towards the site of injury and polarized towards a pro-inflammatory M1 phenotype [43]. These M1 macrophages produce pro-inflammatory cytokines such as IL-1 and inducible nitric oxide synthase [42, 43]. With the initiation of the repair phase a shift from M1 to M2 takes place. Although different subtypes of M2 macrophages carry distinct functions, general properties are mainly anti-inflammatory with regulation of tissue repair and ECM remodeling [42, 43]. M2 macrophages have shown to accumulate in both fibrotic animal models and human CKD tissue biopsies [43]. In IRI and UUO mice models, depletion of this M2 phenotype reduced collagen deposition and protected against renal fibrosis [43]. These effects may be explained through elimination of the profibrotic M2 secretome which enhances proliferation and activation of fibroblasts thereby promoting fibrogenesis [15, 43]. Furthermore, an infiltrating subpopulation of neutrophils expressing eosinophil marker Siglec-F has shown to contribute to fibrogenesis in an UUO injury model [48]. These neutrophils express proinflammatory cytokines such as TGF-β1, IL-1β and TNF-α, and carry enhanced ability to form neutrophil extracellular traps (NETs) [48]. Also, renal biopsies from patients with DN and focal segmental glomerulosclerosis show that the human counterpart population of Siglec-F neutrophils increases in CKD [48].

Cellular and secretory crosstalk enhance endMT

To date, studies focusing on cell–cell interactions in the KME and their effects on renal endMT are sparse. However, evidence from specific cellular secretomes and ligand–receptor analyses suggest the contribution of these signals in inducing and/or supporting endMT during AKI–CKD transition. Additionally, release of matricellular ligands through ECM remodeling can further enhance endMT induction [11, 14]. Figure 2 summarizes relevant cellular interactions and related paracrine signals in the KME in both healthy and injured contexts.

So far, a single study has reported a direct link between renal endMT and defective PTC–REC interactions [34]. PTCs display changes in metabolism upon injury with the downregulation of FAO and upregulation of glycolytic processes [44]. Inducible endothelial-specific deletion of *Twist1* or *Snai1* as pro-mesenchymal transcription factors curtailed endMT, vascular leakage and kidney fibrosis in a UUO model [34]. Furthermore, this conditional deletion decreased the expression of carbonic anhydrase IX, an indicator for hypoxia, in PTCs and increased preservation of FAO through diminishing epithelial Myc signaling, a transcription factor known to enhance glycolytic adaptations [34]. Interestingly, PTCs with a conditional *Myc* knock-out displayed similar metabolic changes and reduced fibrogenesis as seen in the endothelial *Snai1/Twist1* knock-out model, while *Myc* deletion in α-SMA+ myofibroblasts did not induce the same effects [34]. Hence, metabolic changes in tubular epithelial cells and renal endMT are closely related and critical drivers of kidney fibrosis.

TGF-β signaling exerts fibrogenic effects on multiple cell types in fibrotic kidney disease. As previously mentioned, renal endothelial cells can undergo endMT following TβRII stimulation [13]. G2/M-arrested, senescent PTCs show prolonged secretion of TGF-β up to 6 weeks in mouse IRI and aristolochic acid toxic nephropathy [45]. Also, ligand–receptor analysis revealed increased TGF-β2–endoglin signaling between VCAM1+ FR-PTCs and RECs 6 weeks after mouse IRI [40]. Endoglin, a type III accessory TGF-β receptor, carries a more indirect, modulatory effect on TGF-β signaling by balancing ALK1/ALK5 signaling [8, 12]. Although endoglin can negatively regulate ALK5 signaling and therefore inhibit endMT, prolonged TGF-β binding can also favor ALK5 signaling and induce endMT [6]. These specific regulatory effects of endoglin signaling on EC angiogenic potential are dependent on relative abundance of endoglin protein isoforms which was not specified in the ligand–receptor data on FR-PTC and REC interaction [12, 13, 40]. Thus, TGF-β-mediated endMT in RECs through paracrine signaling by senescent PTCs may occur but warrants further experimental validation.

Another potentially relevant interaction between VCAM1+ FR-PTCs and RECs 6 weeks after mouse IRI involves the binding of ET-1 ligand to endothelin receptor type B on RECs [40]. ET-1 induces endMT *in vitro* in human and murine ECs [2, 49]. Similar capacities of ET-1 were also demonstrated *in vivo* in a skin and lung fibrosis mice model [2, 49]. Although ET-1 alone was able to induce endMT, its effects also relied on amplification of an autocrine TGF-β signaling loop [2, 49]. The increased interaction between FR-PTCs and RECs through ET-1 signaling may represent a similar mechanism in renal fibrosis.

Furthermore, a characteristic of senescent, G2/M-arrested PTCs in CKD includes secretion of pro-inflammatory Wnt ligands [41]. Activation of Wnt/β-catenin pathways trough DKK-3 signaling showed ability to induce endMT in RMVECs [16]. Both defective ECs and senescent PTCs can acquire pro-inflammatory phenotypes characterized by Wnt and Notch ligand excretion with known profibrotic effects on myofibroblasts and pericytes [7, 16, 41]. The specific ECM composition within the fibrogenic KME further promotes the profibrotic signaling of these soluble factors [14].

The secretome of immunocytes such as M2 macrophages and neutrophils can directly exert profibrotic effects on fibroblasts [43]. The production of IL-1β, TGF-β and diverse matrix metalloproteinases (MMPs) likely also affects ECs and renal endMT [43]. A previous study reported *in vitro* treatment of HUVECs with IL-1β to induce endMT [2]. Also, MMP9, one of the MMPs produced by M2 macrophages [43], enhances endMT in glomerular ECs via induction of Notch signaling, possibly linking the M2 secretome to renal endMT [50]. As for neutrophils, release of NETs, which includes a characteristic of expanding Siglec-F neutrophil populations after injury, has been shown to induce glomerular endMT *in vitro* via β-catenin signaling, thereby providing another possible mechanism for renal endMT induction [51].

CLINICAL TRANSLATION AND FUTURE PROSPECTS

From a clinical perspective, broadly curtailing TGF-β signaling to halt endMT would elicit unwanted side effects due to its important role in many physiological processes. Abolishment of profibrotic Wnt/β-catenin ligands or MMP9 as part of the M2 secretome for instance could potentially offer more promising strategies. However, current preclinical evidence for this is lacking. Recent studies have shown that readily approved drugs within the antidiabetic and cardiovascular field may prevent endMT through partial abolishment of TGF-β activation or reduction of less potent, non-TGF-β-mediated mechanisms [2, 52–54]. This partial inhibition may already delay AKI–CKD progression through interrupting the pro-inflammatory vicious cycle in the KME.

Dipeptyl-peptidase IV (DPP-4) inhibitors and glucagon-like-peptide-1 (GLP-1) agonists are well-known anti-diabetic drugs which increase insulin/glucagon ratios through (in)directly promoting incretin activity [52]. Increased GLP-1 receptor activity reduces TGF-β1 signaling, with downregulation of TβRI, Smad3 and Snail proteins, and upregulates BMP-receptor 2 (BMPR2) expression causing inhibition of endMT in monocrotaline-induced kidney injury in rats [52]. This balance between TβR and BMP2R has also been implicated to regulate endMT in human pulmonary ECs, offering perspective for other fibrotic diseases as well [2]. Additionally, linagliptin treatment in 5/6 nephrectomy rats decreased the pro-inflammatory, endMT-associated urinary EV signature [39]. However, clinical use of linagliptin in diabetes mellitus type II patients with high risk on kidney disease development did not benefit renal function [55]. As for statins, TGF-β1 signaling via Smad2/3 phosphorylation was abrogated in STZ-treated mice through treatment with lovastatin, thereby reducing glomerular endMT [53]. Within the cardiovascular field, a recent study shows a different mechanism through which statins inhibit endMT under normoglycemic and hyperglycemic conditions [54]. Both in induced pluripotent stem cell-derived ECs and an *in vivo* mouse model, statin treatment reduced endMT in ECs through inhibition of non-TGF-β-mediated YAP nuclear translocation and reducing activity of downstream SOX9 enhancer [54]. As for renal disease, YAP was identified as a regulator of endMT in UUO-induced kidney fibrosis [37]. Further investigation is warranted to elucidate similar mechanisms in human AKI–CKD transition and to explore the potential of these anti-diabetic and cardiovascular drugs to improve renal endMT phenotypes in a clinical context.

In addition to therapeutic strategies to halt endMT, enhancement of neovascularization through stimulating the reverse process, mesenchymal-to-endothelial transition, may also offer therapeutic potential [56]. Cardiac fibroblasts have shown the ability to adopt EC characteristics and improve vascularity after injury through a p53-dependent fate transition [56]. Similar reverse processes in kidney disease remain to be investigated [7].

To summarize, endMT includes a complex phenotypic transdifferentiation that aggravates fibrotic disease by enhancing local tissue hypoxia and inflammation and possibly expanding the pool of fibrogenic myofibroblasts. Reduction or even reversal of endMT offers therapeutic potential by reducing organ structure disruption and dysfunction. As for renal disease, inhibition of endMT could delay AKI–CKD progression through favorable effects on tubular health and reduce local inflammation in the KME. Animal studies propose various strategies to inhibit endMT through abrogation of (non)-TGF-β-mediated pathways with promising results for statins and DPP-4- and GLP-1-mediated antidiabetic drugs in terms of clinical translation.

ACKNOWLEDGEMENTS

We kindly thank Manon Zuurmond (LUMC, Leiden, The Netherlands) for providing us with the figures.

FUNDING

M.E.J., M.A.E., S.J.D. and T.J.R. are supported by The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW); The Novo Nordisk Foundation Center for Stem Cell Medicine is supported by Novo Nordisk Foundation grants (NNF21CC0073729).

AUTHORS' CONTRIBUTIONS

Review conception, design and manuscript preparation: M.E.J., S.J.D. and T.J.R. Manuscript preparation and revision: D.K.V. and M.A.E. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

No new data were generated or analyzed in support of this research.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Rosenbloom J, Macarak E, Piera-Velazquez S *et al.* Human fibrotic diseases: current challenges in fibrosis research. *Methods Mol Biol* 2017;**1627**:1–23. https://doi.org/10.1007/978-1-4939-7113-8_1
2. Piera-Velazquez S, Jimenez SA. Endothelial to mesenchymal transition: role in physiology and in the pathogenesis of human diseases. *Physiol Rev* 2019;**99**:1281–324. https://doi.org/10.1152/physrev.00021.2018
3. Ferenbach DA, Bonventre JV. Mechanisms of maladaptive repair after AKI leading to accelerated kidney ageing and CKD. *Nat Rev Nephrol* 2015;**11**:264–76. https://doi.org/10.1038/nrneph.2015.3
4. Kramann R, Kusaba T, Humphreys BD. Who regenerates the kidney tubule? *Nephrol Dial Transplant* 2015;**30**:903–10. https://doi.org/10.1093/ndt/gfu281
5. Rayego-Mateos S, Marquez-Expósito L, Rodrigues-Diez R *et al.* Molecular mechanisms of kidney injury and repair. *Int J Mol Sci* 2022;**23**:1542. https://doi.org/10.3390/ijms23031542
6. Goligorsky MS. Pathogenesis of endothelial cell dysfunction in chronic kidney disease: a retrospective and what the future may hold. *Kidney Res Clin Pract* 2015;**34**:76–82. https://doi.org/10.1016/j.krcp.2015.05.003
7. Lipphardt M, Song JW, Matsumoto K *et al.* The third path of tubulointerstitial fibrosis: aberrant endothelial secretome. *Kidney Int* 2017;**92**:558–68. https://doi.org/10.1016/j.kint.2017.02.033
8. He J, Xu Y, Koya D *et al.* Role of the endothelial-to-mesenchymal transition in renal fibrosis of chronic kidney disease. *Clin Exp Nephrol* 2013;**17**:488–97. https://doi.org/10.1007/s10157-013-0781-0
9. Yoshimatsu Y, Watabe T. Emerging roles of inflammation-mediated endothelial-mesenchymal transition in health and disease. *Inflamm Regen* 2022;**42**:9. https://doi.org/10.1186/s41232-021-00186-3
10. Zeisberg EM, Tarnavski O, Zeisberg M *et al.* Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* 2007;**13**:952–61. https://doi.org/10.1038/nm1613

11. Witjas FMR, van den Berg BM, van den Berg CW *et al.* Concise review: the endothelial cell extracellular matrix regulates tissue homeostasis and repair. *Stem Cells Transl Med* 2019;**8**:375–82. https://doi.org/10.1002/sctm.18-0155
12. Schoonderwoerd MJA, Goumans MTH, Hawinkels L. Endoglin: beyond the endothelium. *Biomolecules* 2020;**10**:289. https://doi.org/10.3390/biom10020289
13. Xavier S, Vasko R, Matsumoto K *et al.* Curtailing endothelial TGF-β signaling is sufficient to reduce endothelial-mesenchymal transition and fibrosis in CKD. *J Am Soc Nephrol* 2015;**26**:817–29. https://doi.org/10.1681/ASN.2013101137
14. Li L, Fu H, Liu Y. The fibrogenic niche in kidney fibrosis: components and mechanisms. *Nat Rev Nephrol* 2022;**18**:545–57. https://doi.org/10.1038/s41581-022-00590-z
15. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-β: the master regulator of fibrosis. *Nat Rev Nephrol* 2016;**12**:325–38. https://doi.org/10.1038/nrneph.2016.48
16. Lipphardt M, Dihazi H, Jeon NL *et al.* Dickkopf-3 in aberrant endothelial secretome triggers renal fibroblast activation and endothelial–mesenchymal transition. *Nephrol Dial Transplant* 2018;**34**:49–62. https://doi.org/10.1093/ndt/gfy100
17. Franzin R, Stasi A, Sallustio F *et al.* Extracellular vesicles derived from patients with antibody-mediated rejection induce tubular senescence and endothelial to mesenchymal transition in renal cells. *Am J Transplant* 2022;**22**:2139–57. https://doi.org/10.1111/ajt.17097
18. Mathiesen A, Hamilton T, Carter N *et al.* Endothelial extracellular vesicles: from keepers of health to messengers of disease. *Int J Mol Sci* 2021;**22**:4640. https://doi.org/10.3390/ijms22094640
19. Dumas SJ, García-Caballero M, Carmeliet P. Metabolic signatures of distinct endothelial phenotypes. *Trends Endocrinol Metab* 2020;**31**:580–95. https://doi.org/10.1016/j.tem.2020.05.009
20. Wong BW, Wang X, Zecchin A *et al.* The role of fatty acid β-oxidation in lymphangiogenesis. *Nature* 2017;**542**:49–54. https://doi.org/10.1038/nature21028
21. Xiong J, Kawagishi H, Yan Y *et al.* A metabolic basis for endothelial-to-mesenchymal transition. *Mol Cell* 2018;**69**:689–98.e7. https://doi.org/10.1016/j.molcel.2018.01.010
22. Zhu X, Wang Y, Soaita I *et al.* Acetate controls endothelial-to-mesenchymal transition. *Cell Metab* 2023;**35**:1163–78.e10. https://doi.org/10.1016/j.cmet.2023.05.010
23. Wang FF, Zhang JL, Ji Y *et al.* KLF2 mediates the suppressive effect of BDNF on diabetic intimal calcification by inhibiting HK1 induced endothelial-to-mesenchymal transition. *Cell Signal* 2022;**94**:110324. https://doi.org/10.1016/j.cellsig.2022.110324
24. Zeng H, Pan T, Zhan M *et al.* Suppression of PFKFB3-driven glycolysis restrains endothelial-to-mesenchymal transition and fibrotic response. *Signal Transduct Target Ther* 2022;**7**:303. https://doi.org/10.1038/s41392-022-01097-6
25. Dumas SJ, Meta E, Borri M *et al.* Phenotypic diversity and metabolic specialization of renal endothelial cells. *Nat Rev Nephrol* 2021;**17**:441–64. https://doi.org/10.1038/s41581-021-00411-9
26. Jourde-Chiche N, Fakhouri F, Dou L *et al.* Endothelium structure and function in kidney health and disease. *Nat Rev Nephrol* 2019;**15**:87–108. https://doi.org/10.1038/s41581-018-0098-z
27. Deng Y, Guo Y, Liu P *et al.* Blocking protein phosphatase 2A signaling prevents endothelial-to-mesenchymal transition and renal fibrosis: a peptide-based drug therapy. *Sci Rep* 2016;**6**:19821. https://doi.org/10.1038/srep19821
28. O'Riordan E, Mendelev N, Patschan S *et al.* Chronic NOS inhibition actuates endothelial-mesenchymal transformation. *Am J Physiol Heart Circ Physiol* 2007;**292**:H285–94. https://doi.org/10.1152/ajpheart.00560.2006
29. Zeisberg EM, Potenta SE, Sugimoto H *et al.* Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J Am Soc Nephrol* 2008;**19**:2282–7. https://doi.org/10.1681/ASN.2008050513
30. LeBleu VS, Taduri G, O'Connell J *et al.* Origin and function of myofibroblasts in kidney fibrosis. *Nat Med* 2013;**19**:1047–53. https://doi.org/10.1038/nm.3218
31. Basile DP, Friedrich JL, Spahic J *et al.* Impaired endothelial proliferation and mesenchymal transition contribute to vascular rarefaction following acute kidney injury. *Am J Physiol Renal Physiol* 2011;**300**:F721–33. https://doi.org/10.1152/ajprenal.00546.2010
32. Li J, Qu X, Bertram JF. Endothelial-myofibroblast transition contributes to the early development of diabetic renal interstitial fibrosis in streptozotocin-induced diabetic mice. *Am J Pathol* 2009;**175**:1380–8. https://doi.org/10.2353/ajpath.2009.090096
33. Chang K, Xie Q, Niu J *et al.* Heparanase promotes endothelial-to-mesenchymal transition in diabetic glomerular endothelial cells through mediating ERK signaling. *Cell Death Discov* 2022;**8**:67. https://doi.org/10.1038/s41420-022-00858-0
34. Lovisa S, Fletcher-Sananikone E, Sugimoto H *et al.* Endothelial-to-mesenchymal transition compromises vascular integrity to induce Myc-mediated metabolic reprogramming in kidney fibrosis. *Sci Signal* 2020;**13**:eaaz2597. https://doi.org/10.1126/scisignal.aaz2597
35. Curci C, Castellano G, Stasi A *et al.* Endothelial-to-mesenchymal transition and renal fibrosis in ischaemia/reperfusion injury are mediated by complement anaphylatoxins and Akt pathway. *Nephrol Dial Transplant* 2014;**29**:799–808. https://doi.org/10.1093/ndt/gft516
36. Kuppe C, Ibrahim MM, Kranz J *et al.* Decoding myofibroblast origins in human kidney fibrosis. *Nature* 2021;**589**:281–6. https://doi.org/10.1038/s41586-020-2941-1
37. Ren Y, Zhang Y, Wang L *et al.* Selective targeting of vascular endothelial YAP activity blocks EndMT and ameliorates unilateral ureteral obstruction-induced kidney fibrosis. *ACS Pharmacol Transl Sci* 2021;**4**:1066–74. https://doi.org/10.1021/acsptsci.1c00010
38. Takagi K, Yamakuchi M, Matsuyama T *et al.* IL-13 enhances mesenchymal transition of pulmonary artery endothelial cells via down-regulation of miR-424/503 in vitro. *Cell Signalling* 2018;**42**:270–80. https://doi.org/10.1016/j.cellsig.2017.10.019
39. Delić D, Wiech F, Urquhart R *et al.* Linagliptin and telmisartan induced effects on renal and urinary exosomal miRNA expression in rats with 5/6 nephrectomy. *Sci Rep* 2020;**10**:3373. https://doi.org/10.1038/s41598-020-60336-4
40. Kirita Y, Wu H, Uchimura K *et al.* Cell profiling of mouse acute kidney injury reveals conserved cellular responses to injury. *Proc Natl Acad Sci USA* 2020;**117**:15874–83. https://doi.org/10.1073/pnas.2005477117
41. Humphreys BD. Mechanisms of renal fibrosis. *Annu Rev Physiol* 2018;**80**:309–26. https://doi.org/10.1146/annurev-physiol-022516-034227
42. Kumar S. Cellular and molecular pathways of renal repair after acute kidney injury. *Kidney Int* 2018;**93**:27–40. https://doi.org/10.1016/j.kint.2017.07.030
43. Tang PM, Nikolic-Paterson DJ, Lan HY. Macrophages: versatile players in renal inflammation and fibrosis. *Nat Rev Nephrol* 2019;**15**:144–58. https://doi.org/10.1038/s41581-019-0110-2
44. van der Rijt S, Leemans JC, Florquin S *et al.* Immunometabolic rewiring of tubular epithelial cells in kidney disease.

Nat Rev Nephrol 2022;**18**:588–603. https://doi.org/10.1038/s41581-022-00592-x

45. Yang L, Besschetnova TY, Brooks CR *et al.* Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. *Nat Med* 2010;**16**:535–43, 1p following 143. https://doi.org/10.1038/nm.2144
46. Humphreys BD, Lin S-L, Kobayashi A *et al.* Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol* 2010;**176**:85–97. https://doi.org/10.2353/ajpath.2010.090517
47. Kramann R, Schneider RK, DiRocco DP *et al.* Perivascular Gli1+ progenitors are key contributors to injury-induced organ fibrosis. *Cell Stem Cell* 2015;**16**:51–66. https://doi.org/10.1016/j.stem.2014.11.004
48. Ryu S, Shin JW, Kwon S *et al.* Siglec-F-expressing neutrophils are essential for creating a profibrotic microenvironment in renal fibrosis. *J Clin Invest* 2022;**132**. https://doi.org/10.1172/JCI156876
49. Wermuth PJ, Li Z, Mendoza FA *et al.* Stimulation of transforming growth factor-β1-induced endothelial-to-mesenchymal transition and tissue fibrosis by Endothelin-1 (ET-1): a novel profibrotic effect of ET-1. *PLoS One* 2016;**11**:e0161988. https://doi.org/10.1371/journal.pone.0161988
50. Zhao Y, Qiao X, Wang L *et al.* Matrix metalloproteinase 9 induces endothelial-mesenchymal transition via Notch activation in human kidney glomerular endothelial cells. *BMC Cell Biol* 2016;**17**:21. https://doi.org/10.1186/s12860-016-0101-0
51. Pieterse E, Rother N, Garsen M *et al.* Neutrophil extracellular traps drive endothelial-to-mesenchymal transition. *Arterioscler Thromb Vasc Biol* 2017;**37**:1371–9. https://doi.org/10.1161/ATVBAHA.117.309002
52. Xu J, Wang J, Cheng Y *et al.* Glucagon-like peptide-1 mediates the protective effect of the dipeptidyl peptidase IV inhibitor on renal fibrosis via reducing the phenotypic conversion of renal microvascular cells in monocrotaline-treated rats. *Biomed Res Int* 2018;**2018**:1864107. https://doi.org/10.1155/2018/1864107
53. Ma Z, Zhu L, Liu Y *et al.* Lovastatin alleviates endothelial-to-mesenchymal transition in glomeruli via suppression of oxidative stress and TGF-β1 signaling. *Front Pharmacol* 2017;**8**:473. https://doi.org/10.3389/fphar.2017.00473
54. Liu C, Shen M, Tan WLW *et al.* Statins improve endothelial function via suppression of epigenetic-driven EndMT. *Nat Cardiovasc Res* 2023;**2**:467–85. https://doi.org/10.1038/s44161-023-00267-1
55. Rosenstock J, Perkovic V, Johansen OE *et al.* Effect of linagliptin vs placebo on major cardiovascular events in adults with type 2 diabetes and high cardiovascular and renal risk: the CARMELINA randomized clinical trial. *JAMA* 2019;**321**:69–79. https://doi.org/10.1001/jama.2018.18269
56. Ubil E, Duan J, Pillai ICL *et al.* Mesenchymal–endothelial transition contributes to cardiac neovascularization. *Nature* 2014;**514**:585–90. https://doi.org/10.1038/nature13839

Received: September 4, 2023; Editorial decision: November 5, 2023