Targeting the progression of chronic kidney disease

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Abstract: Chronic kidney disease (CKD) is a devastating condition that is reaching epidemic levels owing to the increasing incidences of diabetes mellitus, hypertension and obesity as well as ageing of the global population. Regardless of the underlying aetiology, CKD is slowly progressive and leads to irreversible nephron loss, end-stage renal disease and/or premature death. Factors that contribute to CKD progression include parenchymal cell loss, chronic inflammation, fibrosis and the reduced regenerative capacity of the kidney. Current therapies have limited effectiveness and only delay disease progression, underscoring the need to develop novel therapeutic approaches to either stop or reverse progression. Preclinical studies have identified several approaches that reduce fibrosis in experimental models, including targeting cytokines, transcription factors, developmental and signalling pathways and epigenetic modulators, particularly non-coding RNAs. Some of these nephroprotective strategies are now being tested in clinical trials. Lessons learned from TGFβ1 blockade underscore the need for a holistic approach to CKD therapy as approaches that target a single pathogenic process may result in unexpected negative effects on simultaneously occurring processes. Additional promising approaches include preventing tubular cell injury and anti-fibrotic strategies targeting activated myofibroblasts, the main collagen producing cells.

Key points

- Current therapies for chronic kidney disease (CKD) target multiple pathogenic pathways but only slow disease progression; an improved understanding of CKD pathogenesis is needed to optimize treatment approaches
- Partial epithelial to mesenchymal transition contributes to renal fibrosis through epithelial G2/M cell cycle arrest and induction of a senescence-related phenotype; these processes are potential therapeutic targets

- Strategies that target activated myofibroblasts (the main collagen producing cells) or enzymes that are involved in collagen degradation have the potential to improve or even reverse renal fibrosis
- Kidney injury results in the reactivation of developmental pathways that contribute to CKD progression; these pathways are also potential therapeutic targets
- Growth factors such as PDGF, CTGF and gremlin promote both inflammation and fibrosis in kidney disease; these factors are promising therapeutic targets for CKD.
- Epigenetic modulators are potential targets to prevent or reduce kidney damage; microRNA therapies and BET inhibitors are renoprotective in preclinical models and are now undergoing clinical trials with CKD end points

Introduction

The contribution of CKD to worldwide mortality is rapidly increasing, illustrating the shortcomings of current therapeutic approaches[4]. The incidence of CKD and its absolute contribution to cardiovascular disease and mortality increases with age and over 60% of those aged over 80 years have CKD. Patients with CKD have an increased risk of acute kidney injury (AKI) and repeated episodes of AKI contribute to CKD progression. Similarly, AKI can lead to the development of CKD. Independent of its cause, CKD is characterized by progressive and irreversible nephron loss, reduced renal regenerative capacity, microvascular damage, metabolic changes, oxidative stress and inflammation, ultimately resulting in fibrosis[1,5–10].

Fibrosis is part of the normal repair process that is triggered in response to injury and preserves the architecture and functional integrity of the tissue. However, deregulation of this process leads to pathological accumulation of extracellular matrix (ECM) proteins, mainly collagens[1]. In CKD, loss of podocytes and their replacement by ECM (termed glomerulosclerosis), tubular cell injury and subsequent tubulointerstitial fibrosis contribute to nephron loss[7,9]. These processes result in the replacement of parenchymal tissue by ECM — the pathological hallmark of fibrosis – and concomitant irreversible damage. Thus, regardless of aetiology, CKD progresses to tubulointerstitial fibrosis, which correlates more strongly with proteinuria and disease severity than does histological glomerular injury[11].Tubulointerstitial diseases also lead to glomerulosclerosis, although the molecular pathways are less well characterized than those that underlie the link between glomerular injury and tubulointerstitial fibrosis.

Studies done in the last 10 years has focused on the molecular and cellular pathways that drive ECM accumulation, a process known as fibrogenesis. Data from preclinical studies that have tested anti-fibrotic therapies in multiple models of organ fibrosis and the results of clinical trials have provided information on pathways that are relevant for fibrogenesis and highlighted the need to implement novel therapeutic strategies. These strategies include modulating inflammation, inhibiting profibrotic growth factors, and targeting epigenetic alterations[2]. The nephroprotective effect of specific targeting of fibrosis is unclear owing to a lack of clinical data despite promising findings in preclinical models. Moreover, some therapeutic approaches that were initially thought to primarily modulate molecular pathways that are directly involved in fibrogenesis have been found to also modulate additional processes, such as inflammation, making it difficult to discern which protective pathway predominates in vivo.

In this Review, we discuss drivers of fibrogenesis and the contributions of epithelial cell injury, inflammation, regeneration pathways and factors that promote the AKI to CKD transition. We believe that a holistic approach is the only rational strategy to prevent CKD progression owing to the simultaneous contributions of several pathogenic processes and the potential for single mediators to differentially modulate key mechanisms such as cell injury, inflammation and fibrogenesis. Hence, we take a broad approach to the topic of preventing CKD progression, not only discussing direct targeting of fibrotic pathways but also other therapeutic approaches that have been reported to lead to a decrease in kidney fibrosis in preclinical and/or clinical studies. In the nephrology literature, the term fibrosis is traditionally used to refer to interstitial fibrosis, whereas sclerosis is used to refer to processes taking place in the glomerulus. In both cases the outcome is the replacement of parenchymal renal cells by ECM, therefore, in this article we refer to both processes when discussing kidney fibrosis.

Current therapies for kidney disease

The current mainstay of CKD therapy is renin-angiotensin system (RAS) blockade using angiotensin converting enzyme (ACE) inhibitors and/or angiotensin receptor blockers (ARBs). However, RAS blockade is indicated only in proteinuric or hypertensive nephropathies and delays but does not prevent CKD progression[12]. Both ACE inhibitors and ARBs slow CKD progression in proteinuric kidney diseases. Mineralocorticoid receptor blockers also have an antiproteinuric effect but whether they delay CKD progression when used in combination with ACE inhibitors or ARBs remains to be proven[13]. No current therapy can prevent AKI or the AKI-to-CKD transition.

Clinical trials have not specifically addressed the question of whether RAS blockade decreases renal fibrosis, but indirect clinical evidence (for example, normalization of the urinary collagen fragment peptidome and sample preclinical evidence support a beneficial effect[12,14]. Studies in cultured cells and animal models have identified multiple profibrotic actions of angiotensin II (AngII) and aldosterone, substantially beyond their antiproteinuric, systemic and glomerular haemodynamic effects (**Figure 1)**[12,15]. AngII is now considered to be a cytokine that regulates renal cell responses and contributes to several processes that are involved in kidney disease, including cell injury, inflammation and fibrosis[15].

In 2019, the endothelin-1 receptor antagonist atrasentan and sodium-glucose transporter 2 (SGLT2) inhibitors were shown to prevent CKD progression in patients with DKD[19,20]. These findings represent a major breakthrough in the treatment of kidney disease. SGLT2 inhibitors are already part of the therapeutic armamentarium against DKD but atrasentan is not yet available in the clinic. Both canagliflozin and atrasentan have pleiotropic effects that may directly impact modulators of fibrosis. Canagliflozin has been observed to have anti-fibrotic effects in experimental DKD, adenine-induced CKD and human liver disease[249–251], whereas atrasentan reduced kidney fibrosis in experimental DKD[252–254]. Whether SGLT2 inhibitors slow kidney fibrosis in humans is not yet known.

Prevention or reversal of fibrosis

The current approach to the clinical problem of irreversible kidney fibrosis is prevention, which should be targeted at preventing AKI-to-CKD transition and/or the progression of existing fibrosis. This approach encompasses a wide array of strategies that include preventing tubular cell injury, decreasing potentially injurious inflammatory responses and targeting mechanisms that directly cause fibrosis. Certain therapeutic targets may regulate multiple processes, making it difficult to dissect these interacting mechanisms. For example, AngII promotes inflammation and fibrosis through the activation of multiple signaling systems **(Figure 1).**

The ultimate aim of fibrosis research is reversal of fibrosis but little progress has been made toward this goal. Regression of glomerulosclerosis has been observed in rats on RAS blockade, but this process is not thought to occur in humans[16]. In patients with diabetic kidney disease (DKD) in whom the injurious stimulus was completely removed by pancreas transplantation, reversal of **kidney** fibrosis, as assessed by basement membrane thickening, took 10 years[17]. Thus, reversal of fibrosis is achievable in humans, but the timelines should be measured in decades, at least when the therapeutic maneuver is limited to removal of the pro-fibrotic stimulus[18]. Furthermore, whether interstitial type I and III collagen deposition is as reversible as basement membrane type IV collagen deposition is unclear.

An unmet need exists for therapies that prevent CKD progression or promote kidney regeneration. The development of such therapies requires an in-depth understanding of the molecular mechanisms of kidney injury and of why kidney repair fails**.**

Tubular cell injury and regeneration

The tubular epithelium is metabolically very active and has a high mitochondrial content to fuel active transport but is very vulnerable to injury owing to hypoxia, toxic compounds, proteinuria, metabolic disorders and senescence. In response to an insult, tubular epithelial cells undergo several changes, including loss of cell-to-cell contacts and the polarized epithelial phenotype, leading to impairment of tubular function. Tubular cell injury can be lethal (resulting in cell death) or sublethal (leading to surviving but injuried cells). The initial insult is followed by a recovery phase characterized by activation of protective and regenerative mechanisms in the surviving cells that restore epithelial cell properties and functions. Failure of regenerative mechanisms may promote kidney fibrosis and CKD progression (**Figure 2**). The mechanisms of tubular damage are complex and **involve** mitochondrial dysfunction, oxidative stress, metabolic changes, cell cycle arrest, dedifferentiation, induction of a senescence-related phenotype, secretion of inflammatory mediators, RAS activation and epigenetic changes.

Mechanisms of tubular cell death

The mechanisms of tubular cell death are complex and differ with aetiology, magnitude of the initial insult and timing during kidney injury (**Figure 2**). Multiple forms of cell death contribute to kidney injury, including apoptosis, regulated necrosis.

Apoptosis. Initial studies of the therapeutic modulation of cell death in the kidney focused on apoptosis in experimental AKI[22]. The results of some studies were promising and others disappointing. For example, key modulators of apoptosis, such as caspase inhibitors, were shown to potentially increase the severity of kidney injury and clinical development for this indication was abandoned[23,24]

Currently, only one apoptosis-modulating agent, QPI-1002, a siRNA targeting p53, is undergoing phase 3 clinical trials to prevent AKI following cardiac surgery (NCT03510897). P53 targeting may be nephroprotective through several pathways, including protection from tubular cell apoptosis and from G2/M arrest (discussed below). However, preclinical studies have raised the issue of timing of p53 targeting, which if not optimized, might enhance kidney fibrosis despite protecting from the initial AKI episode[25,26]. This dissociation between the acute and chronic impact of therapeutic interventions is illustrated by the increased severity of ischaemia-reperfusion injury (IRI) attributed to increased necroptosis in caspase 3 deficient mice compared with wild-type controls, which is followed by milder microvascular rarefaction and renal fibrosis[27]. In this regard, fibroblast abundance may also be regulated through apoptosis induction[28].

Regulated necrosis. Accumulating evidence suggests a key role of regulated necrosis in AKI. Strategies that target ferroptosis or necroptosis protect against diverse forms of AKI[29]. These forms of cell death are more attractive targets than apoptosis, given their potential to boost inflammatory and immune responses, and may be sequentially recruited, potentially impacting on the optimal timing of specific therapeutic interventions[23,30,31]. Targeting of the necroptosis pathway molecules receptor-interacting serine/threonine-protein kinase 1 (RIPK1) or RIPK3 prevented fibrosis in mice with unilateral uretheral obstruction (UUO) and/or adenine nephrotoxicity, at least in part through actions in fibroblasts [32,33]. Based on descriptive data, sustained activation of regulated necrosis pathways was also hypothesized to promote the AKI-to-CKD transition in cisplatin nephrotoxicity but, strikingly, in the absence of fibrosis[32].

Autophagy. Autophagy is a tightly regulated catabolic process that delivers damaged organelles or cytoplasmic components to lysosomes for degradation and recycling to maintain cellular homeostasis[34]. In mice, tubular-specific deficiency of autophagy-related proteins (e.g. autophagy protein 5 (atg5), atg7 and microtubule-associated protein 1A/1B-light chain 3 (LC3) increases the accumulation of damaged mitochondria, tubular cell apoptosis and CKD progression[34]. Studies using pharmacological inhibition of autophagy and deletion of autophagy genes confirmed its protective role in AKI, CKD and podocyte injury. Although excess autophagy may be deleterious, therapeutic optimization of autophagy using various strategies, including chemical chaperones to

normalize endoplasmic reticulum stress and activation of the transcription factor EB, should be investigated as approaches to prevent fibrosis.

Cell cycle arrest

Cell cycle arrest in the G1 phase following AKI is a protective mechanism that prevents replication of damaged DNA[35,36]. However, sustained cell cycle arrest can lead to cell senescence and maladaptive repair (**Figure 2**). For example, proximal tubular cell G2/M arrest during AKI leads to fibrosis through activation of c-jun N-terminal kinase (JNK) signaling and upregulation of the production of profibrotic cytokines[37]. In mice with IRI, treatment with a p53 inhibitor prevented G2/M arrest and subsequent fibrosis[37]. Epithelial cells in the G2/M phase form target of rapamycin (TOR)-autophagy spatial coupling compartments (TASCCs) that promote a profibrotic secretory phenotype similar to the senescence-associated secretory phenotype. Deletion of cyclin G1, an atypical cyclin that promotes G2/M arrest, or deletion of key TASCC components reduced kidney fibrosis in mice[38].

As discussed above, induction of autophagy suppresses renal fibrosis[39]. Interestingly, pharmacological enhancement of autophagy by rapamycin or epithelial cell-specific deletion of Atg5 suppressed collagen accumulation by blocking G2-M arrest in cultured tubular cells and **in a mouse model of tubulointerstitial** fibrosis[40]. Treatment with an epidermal growth factor receptor (EGFR) kinase inhibitor downregulated the expression of cyclin B1, cyclin-dependent kinase 1 (Cdk1), Cdk2 and ARK2 and reduced fibrosis[41]. Treatment with other kinase inhibitors, such as JNK inhibitors, also presented similar properties[37].

Inhibition of macrophage migration inhibitory factor (MIF) increased the severity of experimental fibrosis and inflammation in various models of renal injury[42]. The renoprotective effects of MIF are mediated by regulation of the cell cycle in tubular cells. MIF-deficient mice had significantly reduced expression of cyclin B1 and increased expression of cyclin-dependent kinase inhibitor 1B, which abrogated cell cycle arrest in tubular cells and thereby improved regeneration and reduced the secretion of profibrotic and proinflammatory factors[42]. MIF inhibition and kinase inhibitors are undergoing clinical trials for use in cancer and autoimmune diseases. If safety is demonstrated, repurposing of these agents may facilitate clinical studies in kidney disease[43]. Cell cycle regulation is therefore a potential new therapeutic target in kidney disease.

Senescence

Cellular senescence may result from permanent cell cycle arrest initiated by increased expression of cyclin kinase inhibitors (mainly p53, p21 and p16Ink4a), telomere shortening, oxidative stress, genotoxic injury or profibrotic factors such as TGF-β[44]. Senescent cells have a distinct secretome consisting of pro-fibrotic and pro-inflammatory factors that may contribute to wound healing, embryonic development and tumor suppression or promotion. Thus, the senescence secretome may be beneficial or deleterious, depending on the biological context[45].

In the kidney, senescent cells accumulate with advancing age and CKD, and genetic clearance of these cells leads to improved preservation of kidney function during ageing[44]. Targeting senescence using a forkhead box protein O4 (FOXO4) peptide that selectively interacts with p53 in senescent cells reduced experimental kidney fibrosis[46]. Similarly, blocking p16Ink4a-regulated genes or treatment with senolytic agents was nephroprotective in obese mice[47]. Knockout of myeloid differentiation 88 (Myd88) also reduced senescent cell accumulation and fibrosis in mice, indicating a role of epithelial innate immunity in these processes[48]. Future studies should clearly define the scope of the anti-fibrotic actions of senolytic drugs in preclinical kidney injury.

Loss of klotho expression

Klotho is predominantly expressed in the kidney and has anti-oxidant, anti-inflammatory, anti-fibrotic and anti-ageing properties[49]. Inflammation, albuminuria and epigenetic mechanisms contribute to the loss of Klotho expression very early in the course of both AKI and CKD[50,51]. Acquired Klotho deficiency is thought to contribute to the ageing-like features of human CKD and to CKD progression. Nephroprotective effects of Klotho have been demonstrated in several experimental models of kidney injury. Among other potential mechanisms, Klotho modulation of TGF-β1 and Wnt/β-catenin signaling protected against kidney fibrosis triggered by tubular injury (UUO) or glomerular (adriamycin-induced) injury in mice[52,53]. *In vivo* target gene activation via CRISPR/Cas9-mediated trans-epigenetic modulation of Klotho expression[54] protected against cisplatin-induced AKI. Direct administration of recombinant Klotho is not yet a clinical option. However, a secondary analysis of a successful clinical trial of a repurposed drug, pentoxifylline, showed that this drug increased klotho levels in patients with DKD, likely through prevention of Klotho downregulation as a result of albuminuria or inflammatory cytokines[50,51,55].

Loss of epithelial properties

Phenotypic changes are quintessential footprints of tubular epithelial damage. The loss of epithelial cell properties is associated with the acquisition of mesenchymal markers, leading to an aberrant secretome that includes pro-inflammatory and profibrotic proteins, eventually culminating in epithelial to mesenchymal transition (EMT) into myofibroblasts (**Figure 2**). Although the concept of complete EMT is highly debated in the context of renal fibrosis[6-8], in vivo evidence supports a contribution of partial EMT to kidney fibrosis. Mice with knockdown of the transcription factors *Twist1* or *Snai1* were protected from interstitial fibrosis in the UUO and folic acid-induced renal injury models [56,57]. The proposed mechanism involves p21–mediated G2/M cell cycle arrest of tubular cells, which limits their potential for repair and regeneration[56]. Snai1 also induces partial EMT and activation of the nuclear factor kappa B (NF-κB) pathway, which results in the release of inflammatory cytokines without directly generating myofibroblasts[58]. This mechanism links partial EMT to a senescence-related phenotype and sustained inflammation. In development and cancer, Snail1 regulates epithelial plasticity between EMT and the reverse process, but whether partial EMT and its consequences can be reversed in kidney disease is unknown. An improved

understanding of the mechanisms of regeneration versus EMT in epithelial cells may provide clues for novel therapies.

Drivers of fibrosis

Myofibroblasts

Intensive research on the origin of kidney myofibroblasts using lineage tracing and immunofluorescent microscopy technologies has suggested that they may derive from multiple cell types, such as activated renal fibroblasts, pericytes, epithelial cells (via EMT), endothelial cells (via endothelial-to-mesenchymal transition), bone marrow derived cells and fibrocytes[8,59]. Mesenchymal resident kidney cells are now thought to be key precursors of myofibroblasts **(Figure 2).** Genetic lineage analysis identified perivascular Gli1+ mesenchymal cells as precursors of <50% of myofibroblasts and their genetic ablation reduced fibrosis by the same percentage[60] Thus, although partial EMT contributes to tubular cell injury and kidney fibrosis, epithelial cells have a limited, if any, impact as a direct source of myofibroblasts. As persistently activated myofibroblasts are the major source of collagen accumulation in the kidney[8,59], specific myofibroblast-targeting strategies should be investigated. Some anti-inflammatory strategies, such as TWEAK-Fn14 targeting, decreased fibroblast proliferation and final myofibroblast numbers as well as fibrosis in preclinical studies[61]. As Gli1-positive cells are a key source of myofibroblasts, their selective targeting should also be explored for clinical translation.

Extracellular matrix

ECM is arguably not only the major component that is modified in renal fibrosis but also an active contributor to fibrosis development[62]. The balance between deposition and degradation of ECM ultimately dictates the rate of fibrosis progression in individual organs, including in the kidney. Specific ECM components also contribute to kidney fibrosis progression. For example, the glycoprotein tenascin-C promotes myofibroblast proliferation so perpetuates fibrogenesis[63]. Matrix stiffness is a key myofibroblast mechanoactivator that is thought to contribute to forms of fibrosis such as scleroderma[64] and should be explored as a therapeutic target in kidney fibrosis.

Matrix metalloproteinases and PAI-1

Two of the most relevant protease systems in the homeostasis and regulation of ECM components are the matrix metalloproteinase (MMP) family and the plasminogen activation system. These protease systems are interconnected because plasmin activates MMPs[65]. Plasminogen activator inhibitor-1 (PAI-1) inhibits fibrinolysis and promotes ECM accumulation. As the levels of PAI-1 are increased in both AKI and CKD, this protein is a potential biomarker and therapeutic target for kidney fibrosis[66,67]. Genetic or pharmacological targeting of PAI-1 protected against experimental UUO, DKD, protein overload nephropathy, and subtotal nephrectomy[68–71], whereas PAI-1 overexpression increased fibrosis in murine UUO[72]. Specific PAI-1 deletion in renal fibroblasts was sufficient to protect mice against UUO-induced

tubulointerstitial fibrosis, highlighting the contribution of fibroblast-derived secretory factors to this process[73]. Individual MMPs may have different impacts on kidney fibrosis. For example, MMP9 deficiency decreased kidney fibrosis in murine UUO[74], whereas MMP2 inhibition had the opposite effect[75,76].

TGF-β1

TGF-β1 has traditionally been considered the master regulator of fibrosis and early preclinical data from the 1990s were very promising regarding its potential as a therapeutic target in kidney fibrosis[77]. In vitro, TGF-β1 promotes the transformation of fibroblasts into myofibroblasts to increase ECM production, inhibits ECM degradation and induces EMT phenotypic changes in tubular cells[77]. Multiple preclinical studies have shown anti-fibrotic effects of various strategies targeting the TGF-β1 pathway; however, adverse effects on the kidney and cardiovascular system were also reported. TGF-β1-neutralizing antibodies increased proteinuria in **models of** DKD and in puromycin-induced nephrosis[77], increased kidney inflammation in the UUO model and in CTGF-induced renal damage in mice [78,79], induced an unstable plaque phenotype characterized by increased inflammatory cells and low collagen content in experimental murine atherosclerosis[80] and caused vascular inflammation after stent implantation **in pigs**[81]. Some of the anti-inflammatory effects of TGF-β1 can be attributed to modulation of regulatory T (Treg) cell responses[82,83].

Clinical development of TGF-β1 signaling blockers is an active field of research in oncology owing to the roles of TGF-β1 in EMT, cell invasion and metastatic colonization of cancer cells[84]. However, clinical development for kidney disease was halted following phase 1/2 trials of anti-TGF-β1 antibodies in focal segmental glomerulosclerosis and DKD[85]. The TGF-β1 story illustrates the need for a holistic approach to the treatment of kidney fibrosis that does not necessarily focus on direct regulators of ECM deposition.

TGF-β1 signaling leads to activation of Smad3, which has a key role in fibrotic responses[77]. In patients with CKD, elevated tissue TGF-β1 expression and activation of Smad3 were associated with glomerular and interstitial ECM accumulation[84,86]. Another member of the TGF-β superfamily, bone morphogenetic protein-7 (BMP-7), and its downstream Smad1/5/8 signalling are down-regulated in CKD. Studies published nearly two decades ago demonstrated anti-fibrotic effects of recombinant BMP-7 or Smad3 blockade using various strategies, including gene silencing or Smad7 overexpression in multiple preclinical models[77,87–89]. However, these strategies have not yet been translated into the clinic. Importantly, the Smad signaling pathway can be activated and promote fibrosis in a TGF-β1-independent manner[90]. Interestingly, Ang II, can activate Smad signalling, suggesting that RAS blockade might have anti-fibrotic effects via inhibition of the Smad pathway (**Figure 1**). In addition, TGF-β1 can signal through non-canonical pathways, including ERK and JAK–STAT[91]. Drugs targeting these intracellular pathways could represent useful approaches to treat kidney fibrosis.

PDGF

PDGF is a growth factor released from platelets that in the kidney promotes the proliferation and/or recruitment of fibroblasts, mesangial cells, pericytes and other cell types[92–95]. Different PDGF isoforms (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD) are formed by disulfide-linked homodimeric and heterodimeric glycoproteins[96], and can be produced by different cell types, including podocytes and tubular epithelial cells. The PDGF receptor (PDGFR) has two different chains (α and β) that can form homodimers and heterodimers. PDGFR-α is expressed in renal interstitial cells **and** mesangial cells, and PDGFR-β in mesangial and glomerular parietal epithelial cells[97,98].

The contribution of PDGF to CKD progression has been extensively characterized. The expression of PDGF ligands and receptors is increased in human glomerulonephritis, including in mesangial proliferative glomerulopathy, IgA nephropathy, crescentic glomerulonephritis, lupus nephritis, membranous nephropathy and transplant glomerulopathy[98–105]. Many preclinical studies have observed a beneficial impact of PDGF modulation in kidney diseases of diverse aetiology. PDGFR kinase inhibitors decreased kidney inflammation and fibrosis in **models of** immune glomerulonephritis, DKD, hypertension, chronic allograft nephropathy, IRI and UUO[106–114]. Podocyte-specific overexpression of PDGF-D in transgenic mice induced glomerulosclerosis, tubular damage, and loss of renal function[101]. In these mice, the expression of genes encoding PDGF-A, PDGF-B, PDGFR–α, PDGFR-β as well as proinflammatory and profibrotic genes were increased. In the anti-Thy-1 glomerulonephritis model, a PDGFR/Fc chimeric molecule inhibited cellular proliferation and profibrotic factors[115]. In the same model, treatment with a PDGF-DD-specific neutralizing antibody also had beneficial effects, reducing cell proliferation, glomerular infiltration o0f monocytes and macrophages and ECM accumulation[116]. In experimental DKD, PDGF-β gene deletion decreased the urinary albumin/creatinine ratio and oxidative stress[117]. These results suggest that PDGF isoforms and receptors could be good therapeutic targets to modulate inflammation and fibrosis in kidney disease. However, implementation in clinical studies has lagged behind preclinical knowledge.

Developmental pathways and genes

Kidney injury results in the reactivation of developmental pathways (e.g. Notch, Wnt, hedgehog and SOX9)[118] and the de novo induction of genes (including growth factors such as gremlin and connective tissue growth factor (CTGF)) that have roles in both the recovery and progression of kidney injury (**Figure 2**). These pathways and genes have been investigated as potential therapeutic targets for kidney disease and fibrosis.

Developmental pathways

Notch. The Notch pathway has a critical role in proximal tubule cell and podocyte differentiation during kidney development and contributes to kidney regeneration and damage[119]. Studies using genetic modulation of Notch components or Notch inhibition by γ-secretase inhibitors have

demonstrated a role of Notch activation in CKD-related processes such as podocyte apoptosis, fibroblast activation and kidney inflammation[119,120]. Notch blockade has been suggested as a potential approach to treat kidney disease based on promising findings in models of kidney fibrosis[121,122].

Wnt/β-catenin. The Wnt/β-catenin signaling pathway is required for tubular progenitor epithelial repair in AKI[123]. However, Wnt/β-catenin signaling also contributes to kidney fibrosis by regulating interstitial fibroblast and pericyte ECM and is a potential therapeutic target. In experimental UUO, subtotal nephrectomy, IRI and salt-sensitive hypertension, kidney fibrosis was linked to activation of the Wnt/β-catenin pathway[124–127]. The peptidomimetic small molecule ICG-001, which selectively inhibits β-catenin-mediated gene transcription, decreased kidney fibrosis in murine UUO. Moreover, in cultured tubular cells, ICG-001 inhibited TGF-β1-induced *PAI-1* and *Snail1* mRNA expression in a Smad-independent manner[128]. ICG-001 also prevented AKI-to-CKD progression in mice following IRI[129].

Sox9. The Sox9 transcription factor has a role in tubular regeneration. Sox9+ proximal tubular cells display progenitor-like properties *in vitro* and can proliferate, expand and differentiate into tubular and glomerular parietal cells to replace damaged tissue in AKI[130–132]. In addition, Sox9 is a key transcriptional regulator of genes with roles in ECM formation. Sox9 blockade impaired regeneration in liver fibrosis-associated AKI, leading to fibrosis[133], whereas in unilateral renal IRI, treatment with human adipose-derived mesenchymal stem cells increased Sox9 expression in tubular cells, promoting tubular regeneration and decreasing renal fibrosis[134].

Sonic hedgehog. The Sonic hedgehog (Shh) pathway participates in renal morphogenesis and regulates the gene expression of cell cycle modulators that control proliferation and differentiation[135]. The Shh pathway also interacts with other signaling pathways such as BMP, Wnt and FGF during development[135], and with TGF-β1, Wtn and Notch in fibrosis[141], illustrating the complexity of these processes. In experimental fibrosis, the Shh pathway is activated and downstream induction of Gli1 and Gli2 expression is observed in tubular cells, PDGFR-β-positive pericytes, interstitial fibroblasts and myofibroblast progenitors[136–141]. Upregulation of the sensor receptor protein patched homolog 1 (Ptch1), Gli1 and Gli2 has been reported in human fibrotic kidneys [137,142]. In cultured mesenchymal stem cell-like progenitor cells, Gli2 drives myofibroblast cell-cycle progression[137].

Pharmacological inhibitors of the Shh pathway, such as the natural compound cyclopamine, Smo modulators or Gli inhibitors, as well as genetic modulation of Shh pathway components reduce experimental kidney fibrosis and inflammation[141], whereas Shh overexpression promotes the proliferation of renal interstitial cells and accelerates AKI-to-CKD progression in various murine models[142]. In experimental HIV-associated nephropathy, Ptch expression was mainly localized in podocytes. Interestingly, Shh blockade using the small molecule darinaparsin (an arsenical currently in clinical trials or the Gli inhibitor GANT61, induced cell-cycle arrest in myofibroblasts, indicating that it is an anti-fibrotic drug directly targeting activated fibroblasts[137]. As tubular cells

are the main source of Shh, but Shh proteins mainly act on myofibroblasts, drugs targeting Shh are promising anti-fibrotic therapies. In addition to tubulointerstitial events, in vitro gain-of-function and loss-of-function experiments modulating Shh or Gli1 in podocytes showed that these proteins regulate the EMT phenotype and changes in permeability[143]. However, the interaction of podocyte Shh with other glomerular cells requires further investigation.

Developmental genes

Gremlin. Gremlin has a role in fibrotic disorders, including renal fibrosis, and both shares profibrotic properties with and directly upregulates TGF-β1[144]. *In vitro,* Gremlin promotes ECM production in renal fibroblasts and induces EMT of tubular cells[145]. Moreover, *Grem1* gene silencing inhibited TGF-β1-mediated profibrotic events, including ECM production in cultured fibroblasts and induction of tubular EMT in the HK2 cell line [145]. In human CKD, re-expression of Gremlin was associated with fibrosis, TGF-β1 upregulation and Smad activation[146,147]. Interestingly, Gremlin directly activated the Smad pathway in cultured renal cells, and Smad7 overexpression blocked its profibrotic effects[144]. Gremlin also acts as a BMP antagonist during development and in fibrotic disorders[144]. In addition, Gremlin can bind to vascular endothelial growth factor receptor-2 (VEGFR2) in tubular cells *in vivo* and *in vitro* to induce inflammation and fibrotic-related responses[148,149]. However, VEGFR2 binds other ligands, mainly VEGFs. Future studies should explore whether these factors and receptors are therapeutic targets in kidney fibrosis. Gremlin can also bind macrophage migration inhibitory factos (MIF) and behave as an endogenous antagonist that inhibits monocyte migration[150] and improves experimental atherosclerosis[151].

We found that Gremlin is a potential urinary biomarker of ANCA-associated crescentic glomerulonephritis, which spontaneously progresses to glomerular and interstitial fibrosis[152]. Gremlin is expressed in glomerular crescents that are associated with EMT[153] and has been reported to colocalize with infiltrating cells that express macrophage markers (CD68, CD163 and CCL18)[152], suggesting a role for Gremlin in the formation of these crescents. These findings suggest that Gremlin is a potential therapeutic target for crescentic glomerulonephitides but this hypothesis needs to be tested in **preclinical** interventional studies.

Connective tissue growth factor. CTGF (also known as CCN2) is a matricellular protein that has been implicated in diverse biological processes such as cell proliferation, angiogenesis, migration and ECM remodeling[154]. Traditionally, CTGF has been described as a downstream profibrotic mediator of TGF-β1[155] and AngII[156,157]. Inhibition of CTGF decreased fibrosis in diverse preclinical models of disease[160]. In CKD, CTGF is overexpressed in close proximity to areas of fibrosis. In cultured mesangial cells, both CTGF and its C-terminal cystine-knot module promoted tubular EMT and increased collagen production. By contrast, CTGF gene knockdown using gene silencing, antisense oligonucleotides or in conditional knockout mice decreased kidney fibrosis *in vivo*[154]. In addition, CTGF directly regulates renal inflammatory responses, including NF-κB pathway activation and Th17 responses[158,159]. CTGF is also a non-canonical EGFR ligand[160] and

binds EGFR in proximal tubular cells via its C-terminal module, promoting kidney proinflammatory and profibrotic responses[154]. Thus, EGFR is a therapeutic target of potential interest in kidney fibrosis and CKD. An anti-CTGF antibody, pamrevlumab (FG-3019), was initially tested in phase I RCTs for diabetic kidney disease and FSGS[161] (NCT00782561). However, clinical development for CKD was terminated without clear explanation.

Immune cells and inflammation

Kidney inflammation involves immune cells, including neutrophils, monocytes, macrophages, CD4+ and CD8+ lymphocytes and natural killer (NK) cells[9,162]. In AKI, i nflammation is required for kidney repair but can also amplify tissue injury and contribute to the AKI-to-CKD transition (**Figure 2**). Persistent inflammation is also a key characteristic of CKD that leads to progressive renal fibrosis. Kidney inflammation is associated with endothelial dysfunction and activation of glomerular and tubular epithelial cells, with the consequent release of inflammatory molecules, which further recruit immune cells into damaged kidneys (**Figure 3**). Targeting immune cells and cytokines to prevent kidney fibrosis has been investigated in preclinical studies.

Macrophages

Although debated, the division of macrophages in two archetypal populations, M1 and M2, is widely accepted[163,164]. M1 macrophages produce proinflammatory cytokines, including IL-1β, IL-6 and TNF, and have cytotoxic properties, whereas M2 macrophages are subdivided by their roles in wound-healing (tissue repair) (M2a), immune regulation (M2b) and anti-inflammatory effects (M2c), the latter induced by IL-10 and TGF-β1[163].

Macrophages are important contributors to the inflammatory response during AKI. Early in AKI, M1 macrophages promote inflammation and cell death[165,166], whereas a phenotypic switch to M2 macrophages generally correlates with renal repair during the recovery phase (**Figure 3**). Failed resolution of inflammation is characterized by persistent infiltration of inflammatory macrophages, which impairs kidney regeneration and contributes to the AKI-to-CKD transition[9,165]. Therefore, the ratio of M1/M2 macrophage markers or **the levels of** secretome-derived factors are potentially useful tools to monitor disease progression and/or remission.

Macrophages also contribute to CKD progression and genetically modified macrophages have been shown to prevent the progression of experimental CKD[166]. IL-10 inhibits macrophage activation and effector functions *in vitro* by inducing the production of anti-inflammatory molecules (IL-1 receptor antagonists and scavenger receptors) and reducing the production of proinflammatory cytokines[167]. Infusion of IL-10-transfected macrophages improved renal function in rat anti-glomerular basement membrane nephritis and in IRI[168,169]. The nephroprotective effect was partly mediated by lipocalin-2 induction in a process that was regulated by intracellular iron [169]. Injection of IL-4-transfected macrophages into the kidney, but not their systemic administration, had similar anti-inflammatory and anti-proteinuric effects in experimental glomerulonephritis[170]. In mice with adriamycin-induced podocyte injury or streptozotocin-induced

diabetes, adoptive transfer of activated M2a (induced using IL-4 and IL-13) or M2c macrophages (induced using IL-10 and TGF-β1) reduced structural and functional injury[171,172].

Modulation of STAT, interferon (**IFN)** and Toll-like receptor (TLR) signaling pathways have been explored as approaches to modulate the balance of macrophage populations[173–176]. TLRs and IFN-γ trigger STAT1 activation to induce interferon regulatory factor (IRF)–STAT-mediated M1 responses. M1 macrophages increased the expression of IRF5, which is required for the induction of cytokines (IL-12, IL-23) that modulate Th1 and Th17 responses[177].

Stimulation of macrophages with IL-4 activates STAT6 to induce M2 polarization responses, such as the expression of mannose receptor, resistin-like α and chitinase 3–like protein[178]. Similarly, stimulation of macrophages with IL-10 activates the STAT3 signalling pathway and induces the expression of IL-10 and TGF-β1, which are also associated with an M2-like phenotype[179,180]. The existence of overlapping macrophage phenotypes and the differences between human, rat and murine macrophage surface markers, suggests that extrapolation of preclinical data to human kidney diseases may not be straightforward.

Other mediators of inflammation

The main inflammatory mechanisms that are involved in renal damage are driven by the NF-κB pathway, reactive oxygen species (ROS) and activation of protein kinases, including the mitogen-activated protein kinase (MAPK) cascade and the JAK–STAT signaling pathway. Knowledge of the intracellular signaling pathways that are involved in kidney inflammation and fibrosis has grown exponentially but has so far failed to benefit patients with kidney disease. This failure is exemplified by the lack of licensed drugs targeting these pathways in DKD despite multiple RCTs[181]. In preclinical studies, inhibitors of cytokines (e.g. TNF, CCL2 and IL-1β) and intracellular pathways (e.g. JAK–STAT and NF-κB) have shown promise for nephroprotection in DKD[181]. However, clinical studies in patients with DKD have not followed (e.g. TNF), are progressing slowly (e.g. CCL2 targeting therapies) or have failed to confirm a benefit (e.g. Nrf2 activators)[182].

A post hoc analysis of the CANTOS trial of IL-1β inhibition in patients with a history of myocardial infarction demonstrated a reduced risk of major adverse cardiovascular events among participants with CKD (46% of whom had diabetes) but no impact on CKD progression[183]. The Nrf2 activator and NF-κB inhibitor bardoxolone methyl increased eGFR in diabetic patients with advanced CKD but also increased cardiovascular events, prompting termination of the trial[184]. This drug is undergoing phase 3 clinical trials in Japanese patients with DKD and low cardiovascular risk and is in the early stages of clinical development for forms of CKD found in younger patients, such as Alport syndrome and polycystic kidney disease (Table 1). Bardoxolone raises interesting conceptual questions because, in contrast to RAS blockade, it increases GFR in already hyperfiltering kidneys. In the early stages this effect is not associated with decreased fibrosis.

Repositioning of old drugs with a proven safety record is a promising strategy for treatment of kidney disease. For example, pentoxifylline has been shown to have nephroprotective, anti-inflammatory and Klotho-sparing effects and decrease cardiovascular risk in open-label clinical trials in patients with DKD or CKD[55,185]. However, placebo-controlled trials of pentoxifylline are required to confirm these beneficial effects. A panel of inflammatory cytokines, mainly from the TNF-R superfamily, have been identified as markers of progression to ESRD in diabetes[186], supporting the concept of DKD as an inflammatory disease and the potential use of anti-inflammatory drugs. Another emerging field is mesenchymal stem cell therapy in which the beneficial antifibrotic effects observed in preclinical studies seem to be mediated by the release of anti-inflammatory factors[187]. Ongoing phase 1 clinical trials in focal segmental glomerulosclerosis (NCT02693366, NCT02382874) are starting to address the potential of these approaches for kidney diseases.

Novel anti-cytokine strategies

Intensive research in the immunology field has identified novel targets for the treatment of chronic inflammatory diseases, including IL-17A. Early studies suggested a role for IL-17A in pathogen clearance during infection, but it is now considered to be a pleotropic proinflammatory cytokine [188]. IL-17A blockers, such as neutralizing antibodies or soluble receptors, may be beneficial in inflammatory diseases and are undergoing testing in clinical trials for indications such as Crohn disease (NCT00936585), spondyloarthritis (NCT03358134), and psoriasis (NCT03403036). IL-17A also contributes to skin and liver fibrosis[189]. Although IL-17A increased ECM synthesis in several cultured cell lines, including skin fibroblasts[189], it may also contribute to ECM degradation by regulating MMPs[190]. Whether or not MMP-mediated effects are deleterious is currently unknown.

The blockade of IL-17A, using anti-IL17A neutralizing antibodies as well as studies in genetically modified mice lacking IL-17A or its receptor A, have shown protective effects in experimental models of immune-mediated and non immune-mediated kidney injury[158,191–193]. However, preclinical studies did not always recapitulate human disease and therapy was frequently initiated before the onset of kidney injury[194]. In a mouse model of DKD (leptin-deficient BTBR ob/ob mice), we showed that treatment with a IL-17A neutralizing antibody reduced NF-κB activation and inflammation, improved renal dysfunction and prevented progressive glomerular injury[195]. These data support the future evaluation of IL17A neutralization for overt DKD and are aligned with the hypothesis that in this disease anti-inflammatory therapies might be more effective than anti-fibrotic therapies. Studies that have investigated the effects of targeting IL-17A in experimental kidney disease have reported contradictory findings, describing decreased and increased fibrosis[190,196,197]. We found that systemic administration of IL-17A in mice increased blood pressure associated to an inflammatory response in the kidney, but had no effect on renal collagen accumulation [198]. Consistent with these findings, treatment with a IL-17A-neutralizing antibody did not improve AngII-induced experimental kidney fibrosis. Protection against fibrosis

observed in IL-17-receptor knockout mice with UUO seems to be mediated by decreased MMP-2[190].

Glomerular cell injury

The diversity of glomerular diseases requires the development of safe disease-specific therapies. A need exists to better understand the intricate glomerular cross-talk between different cell types and the ECM[199]. Podocytes are terminally differentiated cells with very limited or absent regenerative potential and regeneration from parietal epithelial cells is also limited. Accelerated podocyte loss is thought to be a key driver of glomerulosclerosis in which lost podocytes are replaced by ECM deposition[200]. Podocytes may be lost through dedifferentiation, detachment or death. Sublethal or lethal podocyte injury promotes pathological albuminuria which, in turn, causes proximal tubular cell injury and activates proximal tubular cells to secrete proinflammatory and profibrotic factors (**Figure 3**). Additionally, stressed podocytes express cytokines and Notch receptors and secrete autocrine and paracrine proinflammatory factors (e.g. chemokines) and profibrotic factors, such as TGF-β1 and Gremlin, which increase ECM production, accelerate podocyte cell death and drive EMT-related phenotype changes[201–203].

Several drugs in clinical use for kidney disease directly protect cultured podocytes, including ARBs, steroids and calcineurin inhibitors. The latter may however exert direct pro-inflammatory and pro-fibrotic effects in the tubulointerstitium[204–206]. Additional therapeutic strategies may promote the expression of adaptive endogenous protective factors, such as heat shock protein beta-1 (HSPB1), that help podocytes to withstand stressors such as high ambient glucose or AngII levels[207]. However, the notion that in glomerular diseases tubular dysfunction only occurs as a result of glomerular injury has been challenged. In experimental diabetes, evidence of tubular dysfunction or injury is an early feature that may even precede glomerular dysfunction[195,208,209]. Strategies aimed at preventing early tubular damage, such as those discussed above, should therefore also be tested as anti-fibrotic therapies for glomerular diseases.

Epigenetic mechanisms

Epigenetic regulatory mechanisms participate in AKI, CKD and the AKI-to-CKD transition. Moreover, evidence that alterations of epigenetic signatures, such as DNA methylation, histone modifications and miRNAs, contribute to CKD progression, suggests opportunities for therapeutic epigenetic modulation[210,211]. In fibrosis, DNA methylation regulates fibroblast activators and ECM-related genes, including *RASAL1*, *type IV collagen*, *MMP9* and *Smad3*[212]. Several drugs that target epigenetic regulators are in clinical use or development, mostly for malignancy, but only anecdotal or post hoc data are available in the field of kidney disease. For example, BET proteins are interesting drug candidates with anti-inflammatory and anti-fibrotic properties[210,213]. They contain bromodomains that recognize epigenetic marks, such as acetylated lysines in histones and transcription factors that regulate many gene transcriptional programs. The BET inhibitor apabetalone is in phase 3 trials for atherosclerosis with kidney function as a secondary end point (BETonMACE, NCT02586155). A post-hoc analysis of phase 2 trials of this drug in patients with

coronary atherosclerosis reported an increase in renal function in those with CKD[214]. However, caution should be exercised when extrapolating preclinical data to the clinic owing to the clinical nephrotoxicity of some of these drugs, such as the Dnmt inhibitors decitabine and 5´-azacytidine and the HDAC inhibitors panobinostat and vorinostat[211]. RNA modulation strategies are one of the next frontiers in therapeutics[215]. Here, we focus on microRNA (miRNA) therapeutics.

MicroRNAs

MiRNAs comprise a large family of conserved, small, non-coding RNAs that repress the translation and/or induce the degradation of their mRNA targets[216]. The control of miRNA function depends on many factors, including the subcellular location, relative expression and processing of the miRNA and the regulation of miRNA-target interactions[217]. A single miRNA can potentially target hundreds or thousands of mRNAs, regulating crucial functions in numerous biological processes, including development, differentiation, stress responses and apoptosis[218]. MiRNAs termed fibromiRs have emerged as powerful dynamic regulators of fibrotic processes[219]. Aberrant expression of miRNAs can drive the initiation and progression of fibrosis in response to persistent tissue injury[220], including renal damage[218]. As miRNAs are stable and can be measured reproducibly from various sources, determination of miRNA profiles could be very useful to define novel biomarkers for fibrosis or AKI-to-CKD transition[220]. The last decade has witnessed a dramatic increase in the identification of miRNAs with potential roles in kidney fibrotic transformation (**Table 2**). Given the functional promiscuity of miRNAs, exclusive targeting of each fibrotic pathway by a particular set of miRNAs is not to be expected.

MiR-21. MiR-21 is one of the best characterized fibromiRs in multiple organs and was one of the first to be described in the kidney[221,222]. Initial studies in tubular epithelial cells showed that TGF-β1 increased miR-21 expression via Smad3 but not Smad2, and miR-21 knockdown halted the progression of fibrosis[223]. Upregulation of another fibrotic-related factor, Notch-1, increased miR-21 expression in pancreatic cancer stem cells[224]. Overexpression of miR-21 has also been described in experimental and human CKD, associated with fibrosis[8].

Data from experimental studies support a role of miR21 in renal fibrosis, inflammation and podocyte damage. For example, suppression of miR-21 using a short hairpin RNA blocked renal fibrosis in a UUO model[223]. Initial reports indicated that miR-21 ameliorated TGF-β1-induced and hyperglycaemia-induced glomerular injury in miR-21-deficient TGF-β1-transgenic mice through repression of proapoptotic signals, thereby inhibiting podocyte loss[225]. In diabetic mice, miR-21 antagonism decreased podocyte loss, albuminuria, and interstitial fibrosis at least in part by blocking the direct effects of miR-21 on podocytes[226]. Moreover, the ETA blocker atrasentan directly decreased miR-21 levels and increased klotho expression via modulation of miRNA *in vitro* and decreased kidney fibrosis in experimental DKD[252–254].

In miR-21-knockout mice, many genes were silenced after renal injury, but, surprisingly, these genes were not involved in inflammation or fibrosis, but in metabolic and mitochondrial

functions[227]. These findings illustrate the importance of validating predicted gene targets based on complementary sequences in the 3'UTR of mRNAs in vivo.

MiR-21 overexpression is protective in AKI[228]; therefore a dual role of miR-21 protective in AKI and deleterious in CKD has been suggested. Similar dual roles have been observed for some developmental pathways. In vivo delivery of miRNA mimics or inhibitors has emerged as a promising therapeutic strategy for CKD and the RG-012 anti-miR-21 molecule is undergoing clinical trials in Alport syndrome (NCT03373786). If the results are positive, the efficacy of this agent could be tested in other glomerulonephritides and novel renoprotective miRNAs could enter the clinic in the next decade. The potential negative impact of anti-miR-21 therapy on susceptibility to AKI should, however, be carefully monitored.

miR-29 family members. The miR-29 family members miR-29a, miR-29b and miR-29c regulate ECM production in several organs, including the kidney[221,229]. Many genes that have been identified as targets of the miR-29 family encode ECM proteins, such as collagens, fibrillins, laminins, integrin-β1 and elastin[229]. In tubular cells, miR-29b overexpression abolished the expression of several Ang II-induced genes, including TGF-β, α-SMA, Col1A1 and Col3A1[230]. In retinal pigment epithelial cells miR-29b has been shown to regulate TGF-β-induced EMT[231]. Antifibrotic effects in experimental renal diseases have been reported for all three of the miR-29 family members[232].

Other fibrosis-related miRNAs. Some miRNAs, such as as miR-433[233] and miR-192[234], could promote fibrosis by modulating the TGF-β–Smad3 pathway or related to Smad3-targeting, such as miR-21, miR-192 and the miR-29 and miR-200 families[235]. MiR-23b is upregulated in the kidneys of TGF-β1 transgenic mice and in TGF-β1-stimulated renal epithelial cells; targets of MiR-23b include TGF-β receptor type II, Smad3 and TGF-β1, suggesting a negative feedback loop that regulaties TGF-β1 signaling[236].

The Let-7 family of miRNAs has been associated with the progression of fibrosis in several organs including the kidney. Validated targets of the Let-7 family include collagens (Col1a2 and Col4a1), TGF-β1, TGF-βR1 and Smad2[221]. MiR-214 is highly expressed in the kidney and seems to promote fibrosis in a Smad–TGF-β1-independent manner [237].

MiR-200 family members have been closely related to EMT. Targets of miR-200b include EMT-related factors as the Zinc finger E-box binding homeobox 1 and 2 proteins, which modulate the expression of E-cadherin and fibronectin in tubular cells[238].

Some miRNAs can interfere with regeneration programs that could modulate fibrosis. For example, miR-1247 blocked Sox9-mediated regeneration in a model of liver fibrosis-associated AKI[133]. Downregulation of miR-376b promotes macrophage autophagy by targeting Atg5 in fibrotic mice[239].

Other miRNAs directly target additional profibrotic factors, including CTGF and gremlin. For example, in pathological left ventricle hypertrophy, miR-133 and miR-30c directly downregulate CTGF [240]. Similarly, miR-18 and miR-19 target CTGF in age-related heart failure[241]. Several miRNAs that are involved in the pathogenesis of DKD and AKI also target CTGF and Gremlin[242]. For example, in DKD, miR-26a inhibits CTGF expression and thereby inhibits TGF-β1-induced ECM expression in podocytes[243], and the downregulation of miR-30c promotes renal fibrosis by targeting CTGF [244]. In pulmonary cells, mir-27b directly targets gremlin and a mir-27b mimic decreased the expression of several genes implicated in fibrosis including CTGF[245].

Downregulation of mir-27b has been reported to have a key role in AKI progression, forming a complex axis with the long non-coding RNA (lncRNA) LINC00520 and the secreted cytokine oncostatin M[246]. By contrast, the knockdown of another member of the miR-27 family, miR-27a, prevented DKD progression in mice[247]. These data, which provide only a small example of miRNA involvement in kidney fibrosis, highlight the complexity of miRNA actions. Despite the promising findings of miRNA modulation studies in animal models, to date little evidence exists of efficacy in human fibrotic disease.

LncRNAs

LncRNAs are also potential therapeutic targets for kidney disease. At least 21 novel TGF-β1/Smad3-related lncRNAs have been identified in experimental models of renal damage[248]. However, there are limitations in the translation from mice studies to humans, as most **lncRNAs** are not fully conserved across mammals. Although functional orthologous have been proposed, computational methods have only been partially successful in their identification.

Conclusions

CKD is set to become one of the top global health issues within this century. However, the current therapeutic armamentarium is poor. A holistic approach to CKD progression should target the diverse processes and circumstances that are associated with CKD progression, including inter-current episodes of AKI. Fibrosis is one such process that is difficult to separate from simultaneously ongoing processes such as parenchymal cell injury and loss, and inflammation. Experimental evidence suggests a key role of fibrosis in CKD progression, but no such evidence is available in human CKD. It cannot be excluded that, at least in some circumstances, fibrosis represents an epiphenomenon or byproduct. Therapy to prevent and even reverse kidney fibrosis remains an elusive yet highly attractive goal. So far, therapeutic interventions in late disease, when tubulointerstitial fibrosis is already established, have proved unsatisfactory. Until future drugs capable of reversing renal fibrosis are available, research efforts should focus on unraveling early disease mechanisms to uncover early biomarkers that enable therapeutic monitoring and early therapeutic targets that prevent disease progression.

In our view, the debate concerning whether fibrosis should be directly targeted has been obscured by the need for a holistic approach to CKD therapy, targeting the diverse intertwined processes

and molecular mechanisms that contribute to the generation and progression of kidney fibrosis and CKD. The most extensively characterized example of this holistic approach is the current standard for treating proteinuric CKD, RAS blockade. Multiple actions of the RAS promote kidney cell injury, inflammation, and fibrosis, and it is the combination of these actions that likely makes the RAS such an important contributor to kidney disease and RAS blockade such a successful intervention. Conversely, targeting specific cytokine functions has led to failure. This point is illustrated by the disappointing results of clinical trials of approaches targeting TGF-β1, which promotes fibrosis within a wider function of dampening inflammation and transitioning to a scar stage. These additional functions may lead to adverse effects of TGF-β1-targeting therapies. Downstream mediators of TGF-β1 signalling that promote both inflammation and fibrosis, such as CTGF and Gremlin, might therefore be more attractive therapeutic targets.

Epigenetic regulators that orchestrate complex responses are also promising therapeutic targets. These regulators include miRNAs and histone post-translational modulators. Importantly, unexpected findings in preclinical studies can lead to new understanding of kidney disease pathogenesis. for example, the observation that deletion of miR-21 results in changes in metabolic and mitochondrial functions[227] suggests that reprogramming of metabolism might have a role in fibrosis[255]. A common theme that has emerged in several pathways involved in kidney fibrosis is the differential functions of mediators of AKI versus CKD, illustrating the need for detailed time-course studies and for adapting the timing of therapeutic approaches to the stage of kidney injury.

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Author contributions

All authors researched the data, contributed to discussions of the content, wrote the text and reviewed or edited the article before submission.

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The authors declare no competing interests.

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Table 1 | Clinical trials in renal diseases

Target	Agent(s)	Disease	Comments	Ref(s)
Current therapies				
Renin-aniotensin system	Angiotensin II receptor blockers and angiotensin converting enzyme inhibitors	DKD	Delay CKD progression; impact on kidney fibrosis not yet assessed in placebo-controlled clinical trials	[1,2,3]
SGLT2	Canagliflozin	DKD	Delays CKD progression; impact on kidney fibrosis not yet assessed in placebo-controlled clinical trials	[4]
Vasopressin receptor 2	Tolvaptan	ADPKD	Delays CKD progression; impact on kidney fibrosis not yet assessed in placebo-controlled clinical trials	[5]
Phosphodiesterase	Pentoxifylline	DKD	Delays CKD progression in open-label clinical trials; impact on kidney fibrosis not yet assessed in placebo-controlled clinical trials	[6]
Completed trials				
Endothelin receptor type A	Atrasentan	DKD	Delays CKD progression; impact on kidney fibrosis not assessed in placebo-controlled clinical trials	[7]
Ongoing or planned clinical trials				
p53	QPI-1002 (siRNA)	AKI following cardiac surgery	Would be interesting to follow patients to explore impact on CKD	Phase 3 Clinical trial (NCT03510897)
Nrf2	Bardoxolone methyl	DKD, ADPKD, Alport syndrome	Increased eGFR and cardiovascular events in patients with DKD; currently being tested with eGFR as a primary outcome in Japanese patients with DKD and lower cardiovascular risk; no formal assessment of fibrosis is planned	[8]. Phase 3 Clinical trial (NCT03550443)
BET proteins	Apabetalone	Coronary artery disease, diabetes and eGFR >30 ml/min/1.73 m^2	A post-hoc analysis of patients with CKD in the SUSTAIN and ASSURE trials reported favourable effects on eGFR; the ongoing BETonMACE trial* has eGFR as a secondary end point but no formal assessment of fibrosis is planned	[9], Phase 3 Clinical trial (NCT02586155)
TGF-β1	Pirfenidone	DKD	Decreased fibrosis in preclinical studies; slowed (FSGS) or had no effect (DKD) on eGFR loss over 12 months in phase 2 trials; ongoing trial‡ with primary outcome of eGFR but no formal assessment of fibrosis is planned; this trial is unlikely to provide a breakthrough owing to its small size (n=62) and short follow-up period (12 months)	[10] Phase 3 Clinical trial (NCT02689778)
miR-21	RG012	Alport syndrome		Phase 1 Clinical trial (NCT02586155)

*Estimated primary completion date November 30, 2019. ‡Estimated Primary Completion Date December, 2019. RCT of abandoned therapeutic approaches are not shown; these have been reviewed previously[11]. Abandoned approaches include monoclonal antibodies against TGF-β (fresolimumab and LY2382770) and against CTGF (pamrevlumab, also known as FG-3019), which underwent phase 2 trials with suboptimal results (fresolimumab) or never completed phase 2 trials (LY2382770, Pamrevlumab) in CKD. ADPKD, autosomal dominant polycystic kidney disease; DKD, diabetic kidney disease.

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Table 2 | MicroRNAs with potential roles in kidney fibrosis

microRNA	Main target or interaction	Mechanism	Ref(s)
miR-192	SMAD7	Modulates the TGFβ–Smad pathway	[1]
miR-200,	ZEB1/2, SMAD/FN1, TGFβ2	TGFβ/EMT/ECM	[2–4]
miR-141			
miR-21	SMAD3, Mpv17-like, Reck	TGFβ/PPAR□-Metabolism/ECM	[5,6]
miR-29	SMAD3, Collagens	TGFβ	[7,8]
Let-7c	TGFβRI, HMGA2, Collagens	TGFβ	[9,10]
miR-214	E-Cadherin	EMT	[11,12]
miR-196[a] and miR-196[b]	TGFβRII	ECM	[13]
miR-324	Prolyl endopeptidase, Ac-SDKP	Metabolism/ECM	[14]
miR-30	CTGF, UCP2	Metabolism/ECM	[15,16]
miR-433	TGFβ/SMAD3-AZIN1, GCLs	Metabolism/ TGFβ	[17,18]

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Figure 1 | Potential role of angiotensin II in the development of kidney fibrosis. Experimental and clinical data suggest that binding of angiotensin II (AngII) to the type-1 angiotensin II receptor (AT1) activates intracellular signalling pathways that lead to **the production of reactive oxygen species (ROS)**, activation of transcription factors and modulation miRNA expression. These effects result in modulation of gene expression and the secretion of proinflammatory and profibrotic factors that contribute to renal inflammation and fibrosis. TGF-beta1 mainly regulates EMT and ECM, whereas other factors as CTGF and gremlin, have a dual action, regulating both fibrotic and inflammatory processes. In addition, AngII regulates cell growth and phenotype that are involved in loss of parenchymal cells and fibrosis. Together, fibrosis, parenchymal cell loss and inflammation lead to loss of kidney function and proteinuria. Renin-angiotensin system (RAS) blockade using angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) is the main nephroprotective strategy in clinical use. Although ACE inhibitors and ARBs slow progression of proteinuric kidney disease, whether they can reduce kidney fibrosis has not yet been addressed in clinical trials.

Figure 2. Tubular cell injury in AKI, AKI-to-CKD transition and CKD progression. Kidney tubules consist of polarized epithelial cells located on a basement membrane and surrounded by interstitial tissue. Adjacent cells interact through adherent junctions, desmosomes and tight junctions, creating a continuous cell layer that facilitates solute transport. Kidney tubular epithelium function is highly energy demanding and thus highly dependent on mitochondrial function. Tubular cell injury can result in cell death via mechanisms including apoptosis and regulated necrosis (e.g. necroptosis and ferroptosis) and/or sublethal damage, including loss of brush border and cell-cell contacts, that impairs tubular function. Acute tubular injury can lead to acute kidney injury (AKI), which frequently occurs in the context of chronic kidney disease (CKD). Depending on the severity of the insult, AKI can lead to regeneration and full recovery or can lead to progression of existing CKD or the development of CKD, known as AKI-to-CKD transition. Importantly, stressed and injured tubular cells secrete mediators of inflammation, which, in turn can cause further tubular injury either directly or through the recruitment of inflammatory cells. Oxidative stress is also an integral part of tubular cell injury. Following the acute injury phase, the

remaining cells can regenerate the tubular epithelium. This regeneration may involve several reparative mechanisms, including autophagy (which removes damaged organelles via lysosome-mediated degradation), the reactivation of development pathways (such as Notch, Wnt, hedgehog and SOX-9), the expression of genes that regulate cell differentiation and migration and the secretion of growth factors such as gremlin and CTGF. In AKI-to-CKD transition, epithelial cells undergo phenotypic changes, such as partial epithelial to mesenchymal transition (EMT) and induction of senescence. These changes contribute to the development of tubulointerstitial fibrosis.

Figure 3. Glomerular injury and fibrosis. Glomerular injury is associated with the recruitment of inflammatory cells, including macrophages and lymphocyte populations with characteristic cytokine secretion patterns. Sustained inflammation contributes to the progression of renal damage. Glomerular injury can lead to tubular cell injury and interstitial fibrosis through diverse mechanisms, including the secretion of inflammatory and profibrotic factors, albumin overload and in certain diseases, the glomerular filtration of red blood cells, potentially leading to tubular cell iron overload. Conversely, tubulointerstitial injury can eventually lead to glomerulosclerosis via mechanisms that likely involve inflammatory cells and spill-over of proinflammatory and profibrotic cytokines from the interstitium.

BOX 1: Assessment of kidney fibrosis

Currently, no ideal method to monitor kidney fibrosis exists. Repeat renal biopsies are invasive and the frequently patchy nature of fibrosis leads to potential sample bias. In clinical trials, the readout for drugs that theoretically target fibrosis is estimated glomerular filtration rate (eGFR). Although a key outcome measure in chronic kidney disease (CKD), eGFR is an imperfect surrogate for kidney fibrosis. Thus when eGFR outcomes are not met, it is unclear whether the fibrosis-targeting therapy does not preserve GFR or fails to modulate fibrosis.

Promising methods to non-invasively assess and monitor fibrosis include imaging techniques and urinary proteomics[1–3]. Magnetic resonance imaging (MRI) includes diffusion weighted imaging (DWI), T1 mapping and magnetization transfer[1]. MRI has been used to satisfactorily estimate fibrosis in CKD mice, but further development is needed before it can be used in the clinic. A 2019 study reported use of an elastin-specific MRI agent to detect elastin as a marker of fibrosis in multiple mouse models and ex vivo human kidney samples using T1-weighted TSE (turbo spin echo) imaging ($T1_WI$) and T1 relaxometry[2]. Additional molecular probes for SPECT, PET and MRI are being investigated for monitoring of fibrosis in diverse organs[4].

A urinary proteomics study identified a 273 peptide urinary marker (CKD273) that could be used to predict loss of eGFR in patients without CKD[5]. The CKD273 signature includes reduced levels of type I collagen peptides, suggesting reduced collagen degradation, which might provide evidence of kidney fibrosis[6]. CKD273, but nor eGFR or albuminuria, was subsequently shown to correlate with the degree of fibrosis in human kidney biopsies samples[3].

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