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REVIEW

The future of diabetic kidney disease management: what to expect from the experimental studies?

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Abstract

Diabetic kidney disease (DKD) is a major cause of end-stage renal disease. Intensive blood glucose and blood pressure control, particularly using inhibitors of the renin-angiotensin system, have long been mainstays of therapy in patients with DKD. Moreover, new anti-hyperglycemic drugs have recently shown renoprotective effects and this represents a major progress in the management of DKD. However, the risk of progression is still substantial and additional drugs are required. Recent preclinical studies have identified novel therapeutic targets that may optimize renoprotection in the near future. Besides strategies aimed to reduce oxidative stress and inflammation in the kidney, novel extra-renal approaches targeting stem cells, extracellular vesicles, and the microbiota are on the horizon with promising preclinical data. Herein, we will review these lines of research and discuss potential clinical applications. Given the poor yield of experimental studies in DKD in the past years, we will also discuss strategies to improve translation of preclinical research to humans.

Keywords Diabetic kidney disease · Oxidative stress · Inflammation · Endocannabinoid system · Mesenchymal stem cells · Microbiota

Introduction

The prevalence of diabetes is increasing worldwide to epidemic proportions. In 2019, approximately 464 Mio. people were living with diabetes and the number is predicted to exceed 700 Mio. by 2045. Diabetic kidney disease (DKD) is the commonest cause of end-stage renal disease (ESRD) and in the United States over 50% of subjects requiring dialysis and/or kidney transplantation have diabetes. Furthermore, the development of DKD dramatically increases the risk of cardiovascular both morbidity and mortality and most patients with DKD die for cardiovascular diseases.

Both albuminuria and relentless renal function decline are characteristic DKD features. The increased glomerular permeability to proteins leading to the development of albuminuria is due to podocyte injury, comprising nephrin and podocin downregulation, foot process effacement, and apoptosis. Progressive accumulation of extracellular matrix components (ECM) in both the mesangium and the tubulointerstitium plays a key role in renal function decline, eventually resulting in ESRD [1]. Besides the classical albuminuric phenotype, a new "non-albuminuric" phenotype characterized by eGFR decline without albuminuria has emerged in the last decades, indicating that DKD progression toward ESRD may occur through distinct albuminuric and non-albuminuric pathways [2]. Nevertheless, higher albuminuria still remains the major risk factor for progressive DKD [3, 4].

Mechanistically, the metabolic insult of hyperglycemia plays a key role in the pathogenesis of DKD by inducing oxidative stress and inflammation and by altering important metabolic [polyol pathway, exosamine pathway, advanced glycation end product (AGE) formation] and intracellular signalling pathways [i.e. protein kinase C (PKC), mitogen-activated protein (MAP) kinases, JAK-STAT, Notch], leading to podocyte injury and renal fibrosis. Cytokines released by both renal resident cells and inflammatory cells infiltrating the kidney are important mediators of the renal damage. Among them the prosclerotic cytokines TGF-β1 and CTGF, the vasoactive peptides angiotensin II and endothelin A, and the proinflammatory cytokines monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-α (TNF-α) are of particular relevance.

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In last 30 years, optimal control of both blood glucose and blood pressure and RAS inhibition have been the only available strategies for the treatment of DKD. In this period, preclinical research in experimental models of DKD has greatly improved our understanding of renal pathophysiology and also identified new promising therapeutic targets. However, new drugs arising from these studies failed to prove efficacy and safety in randomized clinical trials (RCTs) in humans. Recently, RCTs that were performed to prove cardiovascular safety of new anti-hyperglycaemic agents revealed that SGLT2 inhibitors (SGLT2i) [5–7] and GLP-1 receptor agonists [8, 9] have unforeseen anti-proteinuric and/or renoprotective effects in DKD. In particular, treatment with SGLT2i on the top of traditional RAS inhibition has proven to significantly reduce not only albuminuria, but also the decline in renal function and the associated cardiovascular risk in patients with established DKD [6]. In addition, atrasentan, which blocks the potent vasoconstrictor peptide endothelin A, showed renoprotective effects in a carefully selected subgroup of patients with type 2 diabetes [10].

These clinical findings represent a major turning point in the treatment of DKD and suggest greater efficacy of drugs acting upon the hemodynamic abnormalities of DKD that are known to interact with the metabolic pathways to promote renal function loss. Increased glomerular capillary pressure is an early feature in diabetes and an important mechanism of progression in all chronic kidney diseases. The particular efficacy of RAS blockers in the treatment of DKD is due to their ability to reduce not only systemic blood pressure, but also glomerular capillary pressure via vasodilation of the efferent arteriole. Similarly, SGLT2i are believed to slow DKD progression predominantly by reducing glomerular capillary pressure via vasoconstriction of the afferent arteriole [11]. Therefore, RAS blockers and SGLT2i appears to have complementary positive effects on renal hemodynamic.

Although results from recent RCTs on DKD represent a major step forward in the effort to retard the progression of DKD, a residual risk still remains and it has been calculated that 2% of patients will still progress to ESRD every year even after addition of SGLT2i to current therapy [12]. There is thus great interest to identify novel strategies for treatment. Herein, we will review novel lines of research that have recently emerged in the field of experimental DKD and their potential clinical applications (Fig. 1). Moreover, given the poor outcome of preclinical research in DKD so far, we

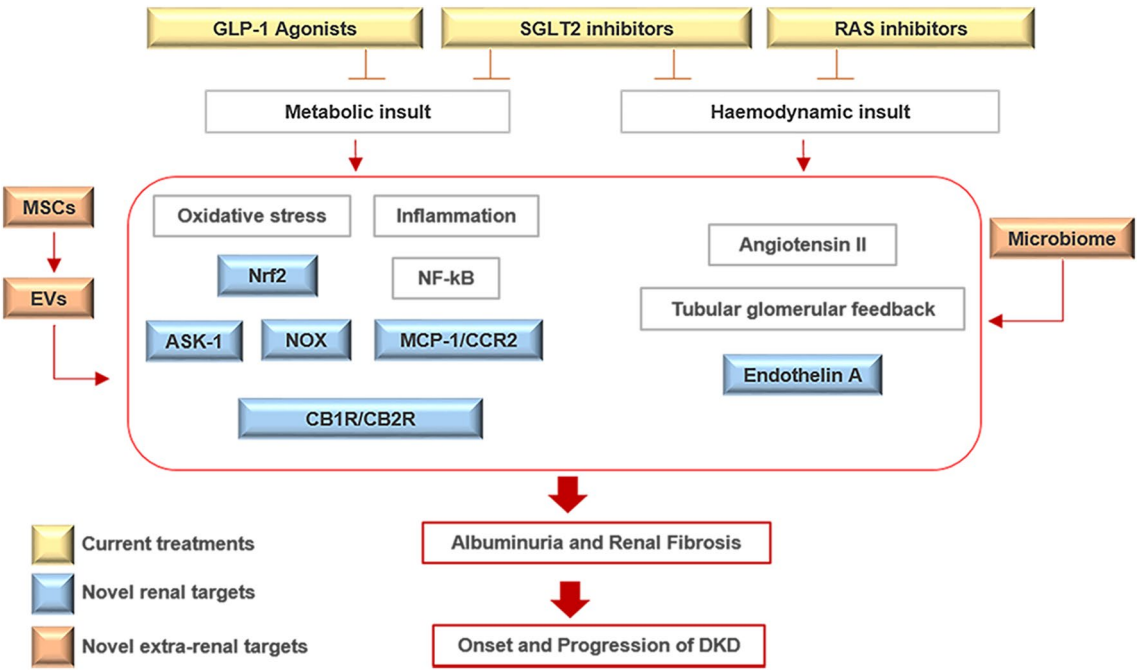

Fig. 1 Current and emerging treatments for diabetic kidney disease. Both the metabolic and the hemodynamic insults play a key role in the pathogenesis of diabetic kidney disease by inducing oxidative stress, inflammation, activation of deleterious signalling pathways and transcription factors, and release of cytokines in the kidney. Currently pharmacological agents for the management of DKD and novel both renal and extra-renal potential targets for treatment are shown. *GLP1* glucagon-like peptide 1, *SGLT2*, sodium–glucose cotransporter 2, *RAS* renin–angiotensin system, *MSCs*mesenchymal stem cells, *EVs* extracellular vesicles, *ASK1* apoptosis signal-regulating kinase 1, *Nrf2* nuclear factor erythroid 2-related factor 2, *NOX* nicotinamide adenine dinucleotide phosphate oxidase, *CB1R* cannabinoid receptor type 1, *CB2R* cannabinoid receptor type 2, *MCP-1* monocyte chemoattractant protein-1, *CCR2* CC chemokine receptor 2, *NF-κB* Nuclear Factor Kappa B

will also discuss strategies to improve translation of animal research to humans.

Oxidative stress

Hyperglycemia causes the formation of an excess of reactive oxygen species (ROS) in both the mitochondria and the cytosol. Although ROS play an important role in intracellular signalling, their accumulation can lead to oxidative stress and induce damage of critical cellular components, such as DNA and proteins. Oxidative stress is considered a leading mechanism of injury in DKD and contributes to both renal fibrosis and function decline [13]. Several antioxidants, such as coenzyme Q10 (ubiquinone), mitochondrial ubiquinone (MitoQ), resveratrol, and ascorbic acid have been tested in experimental DKD, but their efficacy appears modest and there are concerns about potential toxicity [14]. Recently, the NADPH oxidase family and ASK1 have emerged as interesting and promising novel targets in DKD.

NADPH oxidase

NADPH oxidase (Nox) is an enzyme that produces ROS. In neutrophils, Nox plays an important role in antimicrobial host defence and inflammation as the Nox-mediated release of high concentrations of ROS aids in the clearance of invading bacteria [13]. Nox is also present in other cell types, where its role is less understood. Among the five Nox isoforms present in humans Nox4 and Nox5 are the most relevant to DKD.

In animal models of DKD, expression of Nox4 is enhanced in the glomeruli and Nox4 is a major renal source of ROS [15, 16]. Intervention studies in experimental diabetes have shown that administration of Nox4 antisense oligonucleotides [17] as well as both global and podocyte-specific Nox4 deletion [18, 19] protected the kidney from diabetes-induced glomerular damage. Notably, treatment with GKT137831, a specific Nox1/4 inhibitor, markedly reduced albuminuria, oxidative stress, and kidney fibrosis, replicating the beneficial effects of Nox4 deletion [18]. In humans, GKT137831 failed to show a significant reduction in albuminuria. However, rodents differ from humans in the expression of Nox isoforms and do not express Nox5 and this may explain why promising preclinical results could not be replicated in humans. Indeed, preliminary data indicate that Nox4 deletion is no longer protective in transgenic animals expressing humans Nox5.

Although Nox-5 is more difficult to investigate in preclinical studies because it is absent in rodents, recent data highlighted the importance of Nox5 in DKD. In patients with DKD, glomerular Nox5 expression was enhanced predominantly in podocytes and mesangial cells [20, 21]. Exposure of cultured mesangial cells to both high glucose and TGF-β1 induced Nox5, while exposure of podocytes to angiotensin II increased the expression of the Nox5v2 splice variant, providing a possible mechanism for Nox5 upregulation in vivo.

The potential pathogenic role of Nox5 in DKD has been investigated in transgenic animals. Mice expressing human Nox5 exclusively in podocytes developed podocyte injury, albuminuria, oxidative stress, inflammation and this was accelerated in the presence of streptozotocin (STZ)-induced diabetes [20]. In animal models of type 1 diabetes, expression of Nox5 exclusively in vascular muscle cells and mesangial cells exacerbated diabetes-induced renal inflammation, mesangial expansion, and glomerulosclerosis [21, 22]. Moreover, diabetic Akita mice, expressing Nox5 in endothelial cells, showed worsening of albuminuria and inflammation [22]. In vitro studies have cast light on the potential mechanisms of the deleterious effects of the Nox5 in glomerular cells. Exposure to angiotensin II of podocytes expressing Nox5 induced actin cytoskeletal reorganization and activation of Rac GTPase, which promotes cell motility, via a Nox5-dependent mechanism [20]. This is relevant, as podocyte effacement requires cytoskeleton remodelling, foot process movement over the GBM, and slit diaphragm reconstruction and it is thus considered a migratory event. Moreover, mesangial cell exposure to high-glucose and/or TGF-β induced a significant upregulation of MCP-1, NF-κB, PKC-α and PKC-β and this was partially mediated by Nox5 [21]. Besides to be downstream targets of Nox5, PKC-α and PKC-β mediate the phosphorylation of Nox5 and enhance Nox5 activity in a positive feedback loop. Therefore, Nox5 and PKC interact in a bidirectional manner to exacerbate renal injury.

Collectively, these data from studies in experimental diabetes and cultured glomerular cells suggest a beneficial effect of Nox5 blockade in DKD and provide the rationale for the development of specific Nox5 inhibitors. Studies examining Nox5 in animal models where this isoform is endogenously expressed, such as rabbits, are currently on-going.

ASK1

Apoptosis signal-regulating kinase 1 (ASK1) is a redox-sensitive serine threonine kinase that activates the MAP kinases p38 and c-Jun and induces apoptosis, inflammation, and fibrosis in response to oxidative stress.

A recent study has identified ASK1 as a key factor in oxidative stress-induced kidney damage in diabetes [23]. ASK1 activity was enhanced in renal biopsies from patients with DKD. Treatment with the ASK1 inhibitor GS-444217 reduced albuminuria, eGFR decline, and renal structural injury in a mouse model of DKD. Notably, combination

therapy with GS-444217 and enalapril provided even greater renoprotection [23].

Clinical trials of ASK1 inhibitors were recently performed in patients with either nonalcoholic steatohepatitis (NASH) or DKD. In a multicenter phase 2 trial, treatment of patients with NASH and stage 2–3 liver fibrosis with the ASK1 inhibitor selonsertib was effective in reducing liver fibrosis [24]. However, a phase 2 trial evaluating safety and efficacy of selonsertib in adults with type 2 diabetes and moderate-to-advanced DKD failed to meet the primary endpoint as after 48 weeks of treatment eGFR did not differ in patients treated with selonsertib and placebo [25]. Moreover, selonsertib therapy did not modify albuminuria. However, selonsertib had an unexpected acute inhibitory effect on creatinine tubular secretion, leading to an acute and reversible drop in eGFR in the first weeks of the study without changes in measured GFR [25]. This was likely a confounder during the trial and a recent exploratory post-hoc analysis suggested that between 4 and 48 weeks the highest selonsertib dose slowed DKD progression compared to placebo. Further studies are, however, required to dissect between tubular and glomerular effects of selonsertib and to exclude the possibility that selonsertib effect on eGFR is merely related to interference with creatinine tubular handling.

Inflammation

In the past two decades, growing evidence demonstrated a critical role of inflammation in both the pathogenesis and the progression of DKD. Renal monocyte infiltration occurs in both human and experimental DKD. Moreover, expression of cell adhesion molecules, chemokines, and proinflammatory cytokines is increased in the renal tissues of diabetic patients. Several transcription factors (NF-κB, Nrf2) and inflammatory cytokines (MCP-1, TNF-α) were studied in the context of DKD and proposed as potential targets for therapy.

Bardoxolone methyl that activates the potent antioxidant and anti-inflammatory transcription factor Nrf2a was tested in a clinical trial (BEACON) in patients with DKD [26]. The RCT was prematurely terminated because of an excess fluid overload and heart failure (HF) in participants with elevated baseline levels of B-type natriuretic peptide (BNP), likely because of an effect of bardoxolone on the endothelin pathway. However, this line of research has not been completely abandoned and RCTs (TSUBAKI and AYAME) are currently on-going in patients at lower risk of fluid overload based on their both clinical and BNP response to escalating doses of bardoxolone methyl, an enrichment strategy similar to that was also employed in the SONAR trial. Moreover, SGLT2i lower the risks of HF and it would be of interest to explore the effect of a combined therapy with SGLT2i and bardoxolone methyl.

Another inflammatory target that emerged from preclinical research in animal models of DKD is the chemokine MCP-1.

MCP-1

MCP-1 is expressed by both resident glomerular cells and infiltrating monocytes. MCP-1 binds to the CC-chemokine receptor-2 (CCR2) and induces monocyte both recruitment and activation.

Several diabetes-related insults and mediators, such as high glucose, angiotensin II, advanced glycation end products (AGEs), mechanical stretch, TGF-β1, and TNF-α are potent MCP-1 inducers in glomerular cells. Consistent with this, MCP-1 is overexpressed within the glomeruli in experimental diabetes. MCP-1 can contribute to the onset and progression of DKD by fuelling local inflammation through monocyte recruitment [27]. Furthermore, the MCP-1 receptor CCR2 is also expressed by mesangial cells and podocytes [28–30] and MCP-1 may contribute to the development of DKD by directly inducing deleterious effects in resident glomerular cells. Indeed, in mesangial cells MCP-1 enhances ICAM-1 expression, increases fibronectin production, and partially mediates high glucose-induced TGF-β1, fibronectin, and collagen type IV production. Moreover, in podocytes MCP-1 induces nephrin loss, apoptosis via TGF-β1, and podocyte migration [27]. The deleterious effects of MCP-1 in podocytes are likely enhanced in DKD as podocyte CCR2 expression is markedly increased in patients with type 2 diabetes and overt DKD.

Studies in experimental diabetes have convincingly demonstrated a causative role of the MCP-1/CCR2 system in the pathogenesis of DKD. In experimental models of diabetes, MCP-1 deletion prevented the development of albuminuria, nephrin downregulation, and ECM overexpression [29, 31]. Moreover, gene transfer of the 7ND gene, a N-terminal deletion mutant of human MCP-1, improved glomerulosclerosis in iNOS transgenic diabetic mice [32] and diminished diabetes-induced glomerular hypertrophy and glomerulosclerosis in STZ-induced diabetic rats [33].

These promising results led to the development of pharmacological agents blocking the MCP-1/CCR2 system that were then tested in experimental models. In db/db mice, the CCR2 antagonists RS504393 and RS102895 improved insulin resistance and reduced macrophage infiltration and albuminuria [34]. In diabetic mice expressing human CCR2, oral administration of the CCR2 antagonist CCX140-B decreased albuminuria and glomerular hypertrophy, increased podocyte numbers, but also improved glycaemic control [35].

More recently, phase 2 RCTs targeting the MCP-1/CCR2 system have been performed in humans. In patients with

DKD, treatment with the CCR2 antagonist CCX140-B on the top RAS blockade decreased albuminuria, particularly in subjects with overt proteinuria [36]. Similarly, addition to standard therapy of emapticap pegol (NOX-E36), a Spiegelmer that specifically binds and inhibits MCP-1, improved albuminuria without significant side effects [36] and the CCR2 blocker, DMX-200 (propagermanium) is currently under investigation. However, in clinical studies in type 2 diabetes blockade of the MCP-1/CCR2 system also improved HbA1c, likely because of reduced inflammation in the pancreatic islet and/or the adipose tissue. Therefore, the beneficial effects of MCP-1/CCR2 blockade on albuminuria may be partially due to amelioration of blood glucose control. Long-term clinical studies are awaited to clarify the potential clinical relevance of the MCP-1/CCR2 system as novel target in the treatment of DKD. In this regard, it would be important to establish blockade of the MCP-1/CCR2 system still retain benefit when given in addition to SGLT2i/GLP-1RA.

The endocannabinod system

The endogenous cannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), bind to the cannabinoid receptor 1 (CB1R) and 2 (CB2R). The endocannabinoid system (ECS) is predominantly expressed by the central nervous system (CNS) and by peripheral organs involved in the control of metabolism. However, recent studies suggest a role of the ECS in the kidney and in particular in DKD. CB1R is overexpressed by podocytes in both human and experimental DKD, while podocyte CB2R expression undergoes downregulation in patients with advanced DKD. This is likely due to both hyperglycemia and glomerular capillary hypertension as exposure to high glucose or angiotensin II enhances CB1R in podocytes, while mechanical stretch, mimicking glomerular capillary hypertension, decreases CB2R expression [37].

CB1R signalling increases oxidative stress, inflammation, and fibrogenesis, while CB2R has opposite effects. Therefore, an imbalance between glomerular CB1R and CB2R signalling within the glomeruli can contribute to enhance oxidative stress, inflammation, and fibrosis in the context of diabetes. Consistent with this, studies in knockout/transgenic animals have shown that albuminuria and nephrin loss are induced in non-diabetic animals overexpressing CB1R [38], exacerbated in diabetic mice lacking CB2R [39], and ameliorated in diabetic mice lacking CB1R exclusively in podocytes [40].

On the clinical point of view are of particular relevance the results of studies in experimental diabetes using drugs that modulate the ECS. Both peripheral CB1R antagonists and CB2R agonists reduced albuminuria, prevented loss of slit-diaphragm proteins, and ameliorated glomerular fibrosis [41–43]. Moreover, dual therapy with the peripheral CB1R blocker AM6545 and the CB2R agonist AM1241 showed greater efficacy than single therapy in diminishing albuminuria, inflammation, and renal fibrosis [44]. Notably, CB1R blockade also reversed changes in both albuminuria and renal function in animals with established albuminuria [43, 45]. Mechanistically, modulation of the ECS appears to prevent albuminuria by preventing nephrin loss and podocyte apoptosis, while the beneficial effect on renal fibrosis is likely due reduced release of prosclerotic and pro-inflammatory factors [46].

These pre-clinical studies have opened the way to a novel area of research with potential clinical impact in humans. The use of peripherally restricted CB1R antagonists that do not cross the blood–brain barrier and are thus devoid of central side effects, is very promising in human DKD. These compounds are currently under early stage clinical development with the aim of providing proof of safety and tolerability, including CNS safety. Moreover, compounds that are both CB1R antagonists and CB2R agonists may provide additional therapeutic benefits [46]. However, CB1R and CB2R can form heterodimers and their signalling pathways are deeply intertwined; therefore, further research is required to better elucidate their relationship and combined effects in order to avoid undesirable and/or off-target effects.

The gut microbiota

The gut microbiota comprises a set of microorganisms living in the gut in symbiosis with the human host. In recent years, the gut microbiota has gained increasing interest and has been implicated in the pathogenesis of a vast array of diseases, including chronic kidney disease (CKD) and diabetes [47]. Changes in gut microbiota composition, accumulation of microbiota-derived metabolites, interruption of intestinal barrier function, and chronic inflammation have all been reported in CKD. However, evidence linking the gut microbiota to DKD is still limited.

A small case–control study has shown greater relative abundance of *Proteobacteria, Verrucomicrobia,* and *Fusobacteria* phyla in patients with DKD stage 4–5 compared to healthy controls and this was associated with enhanced circulating levels of lipopolysaccharides (LPS) and markers of inflammation [48]. Another study reported that higher faecal amount of *Escherichia-Shigella* and lower amount of *Prevotella* represent a sort of microbiota signature of DKD as it can distinguish among patients with type 2 diabetes those with and without biopsy-proven DKD [49]. *Enterobacteriaceae* and *Proteobacteriaceae* may be linked to DKD because they favour production of toxic metabolites that are normally cleared by the kidney

(uremic toxins). Moreover, they enhance the translocation of bacterial products and LPS into the circulation by increasing gut permeability. On the contrary, *Prevotellaceae* may be protective because they maintain the gut barrier function and reduce local inflammation by enhancing short-chain fatty acid (SCFAs) production [47].

An elegant study has recently described a novel link between the gut microbiota and DKD [50]. Gut microbes, expressing the enzyme tyrosine phenol-lyase (TPL), synthesize phenol from dietary l-tyrosine. After absorption, phenol is metabolized by the liver into phenyl sulfate (PS), which is then secreted by proximal tubular cells. In experimental model of diabetes, treatment with PS induced podocyte hypertrophy, foot-process effacement, GBM thickening, inflammatory, and fibrosis, while treatment with TPL inhibitors (2-aza-tyrosine, L-meta tyrosine) diminished both PS and creatinine levels without significantly altering the microbial taxonomic balance. The observation that podocyte exposure to PS reduced glutathione levels suggests that PS can contribute to DKD by increasing podocyte susceptibility to both oxidative stress and mitochondrial dysfunction. These findings may be relevant to human DKD as in a cohort of 87 patients with type 2 diabetes and microalbuminuria plasma PS levels at baseline were a predictor of albuminuria progression at the 2-years follow up even after adjustment for risk factors.

Other uremic toxins besides PS have been implicated in the progression of renal disease. Indoxyl sulfate (IS) and *p*-cresyl sulfate (PCS) are produced at the intestinal level by proteolytic microbes and then excreted in the urine by active tubular secretion. As kidney function declines, IS and PCS progressively accumulate in blood and may contribute to DKD progression. Indeed, exposure of podocytes to IS decreased both cell viability and expression of podocyte-specific genes, while it increased expression of both cytokine and chemokine expression. Furthermore, exposure of proximal tubular cells to IS and PCS induced epithelial to mesenchymal transition with overexpression of transcription factors associated with interstitial fibrosis and glomerulosclerosis [51]. It is thus temping to speculate that changes in the diet aimed to reduce microbiota-derived uremic toxins may help reduce the rate of progression in advanced stage of DKD.

Although these preliminary data suggest a possible contribution of gut dysbiosis to the development and progression of DKD, further studies are required to gain a better understanding of the relationship between the human microbiome and renal pathophysiology. In particular, studies in larger cohorts of DKD patients using new approaches, such as metagenomics and metaproteomics, are essential to establish whether the gut microbiome may represent a novel target of the treatment of DKD.

Mesenchimal stem cells and extracellular vesicles

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are self-renewing and multipotent progenitors that can differentiate into various lineages. Over the past decade, intervention studies in animal models of DKD have proven the therapeutic potential of MSCs obtained from different sources, such as bone marrow (bm-MSCs), adipose tissue (ad-MSCs) and umbilical cord (uc-MSCs).

Administration of bm-MSCs via the renal artery decreased urinary albuminuria and improved renal function in STZ-induced diabetic rats with established DKD. This effect was paralleled by amelioration of diabetes-induced podocyte loss, foot processes effacement, and downregulation of nephrin and podocin [52]. In a similar experimental model, intravenous infusion of bm-MSCs also reverted glomerulosclerosis, ECM deposition, and diminished macrophage infiltration and pro-inflammatory cytokines levels [53]. More recently, a study performed on type 1 diabetic mice has shown that bm-MSCs limited inflammation-related injury through the phenotypic switch of macrophage from a pro-inflammatory (M1) to anti-inflammatory (M2) phenotype [54]. It is worth noting that with the exception of the paper by Wang et al. in the aforementioned studies an amelioration of blood glucose has been also observed in MSCs-treated diabetic animals. Therefore, it is not clear whether MSCs act directly on the kidney or their beneficial effects are secondary to blood glucose lowering.

Although bone marrow is a rich source of MSCs, harvesting the bone marrow is an invasive procedure. Moreover, quantity, differentiation potential, and maximal life span of MSCs decline with the donor's age. Therefore, several groups have tested the renoprotective effect of MSCs isolated from alternative and more accessible sources. Park et al. demonstrated that treatment with uc-MSCs prevented renal injury and reduced mesangial expansion without affecting blood glucose levels in rats with STZ-induced diabetes [55]. Another study showed that uc-MSCs had a synergic effect in combination with resveratrol in improving renal function and podocyte number in Non-Obese Diabetic (NOD) mice independently by blood glucose levels [56]. Ad-MSCs provide a clear advantage over bm-MSCs as they can be easily and repeatable harvested using minimally invasive techniques with low morbidity and their proliferation capacity does not decline with the age of the donor [57]. Repeated intravenous injections of ad-MSCs attenuated glomerulus hypertrophy and urinary protein excretion in STZ-induced diabetic rats

[58]. Moreover, Takemura et al. showed that transplantation of ad-MSCs under the renal capsule of Spontaneously Diabetic Torii fatty rats attenuates glomerular and tubular injury to a greater extend compared to ad-MSCs administrated per intravenous route [59]. Finally, Lee SE et al. have shown that treatment with ad-MSCs prevented albuminuria and tubular epithelial cell injury via induction of the Arginase-1, a marker of M2 macrophages, and subsequent improvement of mitochondrial function [60]

Although these pre-clinical studies in DKD suggest that MSC-based therapy may represent a novel promising tool for DKD treatment, the experimental design of these studies is heterogeneous in terms of cell types, doses, administration route, diabetes duration, making it difficult to translate these findings into clinical practice. In addition, the use of MSCs has several limitations. As demonstrated by pre-clinical studies, MSCs show minimal homing to the kidney and have limited survival in the kidney environment. Moreover, MSCs have poor expansion capacity in vitro and undergo senescence, which affects cell replication and differentiation capacity. In addition, MSC expansion in vitro for extended periods of time can confer risk of chromosomal instability and malignant transformation [61]. Finally, MSCs can differentiate into undesired lineages as reported by Kunter U et al., showing "maldifferentiation" of intraglomerular MSCs into adipocytes accompanied by glomerular sclerosis [62].

MSC-derived cytokine and extracellular vesicles

Emerging evidence shows that the efficacy of MSCs in ameliorating DKD is mainly due to MSC release of soluble factors and extracellular vesicles (EVs) [63]. Consistent with this, Nagaishi et al. demonstrated that the beneficial effect of bm-MSCs in promoting renal damage repair and reducing macrophage infiltration, interstitial fibrosis, and glomerular injury in experimental DKD was reproduced by treatment with MSC conditioned media [64]. Moreover, LV et al. showed that conditioned media from bm-MSC inhibited the TGF-β/Smad prosclerotic pathway in mesangial cells exposed to a high glucose milieu. This beneficial effect was abrogated by an antibody against bone morphogenetic protein-7 (BMP-7), suggesting that BMP-7 secreted by MSCs was involved. Using a similar experimental approach, the same group also demonstrated that in mesangial cells exposed to high glucose, hepatocyte growth factor (HGF) released by bm-MSC reduced TGF-β up-regulation and inhibited oxidative stress. Furthermore, Li D. et al. found that ad-MSCs prevented high glucose-induced podocyte both injury and apoptosis through secretion of epithelial growth factor (EGF). Finally, glial cell line–derived neurotrophic factor (GDNF) released by ad-MSC prevented podocyte damage by restoring high glucose-induced synaptopodin downregulation [63].

Besides soluble factors, extracellular vesicles (EVs) both exosomes (Exos) and microvesicles (MVs), may also mimic the effect of their parent cells via the horizontal transfer of functional microRNAs (miRNAs), mRNAs, and proteins to target cells. Several studies have shown that treatment with MSC-EVs is safe and has renoprotective effects in models of DKD. In diabetic animals, treatment with bm-MSC-Exos abolished apoptosis and tubular epithelial cells (TEC) damage, improved renal function, and reduced renal fibrosis by acting on the mTOR signalling pathway and autophagy [65]. Urine-derived MSC-Exos prevented both podocyte and TEC apoptosis by suppressing caspase-3 overexpression in STZ-induced diabetic rats [66]. More recently Jin et al. showed that ad-MSC-Exos reduced proteinuria and podocyte apoptosis in db/db mice. Mechanistically, ad-MSC-Exos reversed high glucose-induced apoptosis by enhancing the expression of miR-486, which inhibits the Smad1/mTOR signalling pathway in podocytes [67]. Finally, bm-MSC-EVs and human liver-derived MSC (HLSC) EVs administered in a therapeutic-like regimen inhibit the progression of the functional and morphological dysfunction caused by DKD [68] by reducing high glucose-induced both collagen and TGF overexpression in mesangial cells via the horizontal transfer of functional miR-222 [69].

Overall, these data indicate that EVs may represent an effective genetic information transfer agent that supports a series of biological processes and has therapeutic potential. On the clinical point of view, the use of MSC-derived EVs in place of MSCs has several advantages. EVs overcome concerns posed by MSCs as tumorigenicity, viral infection, cellular rejection, and mal-differentiation. Recent evidence also suggests that EVs can be used as drug delivery vectors to treat and target specific cell types [70]. However, there are still many problems to address before EV therapy could be use in clinical practice. Since the properties of EVs are directly related to the conditions under which they are produced and the cells that produce them, it is essential to establish the characteristics of EVs from different sources for the repeatability and safety of subsequent applications. Moreover, a better understanding of EV mechanism action and complexity of their cargo need to be obtained prior to their use in humans. In addition, regulatory requirements for large-scale manufacturing and quality control are a precondition for clinical trials of EVs.

Implications for translational research

Although the novel lines of research in experimental DKD described above are very interesting, we cannot forget that in the past 20 years drugs that appeared very promising based on preclinical studies failed to prove efficacy and/or safety in RCTs in humans. Paradoxically, novel drugs

for the treatment of DKD have recently come from RCTs designed for a different purpose. Indeed, the first evidence of SGLT2i efficacy in reducing albuminuria and preventing renal function loss was obtained in RCTs aimed to assess the non-inferiority of SGLT2-i compared to traditional anti-hyperglycaemic drugs in terms of cardiovascular outcome [9]. The tradition bench to bedside approach has thus been inverted and we are currently trying to understand the molecular mechanisms underlying the positive effects of SGLT2i and GLP-1RA on the kidney after proving their clinical benefit in humans.

Important limits in the design of experimental studies may explain the low yield of translational research in DKD. To improve translation efficiency, the design of preclinical studies should better match that of RCTs (Fig. 2). Experimental studies should be performed not only in young and lean animals with type 1 diabetes, but also in old and obese animals with type 2 diabetes as most DKD cases are old and obese patients with type 2 diabetes. Moreover, we should choose animal models that develop substantial renal injury and significant renal function loss to better mimic human DKD. Preclinical studies should set strict criteria for randomisation with defined threshold of eGFR, albuminuria, and HbA1c. Safety and efficacy of potential therapeutic agents should be evaluated starting from the onset of DKD and/or albuminuria rather than at the onset of diabetes and secondary prevention/reversal studies should replace primary prevention studies. As eGFR is a major determinant of the primary endpoints used in RCTs and relevant for drug approval, assessment of eGFR in pre-clinical models is crucial and experimental drugs should prove efficacy not only in reducing albuminuria, but also eGFR decline. Finally, interventional drug should prove efficacy when they are given on top of current standard therapy with blood glucose lowering drugs and both RAS and SGLT2 inhibition.

On the other hand, human RCTs also have limitations that contribute to increase the gap between pre-clinical and clinical research. Both structural and ultrastructural abnormalities are usually explored extensively in experimental models, but they are rarely assessed in human RCTs. Indeed, the diagnosis of DKD is purely based on the presence of albuminuria and/or eGFR loss in subjects affected by diabetes. Nonetheless, available histological studies in humans have shown large structural heterogeneity among patients clinically defined as affected by DKD. Particularly in older patients with type 2 diabetes, other insults besides diabetes itself, such hypertension, vascular disease, and previous subclinical renal injury, may contribute to the renal damage and significantly modify the course of the disease and the rate of progression towards ESRD. This heterogeneity is also present among patients recruited in RCTs and may significantly affect the response to treatment. Therefore, renal biopsies should be performed more often in RCTs in DKD (at least in subgroups) to better correlate clinical and structural outcomes and gain a better insight on underlying mechanisms of drug action. Moreover, novel enrichment study designs may be employed to identify drugs that are effective in specific subgroups of patients. In particular, subjects can be selected prior to randomisation based either on their individual response to the drug or on the positivity

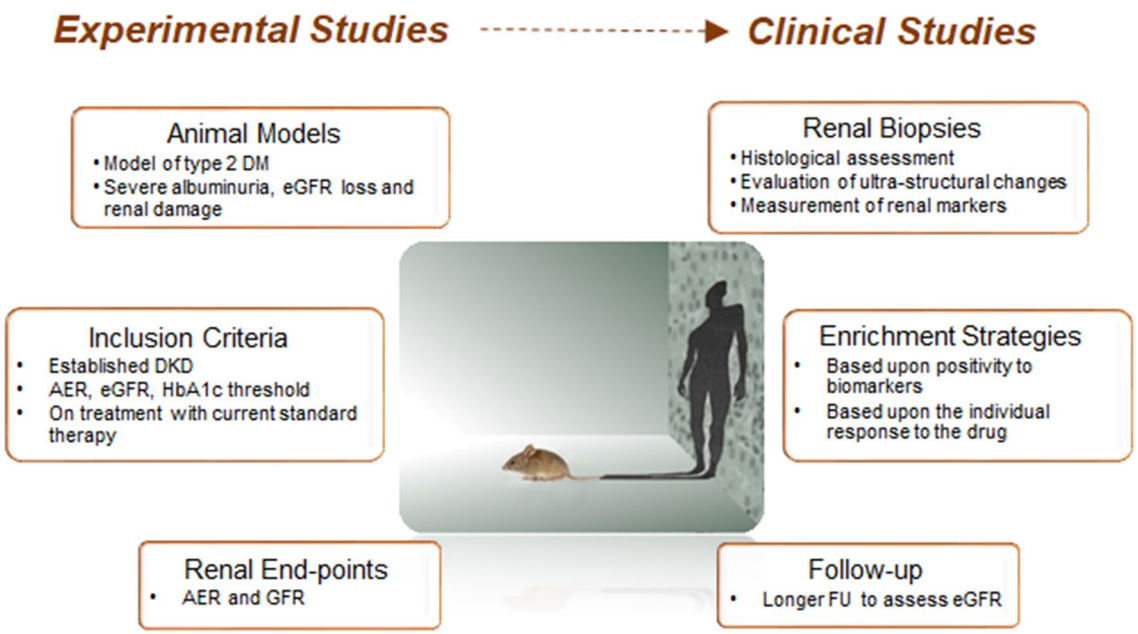

Fig. 2 Changes in the design of both experimental and clinical studies that may help improve translation of animal research to humans

to relevant biomarkers. Finally, clinical studies on SGLT2i have shown that these compounds reduce eGFR in the short-term, but have important reno-protective effects after longer follow-up. Therefore, novel potential drugs should not be dismissed based on preliminary underpowered short-term studies as longer duration of treatment may be required to reveal benefit.

These changes in the design of both experimental and clinical studies may help improve translation of animal research to humans and they are instrumental to further reduce the risk of DKD both onset and progression.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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