Diabetic Kidney Disease: From Pathogenesis to Novel Treatment Possibilities

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Abstract

One of the microvascular complications of diabetes is diabetic kidney disease (DKD), often leading to end stage renal disease (ESRD) in which patients require costly dialysis or transplantation. The silent onset and irreversible progression of DKD are characterized by a steady decline of the estimated glomerular filtration rate, with or without concomitant albuminuria. The diabetic milieu allows the complex pathophysiology of DKD to enter a vicious cycle by inducing the synthesis of excessive amounts of reactive oxygen species (ROS) causing oxidative stress, inflammation, and fibrosis. As no cure is available, intensive research is required to develop novel treatments possibilities. This chapter provides an overview of the important pathomechanisms identified in diabetic kidney disease, the currently established therapies, as well as recently developed novel therapeutic strategies in DKD.

Keywords

Diabetic kidney disease · Diabetic nephropathy · Fibrosis · Inflammation · NADPH oxidase · Oxidative stress

1 Introduction

Diabetic kidney disease (DKD), also referred to as diabetic nephropathy, is a chronic disease of the kidney and one of the most prevalent microvascular complications of diabetes mellitus. Apart from microvascular complications, diabetic patients with and without DKD encounter also increased risk of cardiovascular morbidity and premature mortality (Groop et al. 2009; Penno et al. 2021; McCullough et al. 2007). Of all diabetic patients, up to 30–45% develop DKD, often progressing to end stage renal disease (ESRD). Patients suffering from ESRD become imperatively dependent on dialysis or kidney transplantation, and approximately 45% of all ESRD cases are related to diabetes (Schiffer and Friedrich-Persson 2017; Ostergaard et al. 2020). Although the absence of pathognomonic symptoms impedes early detection of chronic kidney disease (CKD), diagnosis of DKD is based on the presence of

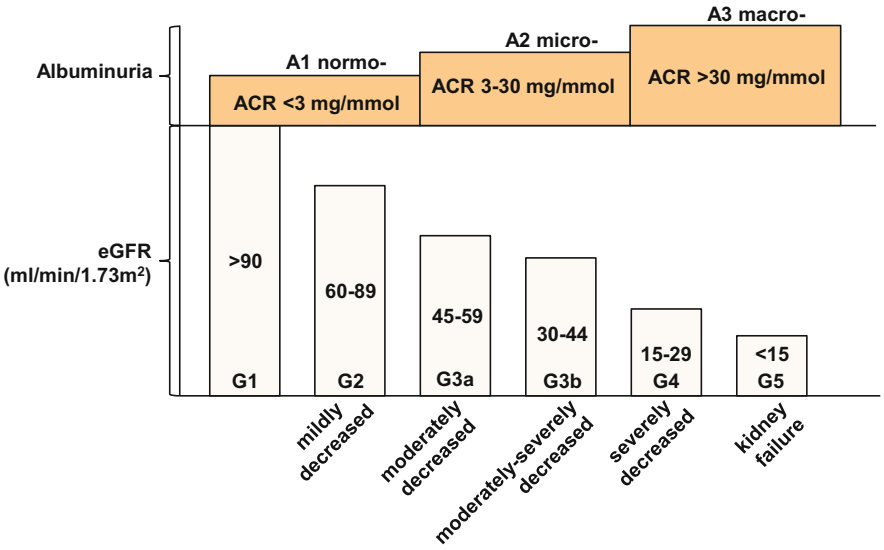

Fig. 1 Definition of diabetic kidney disease. Normoalbuminuria is defined by albumin/creatinine ratios (ACR) of <3 mg/mmol (A1), followed by microalbuminuria, which ranges between 3 and 30 mg/mmol (A2) and macroalbuminuria with an ACR >30 mg/mmol (A3). Kidney function is assessed by eGFR and progresses from normal/healthy (G1, >90 ml/min/1.73 m^2) with a steady decline (G2–G4) ultimately resulting in kidney failure (G5, eGFR < 15 ml/min/1.73 m^2)

micro- or macroalbuminuria with a progressive decline in renal function assessed by the estimated glomerular filtration rate (eGFR) (Fig. 1) (Dagogo-Jack 2021).

1.1 Definition

One characteristic of DKD is albuminuria, which is usually assessed by the albumin-creatinine-ratio (ACR) in a spot urine sample. An ACR of <3 mg/mmol is considered normal (A1), followed by a moderate increase to 3–30 mg/mmol known as microalbuminuria (A2), and a severe increase to >300 mg/mmol, also called macroalbuminuria (A3) (Fig. 1) (Dagogo-Jack 2021). In addition to the ACR, the eGFR is another important clinical indicator of DKD and the decline of eGFR can occur with or without concomitant albuminuria (Tsalamandris et al. 1994). Moreover, glomerular hyperfiltration, that is increased eGFR, is considered as an indicator for the onset of DKD, particularly in type 1 diabetes (Dagogo-Jack 2021). Healthy kidneys show values of ≥90 mL/min/1.73 m^2, followed by an eGFR of 60–89 mL/min/1.73 m^2 considered as mildly decreased, an eGFR of 45–59 mL/min/1.73 m^2 as mildly to moderately decreased, an eGFR of 30–44 mL/min/1.73 m^2 as moderately to severely decreased, an eGFR of 15–29 mL/min/1.73 m^2 as severely decreased, and finally an eGFR of <15 mL/min/1.73 m^2 considered as kidney failure (Fig. 1) (Dagogo-Jack 2021).

1.2 Diagnosis

Early diagnosis is pivotal to mitigate and delay the progression of DKD. Patients with type 1 diabetes should be monitored for ACR and eGFR annually 5 years after diagnosis, while patients with type 2 diabetes should be monitored annually directly when diagnosed with type 2 diabetes as the exact onset of kidney disease is often unclear (Dagogo-Jack 2021). Noteworthy, patients with prediabetes have also been reported to show impaired function of the kidney and/or albuminuria (Plantinga et al. 2010). The mainstay of treatment of DKD includes control of blood glucose. Although these strategies enable a delay of DKD progression, none of these approaches is able to cure kidney disease.

2 Pathogenesis of DKD

The renal physiology is maintained mainly by four cell types involving glomerular endothelial cells, podocytes, mesangial cells, and tubular cells. The well-orchestrated interaction of these cells portrays high complexity of mutual influences making the pathological origin of DKD a challenge to investigate. In the diabetic milieu, several factors including hyperglycemia and associated glucose toxicity, advanced glycation end products (AGEs), growth factors, hemodynamic and hormonal changes contribute to the harmful generation of reactive oxygen species (ROS), which in turn result in renal inflammation and fibrosis (Fig. 2) (Jha et al. 2016a). These alterations cause pathologic functional and structural abnormalities including glomerular basement membrane (GBM) thickening, podocyte loss, mesangial expansion, and eventually glomerulo- and tubulointerstitial sclerosis (Steffes et al. 1992; Kanwar et al. 2008). The glomerular filtration barrier is composed of three layers consisting of fenestrated glomerular endothelial cells, podocytes, and the GBM, which is established by both cell types (Lassen and Daehn 2020). The physiological GBM itself consists mainly of an anionic charged heparan sulfate barrier, followed by a layer of collagen IV, laminin, fibronectin, entactin and proteoglycans called the lamina densa, and another layer of heparan sulfates (Mason and Wahab 2003). These layers ensure passing of selected small molecules while preventing larger molecules from entering the Bowman's space such as albumin (Lassen and Daehn 2020; Lin and Susztak 2016). Under diabetic conditions, the interplay of the filtration barrier components is disturbed, potentially causing albuminuria. Glomerular endothelial cells allow glucose entry independently of insulin, making them specifically vulnerable to the direct exposure of blood glucose levels. In order to metabolize excess intracellular glucose levels due to hyperglycemia, glomerular endothelial cells undergo a phenotypic switch by turning on the polyol-, hexosamine-, AGE/RAGE-, and the PKC pathway, causing the generation of increasing amounts of ROS, which in turn can lead to endothelial nitric oxide synthase uncoupling and reduced nitric oxide bioavailability (Lassen and Daehn 2020; Reidy et al. 2014; Jourde-Chiche et al. 2019). The alterations in their physiology result in the degradation of the heparan sulfate layer, thereby

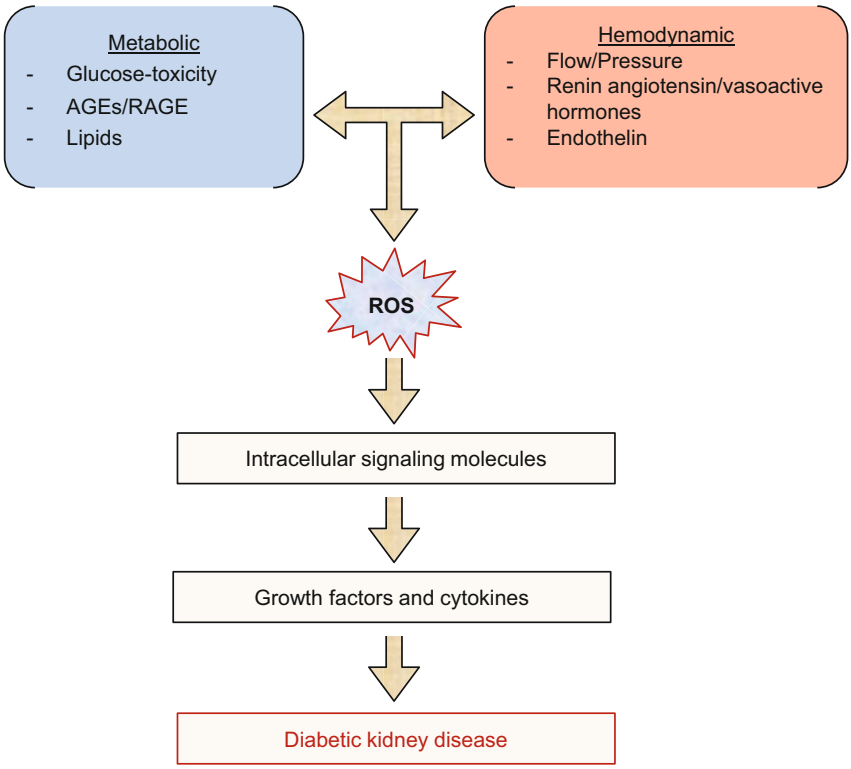

Fig. 2 Metabolic and hemodynamic changes in the diabetic milieu. The diabetic condition leads to altered cellular glucose metabolism resulting in activation of unfavorable signaling pathways. The activation of hemodynamic and metabolic pathways is associated with excessive ROS production, which leads to increased intracellular signaling ensuing the synthesis and recruitment of growth factors and cytokines, leading to a vicious cycle of inflammation and fibrosis in the diabetic kidney

affecting GBM integrity (An et al. 2018; van den Hoven et al. 2009). Maintaining the glomerular filtration function is also dependent on a crosstalk between glomerular endothelial cells and podocytes as endothelial dysfunction impairs podocytes and vice versa (Lassen and Daehn 2020; Cassis et al. 2019). Some mediators of the crosstalk involve vascular endothelial growth factor A (VEGF-A), angiopoietins, endothelin-1, activated protein C, or transforming growth factor-β (TGF-β), which stimulate podocytes to synthesize extracellular matrix (ECM) proteins (reviewed in Lassen and Daehn 2020; Marshall 2016). In diabetes, GBM thickening can also be promoted by the action of AGEs, which in the kidney does not only lead to ROS, glomerular hypertrophy, inflammation and renal fibrosis but also to podocyte injury and apoptosis (Chuang et al. 2007). Podocyte injury leads to cytoskeletal reshaping, a process termed foot process effacement, and weakens the structure of the GBM (Lin and Susztak 2016; Mundel and Shankland 2002). There is contradicting data concerning foot process effacement enabling podocytes to detach from GBM (Lin

and Susztak 2016). Loss of podocytes by apoptosis, or potentially as a result of GBM detachment, can also occur by epithelial-mesenchymal transition (EMT) induced in hyperglycemia by activating TGF-β/Smad, Wnt/β-catenin, integrins/integrin-linked kinase, MAPK, Jagged/Notch, and NFκB signaling pathways. Tubular cells undergo also EMT and together with endothelial-mesenchymal transition (EndoMT), they contribute to the formation of myofibroblasts known to produce ECM proteins and tubulointerstitial fibrosis (Loeffler and Wolf 2015). Mesangial cell injury induced by hyperglycemia additionally enhances the deposition of ECM proteins into the mesangium (Loeffler and Wolf 2015; Tung et al. 2018). The accumulation of ECM proteins, predominantly of different collagens, fibronectin and laminin, leads to scarring of the renal tissue, an important process in the progression of diabetic nephropathy called glomerulosclerosis and interstitial fibrosis, resulting in kidney failure (Loeffler and Wolf 2015; Qian et al. 2008).

3 Current Therapies for DKD

3.1 Lifestyle Changes

The general advice for patients with DKD is to maintain a healthy weight, the cessation of smoking, regular physical activity, and a reduction in dietary sodium. A reduction in dietary sodium to <2,300 mg/day can improve blood pressure control and decrease the risk for cardiovascular disease in patients with CKD (Mills et al. 2016). The advice regarding protein intake suggests a protein intake of 0.6–0.8 g/kg/weight/daily (American Diabetes Association 2021). At this level, a modest protein intake has shown to slow the deterioration of renal function. A higher protein intake is associated with glomerular hyperfiltration, an increase in albuminuria and worsening of renal function. However, a further reduction in protein intake to <0.8 g/kg/weight/daily does not further improve renal function and also carries the risk of malnutrition (Murray et al. 2018). Furthermore, many patients with reduced eGFR have elevated potassium levels due to a reduced excretion of potassium, thus diet advice needs to be adjusted on an individual basis for those patients (Kidney Disease: Improving Global Outcomes Diabetes Work Group 2020).

3.2 Blood Glucose and Blood Pressure Control

Blood glucose and blood pressure control remain the mainstay of risk factor control in the treatment of DKD. Many studies have shown that the optimization of blood pressure and glucose control retards the progression of DKD (UK Prospective Diabetes Study (UKPDS) Group 1998; Holman et al. 2008; ADVANCE Collaborative Group and Patel 2008; DCCT/EDIC Research Group and de Boer 2011; Lewis et al. 1993). However, studies by Fioretto et al. have shown that normalization of blood glucose in type 1 diabetic patients who received a pancreas transplant requires 5–10 years to reverse glomerular and tubulointerstitial changes with arteriolar

hyalinosis remaining unchanged even after 10 years of normoglycemia (Fioretto et al. 1998).

3.2.1 Blood Glucose

There is overwhelming evidence that a reduction in blood glucose is associated with a reduction in microvascular complications, in particular nephropathy. Blood glucose control is usually achieved with oral drugs in type 2 diabetic patients, whereas type 1 diabetic patients require insulin. In terms of oral drugs, metformin, an AMP-activated protein kinase (AMPK) activator, has many beneficial metabolic actions and is recommended as a first-line agent. However, metformin needs to be dose-adjusted in CKD (eGFR <45 mL/min) and is contraindicated if eGFR is <30 mL/min due to the increased risk of lactic acidosis. The target of an HbA1c of 7% or lower has shown to reduce the risk of development and progression of DKD in type 1 and type 2 diabetes (UK Prospective Diabetes Study (UKPDS) Group 1998; Holman et al. 2008; ADVANCE Collaborative Group and Patel 2008; DCCT/EDIC Research Group and de Boer 2011; Diabetes Control and Complications Trial Research Group and Nathan 1993; The Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group and Steffes 2003). In the DCCT trial, patients with type 1 diabetes were allocated to an intensive and a conventional treatment arm and were followed for 6.5 years. The patients in the intensively treated group showed a reduction of albuminuria development of 34% and for macroalbuminuria of 56% (The Diabetes Control and Complications (DCCT) Research Group 1995). The follow-up study (EDIC), which evaluated patients after a median follow-up of 22 years after HbA1c values had converged to approximately 8%, showed a sustained risk reduction of 50% for deterioration of eGFR in the previously intensively treated group (DCCT/EDIC Research Group and de Boer 2011). It has been suggested that this observation, also entitled "metabolic memory", may be at least in part mediated by epigenetic mechanisms (Reddy et al. 2015). In type 2 diabetes, the UKPDS study investigated intensive glycemic control with an HbA1c of 7% versus 7.9% in the conventional treatment group (American Diabetes Association 2000). Intensive glucose control decreased albuminuria risk and had a 37% reduction in the renal endpoint of doubling of serum creatinine (Stratton et al. 2000). Similar to the findings in type 1 diabetes, after 10 years follow-up and with glycemic convergence, benefits persisted on renal and other microvascular outcomes in type 2 diabetes (Holman et al. 2008). These landmark trials show that a glycemic control with an HbA1c of approximately 7% is associated with a significantly reduced risk of DKD. In established CKD, optimization of glycemic control delays the further decline in renal function. Similarly, prospective randomized studies have shown that intensive glycemic control can also delay onset and progression of albuminuria. It should be noted that CKD can increase the half-life of some medications due to reduced renal excretion, which can lead to life-threatening hypoglycemic events. Thus, HbA1c targets need to be adjusted as part of a personalized medicine approach, taking each patient's individual risk factors and concomitant diseases into account.

Table 1 Renal outcomes in studies with SGLT-2 inhibitors or GLP-1 receptor agonists in type 2 diabetes

Study	SGLT2-inhibitors	Renal outcome	HR (95% CI)
CANVAS program (Neal et al. 2017)	Canagliflozin	>40% eGFR loss, ESRD, renal death	0.86 (0.75–0.97)
CREDENCE (Perkovic et al. 2019)	Canagliflozin	Doubling creatinine, ESRD, renal, or CV death	0.70 (0.59–0.82)
DECLARE-TIMI58 (Wiviott et al. 2018)	Dapagliflozin	>40% eGFR loss, ESRD, renal death	0.93 (0.84–1.03)
DAPA-CKD (Heerspink et al. 2021)	Dapagliflozin	>50% decline in eGFR, ESRD, renal or CV death	0.61 (0.51–0.72)
EMPAREG-OUTCOME (Wanner et al. 2016)	Empagliflozin	Doubling creatinine, ESRD, renal death	0.61 (0.53–0.70)
SCORED (Bhatt et al. 2021)	Sotagliflozin	>50% eGFR loss, ESRD	0.61 (0.51–0.72
VERTIS-CV (Cannon et al. 2020)	Ertugliflozin	Doubling creatinine, ESRD, renal death	0.97 (0.85–1.11)
	GLP-1 receptor agonists		
EXSCEL (Holman et al. 2017)	Exenatide	>40% eGFR loss, ESRD, renal death	0.85 (0.74–0.98)
LEADER (Marso et al. 2016a)	Liraglutide	Doubling creatinine, ESRD	0.78 (0.67–0.92)
REWIND (Gerstein et al. 2019)	Dulaglutide	>30% eGFR loss, ESRD, renal death	0.85 (0.77–0.93)

SGLT-2 Inhibitors

Initially, sodium glucose co-transporter 2 (SGLT-2) inhibitors were developed to reduce hyperglycemia, but recent studies have demonstrated additional renoprotective and cardiovascular effects (Table 1) (Neal et al. 2017; Perkovic et al. 2019; Wiviott et al. 2018; Heerspink et al. 2021; Wanner et al. 2016; Bhatt et al. 2021; Cannon et al. 2020). At least part of this effect is independent of glucose control and occurs down to a kidney function of 25 mL/min/1.73 m^2. SGLT-2 inhibitors block the glucose sodium co-transporter in the proximal tubule, thus leading to glucosuria. This is the first pharmacological class, which lowers glucose independent of the actions of insulin. The loss of glucose in the urine also leads to a negative caloric balance and a reduction in total body mass including epicardial fat. A myriad of studies have investigated the potential mechanisms underlying these effects (Fig. 3). These include effects related to the anti-hyperglycemic action but also glucose-independent effects such as a reduction in ROS, inflammation and fibrosis, altogether leading to renal and cardiac protection (Fig. 3) (Filippatos et al. 2019). However, there are some points to consider. The glucose lowering effect of SGLT-2 inhibitors occurs only down to an eGFR of <40 mL/min, but the blood pressure lowering effect is still evident if eGFR is <25 mL/min. The hemodynamic

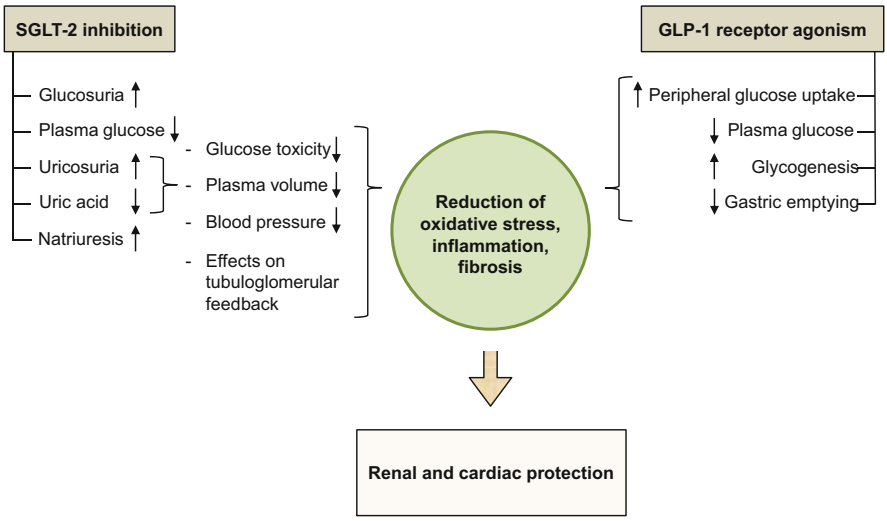

Fig. 3 SGLT-2 inhibition and GLP-1 receptor agonism reduce oxidative stress, inflammation, and fibrosis. Sodium-glucose-linked transporter-2 (SGLT-2) inhibitors increase glucosuria, uricosuria, and natriuresis, and decrease plasma glucose and uric acid levels. Furthermore, SGLT-2 inhibitors decrease glucose toxicity, plasma volume, and blood pressure, and alter the tubuloglomerular feedback. Agonism of glucagon-like peptide-1 (GLP-1) receptor increases peripheral glucose uptake and glycogenesis, while decreasing plasma glucose and gastric emptying. SGLT-2 inhibition and GLP-1 receptor agonism both result in the reduction of oxidative stress, inflammation, and fibrosis

effects often lead to an initial reduction in eGFR in the first week, which then stabilizes toward baseline measurements and provides long-term renoprotection. The cardiac protection may also occur due to inhibitory effects on sympathetic nerve activity (Gueguen et al. 2020). It has been postulated that SGLT-2 inhibitors modulate cardiac metabolism (glucose versus fatty acid consumption), which explains part of the beneficial cardiac effects. Furthermore, either neutral or favorable effects on plasma lipids have been observed. The most recent meta-analysis by McGuire et al. included six trials with this new therapeutic class and despite a degree of heterogeneity across the various compounds concerning cardiovascular outcomes, the authors found consistent protection against renal endpoints and hospitalization for heart failure (McGuire et al. 2021). It should be noted that most of these trials included a relatively healthy renal population with an eGFR of 60–90 mL/min, moderately increased albuminuria and only a very low number of patients with significantly reduced renal function. More recently, two trials have examined the renal outcomes in patients with a significantly reduced eGFR at baseline (CKD3) with macroalbuminuria. The CREDENCE study using canagliflozin included the primary endpoint of ESRD, sustained eGFR <15 mL/min/1.73 m^2 and doubling of serum creatinine or death (Perkovic et al. 2019). The study was terminated early due to clear benefits, with a 30% reduction of the primary endpoint, 32% lower relative risk for ESRD, and significantly lower heart failure and cardiovascular death. There

was no increase in amputations or fracture risk as previously reported with canagliflozin. The other landmark study, DAPA-CKD, investigated dapagliflozin (Perkovic et al. 2019). Again, the SGLT-2 inhibitor reduced the risk of sustained reduction in eGFR with reduced progression to ESRD or death from renal or cardiovascular causes and led to a 29% reduction in risk of death from heart failure and cardiovascular causes irrespective of diabetes. Dapagliflozin is also the only SGLT-2 inhibitor, which reduced all-cause mortality (31% relative risk reduction) (McMurray et al. 2021). There is now increasing evidence that the renoprotective and cardioprotective effects of SGLT-2 inhibitors also may occur in the nondiabetic context, with ongoing studies investigating this issue. In summary, a total of 5 trials demonstrated unequivocal benefits of SGLT-2 inhibitors in primary and secondary kidney disease prevention in diabetes, even in patients with low eGFR. This is now included in the *ADA Standards in Medical Care 2021*, which supports the use of SGLT-2 inhibitors in CKD or heart failure irrespective of glucose control or metformin use (American Diabetes Association 2021).

GLP-1 Receptor Agonists

Another new class of anti-diabetic agents are the glucagon-like peptide (GLP-1) receptor agonists. The mechanism of action includes increased peripheral glucose uptake as well as glycogen synthesis delaying gastric emptying and promoting satiety. This type of drug also confers multiple beneficial renal, cardiac, and metabolic effects (Fig. 3). In particular, due to the effects on gastric emptying and satiety, weight loss is observed. Furthermore, there are reductions in blood pressure and improved lipid profiles. More recent clinical trials have shown attenuation of CKD progression and reduction in cardiovascular mortality. The analysis of renal outcomes of cardiovascular outcome trials has recently shown a slowing of CKD progression (Table 1) (Holman et al. 2017; Marso et al. 2016a; Gerstein et al. 2019; Schnell et al. 2020). However, no trial has investigated renal outcomes as the primary endpoint so far. Post-hoc analyses and recent meta-analysis however suggest that GLP-1 receptor agonists reduce CKD progression. The FLOW study (effect of semaglutide versus placebo on the progression of renal impairment in subjects with type 2 diabetes and CKD) is ongoing and will further investigate this effect (Novo Nordisk A/S 2021). The AWARD-7 trial investigates dulaglutide versus insulin glargine in patients with type 2 diabetes and CKD (Tuttle et al. 2018a). These patients had already a significantly reduced eGFR at baseline. Compared to insulin, dulaglutide was associated with a lower decline in eGFR in two dose groups (-0.7 and -0.5 mL/min/1.73 m^2) compared to -3.3 mL/min/1.73 m^2 eGFR decline in the insulin-treated group. Fewer patients in the high-dose dulaglutide group reached the composite endpoint of ESRD or a >40% decline in eGFR. The LEADER trial (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) and the SUSTAIN-6 study (trial to evaluate cardiovascular and other long-term outcomes with semaglutide in subjects with type 2 diabetes) further supported the cardioprotection by GLP-1 receptor agonists (Marso et al. 2016a, b). The REWIND study was the first study to trial weekly injections with GLP-1 receptor agonists on cardiovascular outcomes (Eli Lilly and Company 2018).

All 3 trials reported significant reductions in the secondary composite renal endpoint of up to 30%. Similar results were observed in the EXSCEL study, which showed a 40% reduction in combined renal endpoints (Bethel et al. 2018). These studies suggest – although mainly in secondary outcome analyses – that GLP-1 receptor agonists confer cardio- and renoprotection. The effects of an exendin-based GLP-1 receptor agonist efpeglenatide was recently investigated on cardiovascular and renal outcomes. Patients had type 2 diabetes with a history of cardiovascular disease and a reduced eGFR with 25–59.9 mL/min/1.73 m^2. Efpeglenatide reduced major adverse cardiovascular events (MACE) by 7% versus 9.2% in the placebo group (Gerstein et al. 2021). The composite renal endpoint occurred in 13% in the efpeglenatide-treated group versus 18.4% in the placebo group (Gerstein et al. 2021). Given the renoprotective effects observed with both drug classes, SGLT-2 inhibitors and GLP-1 receptor agonists, it has been speculated that the combination of both would lead to even better outcomes. There is a potential for synergistic effects given that the mechanism of action only partially overlaps (Fig. 3). This is currently analyzed in the EMPA-SEMA trial (Steno Diabetes Center Copenhagen 2019).

Incretin Therapies

GLP-1 receptor agonists stimulate insulin secretion and suppress glucagon secretion during hyperglycemia. Glucose-dependent insulinotropic polypeptide (GIP) not only stimulates insulin secretion in hyperglycemia, but also stimulates glucagon release during hypoglycemia. It has been hypothesized that a dual agonist for both GLP-1 and GIP receptors could enhance glycemic control and minimize hypoglycemia in patients with type 2 diabetes. Such a dual agonist, tirzepatide, has recently been studied in 2 clinical trials (Rosenstock et al. 2021; Frias et al. 2021). Given subcutaneously once weekly compared to placebo, tirzepatide reduced body weight by 7–9 kg, reduced HbA1c by 2% points, and was not associated with severe hypoglycemia (Rosenstock et al. 2021). In another trial, three doses of tirzepatide were compared to the GLP-1 receptor agonist semaglutide and demonstrated better HbA1c reduction and superior weight loss with tirzepatide (Frias et al. 2021). Whether these improvements also lead to a better renal outcome needs to be analyzed in further studies.

3.2.2 Blood Pressure

Hypertension and diabetes are named the “two bad companions” and both accelerate the development and progression of diabetic nephropathy (Fig. 2). Hypertension per se is a leading cause of CKD. A reduction in systolic blood pressure by 10 mmHg resulted in a 17% risk reduction of mortality, an 11% reduction in cardiovascular events, and 17% reduction in the development of albuminuria (Sleight 2000). In patients with type 1 or type 2 diabetes who have already developed CKD, there is clear evidence that angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) delay the worsening of kidney function and lead to reduction in albuminuria. Albuminuria is both a risk marker and a therapeutic target to reduce the risk of further progression of renal failure. Thus, a reduction in albuminuria achieved by renin-angiotensin-aldosterone system (RAAS) blockade

will ultimately result in better renoprotection (de Zeeuw et al. 2004). However, there is a small group of diabetic patients with nephropathy who progress to ESRD without albuminuria (Macisaac and Jerums 2011). The blood pressure targets in people with diabetes are <140/90 mmHg and lower blood pressure targets are recommended in patients with macroalbuminuria or overt proteinuria (<130/80 mmHg), to reduce not only the risk for renal but also cardiovascular complications. ACE inhibitors and ARBs are the recommended first-line treatment for blood pressure control in patients with hypertension and diabetes, with a reduced kidney function <60 mL/min/1.73 m^2 and macroalbuminuria <300 mg/g (American Diabetes Association 2021).

Angiotensin-Converting Enzyme (ACE) Inhibitors

The initial studies by Lewis et al. demonstrated evidence for a renoprotective effect of ACE inhibitors in type 1 diabetes beyond their blood pressure reducing action (Lewis et al. 1993). A meta-analysis of non-hypertensive patients with type 1 diabetes and microalbuminuria showed that treatment with the ACE inhibitors decreased progression to macroalbuminuria and increased the chance of regression to normoalbuminuria (The ACE Inhibitors in Diabetic Nephropathy Trialist Group 2001).

Angiotensin II Receptor Blockers

The landmark studies, the RENAAL and IDNT studies, support the renoprotective effects of irbesartan and losartan in people with type 2 diabetes with a risk reduction of 25–28% and a 35% decline in proteinuria (Brenner et al. 2001; Lewis et al. 2001). Not only was a reduction in microalbuminuria by 38% observed, but 34% of patients with microalbuminuria regressed to normoalbuminuria (Parving et al. 2001). These renoprotective effects were independent of effects on blood pressure. The MARVAL study also showed a significant benefit of ARBs in patients with significant albuminuria as they reduced the decline in renal function by 4–5 mL/min/1.73 m^2 per year (Viberti et al. 2002). Given that the normal decline in eGFR in a healthy person is 0.8 mL/min/1.73 m^2 per year, there remains a significant residual risk. The combination of an ACE inhibitor and an ARB was investigated in the ONTARGET study, but the combination did not confer additional benefits, and on the contrary was associated with a faster decline in renal function and hyperkalemia (Mann et al. 2008).

Mineralocorticoid Receptor Antagonists

The mineralocorticoid receptor (MR) is the downstream receptor of the renin-angiotensin system (RAS) and is activated by aldosterone. Aldosterone has deleterious effects on sodium retention, blood pressure as well as cardiac and renal inflammation and fibrosis. In patients on long-term RAS blockade, there is evidence for increased aldosterone plasma levels, which is also called "aldosterone escape," resulting in ongoing inflammation and fibrosis despite long-term RAS blockade. In experimental settings, MR antagonism exerts anti-inflammatory and anti-fibrotic effects on the kidney, heart, and vasculature. In patients, MR antagonists have been shown to confer beneficial effects on heart failure but not many studies have

been performed in CKD. Furthermore, spironolactone and to a lesser degree eplerenone have been associated with side effects such as hyperkalemia and gynecomastia. A third generation MR antagonist, finerenone, has stronger affinity and potency compared to spironolactone and eplerenone. The largest study, FIDELIO-CKD is a phase 3 double-blinded randomized study in type 2 diabetic patients with moderate to severe CKD who were on maximally tolerated RAS blockade (Bakris et al. 2020). Finerenone was associated with an 18% relative risk reduction in the primary renal outcome and a 14% relative risk reduction outcome in the secondary cardiac outcome. The beneficial effects on cardiac outcomes were already seen in the first month, whereas the benefits on renal outcomes did not emerge until 12 months of treatment. Despite these advances in treatment options, a large proportion of patients still progresses to ESRD and the target of achieving a near-normal decline in renal function in diabetes has not been achieved. Thus, there remains a significant residual risk and unmet medical need. In contrast, targeting renin directly with aliskiren has not led to superior renoprotection (Parving et al. 2012). However, the endothelin receptor inhibitor avosentan showed promise in experimental animal studies but did not further reduce DKD progression in clinical trials (Mann et al. 2010). Furthermore, these non-specific endothelin receptor blockers were associated with significant side effects such as edema formation or heart failure (Mann et al. 2010). Newer, more selective endothelin receptor antagonists such as atrasentan still hold promise in selected subsets of patients who are not at risk for heart failure and fluid retention. In the recent SONAR study, patients with type 2 diabetes and albuminuria who responded well to initial dosing with a >30% in albuminuria and no substantial fluid retention demonstrated large reductions in albuminuria and reduced the risk of renal events. More recently, a post hoc analysis of the SONAR trial compared 6 weeks of SGLT-2 inhibitor combined with atrasentan versus atrasentan alone. The combination was superior on body weight reduction, fluid retention, and reduced albuminuria further (Heerspink et al. 2021).

3.3 Lipid Management

Elevated cholesterol and triglycerides are part of the diabetic milieu. Current guidelines suggest that patients with CKD, already at increased risk for cardiovascular mortality, should be treated to a low-density lipoprotein (LDL) level below 2.5 mmol/L (Colhoun et al. 2004). In a recent meta-analysis, statins have been shown to reduce albuminuria, specifically in type 2 diabetic nephropathy but the effect on long-term renal function was less clear (Shen et al. 2016). In addition, fibrates have been shown to lower albuminuria in diabetic nephropathy (Davis et al. 2011). A mild increase in serum creatinine has been observed with fibrates, which is not associated with worse renal outcome but related to creatinine excretion. Recently, monoclonal antibodies against proprotein convertase subtilisin kexin 9 (PCSK9) have been developed and these drugs lower LDL by >60%. The LDL lowering capacity is independent of baseline kidney function (Schmit et al. 2019). The effect of PCSK-9 inhibitors on kidney disease and in particular diabetic nephropathy needs to be evaluated in further studies.

4 Novel Experimental Drug Targets for DKD

Multiple signaling pathways are critically involved in the development and the progression of DKD and are potentially suitable to act as novel drug targets for DKD. Here, we provide an overview of studies that particularly aim to target signaling pathways involved in carbonyl and oxidative stress as well as inflammation. Several clinical trials using agents with anti-fibrotic properties have also been performed and many are still ongoing, however, any anti-fibrotic therapy needs to be assessed with respect to potential side effects on the immune system and on wound healing.

4.1 Carbonyl and Oxidative Stress

4.1.1 AGE-RAGE

The diabetic milieu is associated with increased formation of AGEs including early reactive and highly toxic intermediates such as methylglyoxal (MG). AGEs lead to cross-linking of proteins and DNA, thus altering their structure and function (Fig. 4).

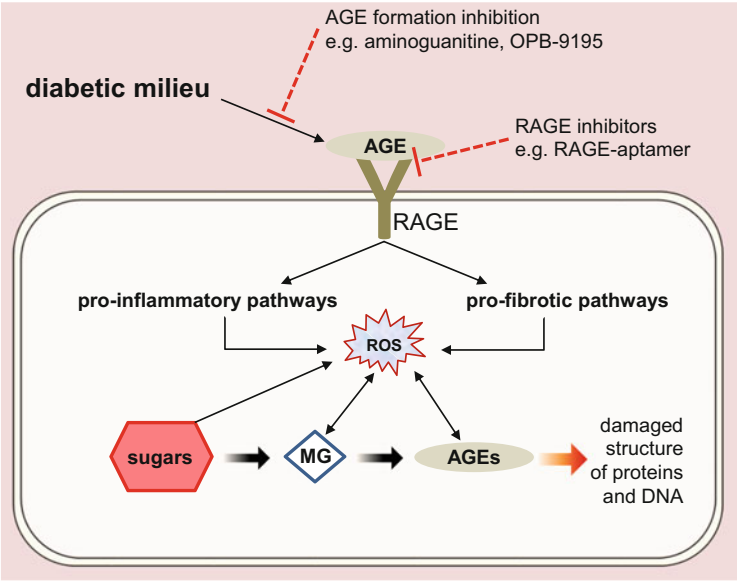

Fig. 4 Activation of the AGE-RAGE axis leads to oxidative stress, inflammation, fibrosis, and apoptosis. Increased advanced glycation end product (AGE) levels in the diabetic milieu bind to the receptor for AGEs (RAGE), resulting in the activation of pro-inflammatory and pro-fibrotic pathways, thereby elevating reactive oxygen species (ROS). Upon cytosolic hyperglycemia, sugars can be transformed to carbonyl intermediates, such as methylglyoxal (MG), and can be further modulated leading to AGEs that bind and cross-link to proteins and DNA, damaging the structure and function of proteins and DNA. The inhibition of AGE formation in the diabetic milieu as well as the blockade of RAGE are two therapeutic approaches aiming to prevent the harmful effects of the AGE–RAGE axis activation on the kidney

Furthermore, AGEs interact with their receptor, receptor for AGE (RAGE), which activates pro-inflammatory and pro-fibrotic signaling pathways. The AGE-RAGE axis plays a critical role in the pathogenesis of diabetic nephropathy as several preclinical studies have demonstrated that its activation induces oxidative stress, inflammation, and apoptosis associated with kidney function impairment (reviewed in Sanajou et al. 2018). Based on these facts, extensive research has been performed to find ways of blocking the AGE-RAGE axis, and two main approaches have evolved, the inhibition of AGE formation and the inhibition of RAGE.

AGE Formation Inhibitors

Several preclinical studies with AGE formation inhibitors, including aminoguanidine, OPB-9195, ALT-946, LR-90, salvianolic acid A or fluorofenidone (AKF-PD), have shown renoprotective effects in experimental animal models of diabetes such as streptozotocin (STZ)-induced diabetic rats, OLETF rats, or Zucker diabetic fatty rats (Nakamura et al. 1997; Wilkinson-Berka et al. 2002; Figarola et al. 2008; Qin et al. 2019; Hou et al. 2017). In all studies, a reduction in albuminuria was the most prominent effect. Aminoguanidine, OPB-9195, LR-90, salvianolic acid A, and AKF-PD furthermore led to reduced formation of glomerulosclerosis, while ALT-946 and LR-90 also demonstrated prevention of tubular fibrosis and damage (Nakamura et al. 1997; Wilkinson-Berka et al. 2002; Figarola et al. 2008; Qin et al. 2019; Hou et al. 2017). Furthermore, vitamins like pyridoxamine (derivative of vitamin B6), thiamine (vitamin B1), and benfotiamine (prodrug of vitamin B1) are known to prevent AGE formation and were also shown to lead to attenuated albuminuria when given to STZ-induced diabetic rats (Degenhardt et al. 2002; Babaei-Jadidi et al. 2003). Thiamine and benfotiamine treatments additionally reduced oxidative stress and inflammatory signaling pathways, such as the activation of protein kinase C (PKC), and benfotiamine alone also inhibited diabetes-induced glomerular hyperfiltration (Babaei-Jadidi et al. 2003). In a pilot trial with type 2 diabetes patients (PYR-210), pyridoxamine treatment showed a trend towards reduced creatinine levels, which is in line with observed decreased serum creatinine levels in diabetic rats treated with pyridoxamine (Degenhardt et al. 2002; Dwyer et al. 2015). Whether pyridoxamine has also positive effects on primary renal end points like albuminuria in humans as it does in animal models needs to be learned from larger future prospective studies with diabetic patients (such as PIONEER-CSG-17). Another approach was conducted in a study using alagebrium, which is an AGE inhibitor and putative AGE-protein crosslink breaker. Its administration to STZ-injected diabetic *ApoE* knockout mice showed similar effects in the kidney like in other studies with AGE formation inhibitors as it reduced albuminuria and glomerulosclerosis formation (Watson et al. 2012). This study moreover highlighted that alagebrium acts also RAGE-independent and that important RAGE-independent signaling pathways are activated by AGEs in the kidney and contribute to DKD, as treatment of *Rage/ApoE* double knockout mice with alagebrium still attenuated glomerulosclerosis, inflammation, and oxidative stress in the renal cortex (Watson et al. 2012).

RAGE Inhibitors

In contrast to the AGE formation inhibitors, few studies have been performed with RAGE inhibitors that prevent binding of AGEs to their receptor (Fig. 4). In an early study, an antibody against RAGE has been applied to STZ-induced diabetic mice, resulting in reduced albumin excretion and improved creatinine clearance when compared to control-treated diabetic mice (Jensen et al. 2006). Other RAGE inhibitors such as RAGE-aptamer or FPS-ZM1 showed similar effects in STZ-induced diabetic rats or AGE-loaded diabetic mice, respectively, and were characterized by reduced albuminuria as well as less inflammation and fibrosis in the kidney (Matsui et al. 2017). The RAGE-aptamer furthermore had preventive effects on oxidative stress generation because NADPH oxidase (NOX) activity was reduced in diabetic rats treated with this RAGE inhibitor (Matsui et al. 2017). More recently, it has been shown that transactivation of RAGE mediates the pro-inflammatory signaling of angiotensin II and mutant RAGE ligands have been able to attenuate this transactivation, opening new avenues for RAGE inhibition (Pickering et al. 2019). Overall, the majority of all conducted preclinical studies have provided evidence for the renoprotective effects of inhibitors that target the AGE-RAGE axis. Clinical trials including diabetic patients will need to be performed to investigate whether inhibitors of the AGE-RAGE axis have similar beneficial effects in humans and could be used as a new therapeutic approach for DKD.

4.1.2 NOX

The NOX family consists of seven isoforms, including NOX1 to NOX5 as well as dual oxidases 1 and 2 (DUOX1 and DUOX2). NOXs are transmembrane proteins and share the ability to transfer an electron across a membrane to reduce oxygen to superoxide or hydrogen peroxide. The biological meaning of NOX-derived ROS compromises microbial defense mechanisms, posttranslational protein processing, cellular signaling, regulation of gene expression, and cell differentiation (Bedard and Krause 2007). NOX activity is predominantly regulated on transcriptional and translational level and has very low or no constitutive activity. Under pathological conditions however, including hypertension and diabetes, enzyme activation can increase and overcome the antioxidative capacity of the cell, causing oxidative stress and hence tissue damage. The human kidney expresses NOX1, NOX2, NOX4, and NOX5, which are found in glomerular, endothelial and mesangial cells, podocytes, proximal and distal tubular cells as well as in interstitial fibroblasts (Jha et al. 2016a; Gorin et al. 2005). Yet, not all NOXs contribute equally to DKD. Studies with deletion of the *Nox1* gene in STZ-induced diabetic *ApoE* knockout mice have not led to improved albuminuria or reduced mesangial expansion, however, were associated with reduced atherosclerosis (Jha et al. 2014). Similarly, *Nox2* deletion in a diabetic mouse model has revealed comparable albuminuria and mesangial expansion levels between diabetic and nondiabetic mice (You et al. 2013), suggesting that the isoforms NOX1 and NOX2 do not critically contribute to DKD. In contrast, several preclinical studies have demonstrated that particularly NOX4 and NOX5 are involved in the development and progression of DKD (Jha et al. 2017).

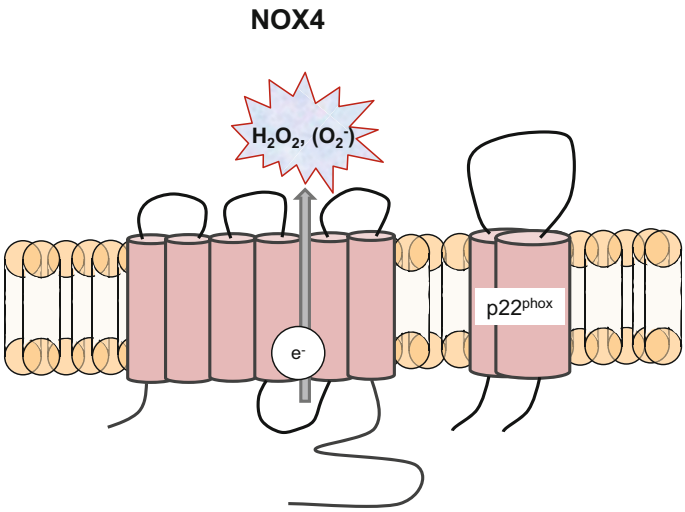

Fig. 5 Simplified model of the NADPH oxidase 4 (NOX4) complex. NOX4 localizes in membranes with six transmembrane domains and requires $p22^{phox}$ for activation. The complex transfers an electron from NADPH across the membrane to produce ROS, such as hydrogen peroxide (H_2O_2) and to a lesser extent superoxide (O_2^-)

NOX4 as a New Target in DKD

Due to its abundance in renal tissue, NOX4 was originally termed Renox (renal oxidase) (Fig. 5) (Geiszt et al. 2000). NOX4 can be found in glomerular endothelial cells, mesangial cells, podocytes, and proximal tubular epithelial cells of the kidney, where it predominantly produces hydrogen peroxide (Fig. 5) (Jha et al. 2016a; Rajaram et al. 2019; Martyn et al. 2006). Different to NOX1 or NOX2 that are located in the plasma membrane and produce extracellular superoxide, NOX4 mainly localizes in intracellular membranes and compartments, such as endoplasmic reticulum, nucleus, mitochondria, and the cytoskeleton, which might explain the challenge of measuring superoxide, hypothesizing that the products of NOX4 are converted to hydrogen peroxide, being able to pass membranes and becoming measurable (Bedard and Krause 2007; Block et al. 2009; Chen et al. 2008; Takac et al. 2011). It has been shown that the E-loop of NOX4 is 28 amino acids longer than that of other NOXs, and minor alterations in that loop can switch superoxide production mode to hydrogen peroxide production mode (Takac et al. 2011). Several studies show upregulation of *Nox4* expression and increased ROS levels in podocytes, mesangial cells, and proximal epithelial tubular cells upon high-glucose conditions (Gorin et al. 2005; Jha et al. 2014; Sedeek et al. 2010). Global *Nox4*-deleted STZ-induced diabetic *ApoE* knockout mice exhibited renoprotective effects, such as reduced albuminuria, attenuated glomerular macrophage infiltration as well as decreased levels of monocyte chemoattractant protein-1 (MCP-1) and nuclear factor-kappa B (NF-κB) (Jha et al. 2014). Consequently, in diabetic mice, NOX4 has been identified as the main source of ROS (Jha et al. 2014). Further studies engaged

in the role of NOX4 in STZ-induced diabetic rats with 2 weeks of treatment with phosphorothioated antisense (AS) nucleotides against *Nox4 (*Gorin et al. 2005*)*. The findings have shown not only downregulated NOX4 levels in the renal cortex in AS-treated diabetic rats but also a reduction of fibronectin as well as whole kidney and glomerular hypertrophy (Gorin et al. 2005). In vitro studies with mesangial cells also showed reduced levels of NOX4 and fibronectin upon AS treatment (Gorin et al. 2005). The beneficial effects of global NOX4 absence have similarly been replicated in podocyte-specific *Nox4*-deleted STZ-induced diabetic mice (Jha et al. 2016b). The absence of NOX4-derived ROS in podocytes led to renoprotective effects including reduction of fibrotic markers such as collagen IV and fibronectin as well as inflammatory markers such as MCP-1 and PKC-α. Additionally, a reduction of mesangial expansion, glomerulosclerosis, GBM thickness, albuminuria, expression of VEGF-A, and restoration of nephrin levels could be measured, which emphasizes the important contribution of NOX4 activity to DKD pathogenesis (Jha et al. 2016b). Interestingly, NOX4 deletion from proximal tubules did not confer renoprotection in diabetes despite significant alterations in mitochondrial ROS formation (Thallas-Bonke et al. 2021), suggesting different pathogenic mechanisms for proximal tubular changes as opposed to glomerular changes.

NOX5 as a New Target in Human DKD

Another important NOX isoform in the context of DKD is NOX5 (Fig. 6). Although NOX5 shares significant homology to NOX1 and NOX2, NOX5 is structurally distinct and expressed in five splice variants (NOX5α-δ and NOX5S) with NOX5α and NOX5β responsible for superoxide production (Fulton 2009; Serrander et al. 2007). The human NOX5 isoform is not endogenously expressed in rodents, and therefore its experimental investigation has been challenging. STZ-induced diabetic transgenic mice expressing human *Nox5* in renal mesangial or endothelial

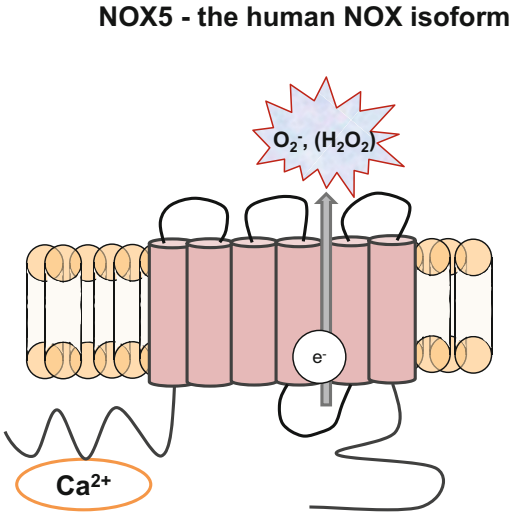

Fig. 6 Simplified model of the NADPH oxidase 5 (NOX5) complex. NOX5 localizes in membranes with six transmembrane domains. Calcium ions (Ca^{2+}) are required to activate NOX5 by binding to the N-terminal side. The complex transfers an electron from NADPH across the membrane to produce ROS, such as superoxide (O_2^-) and potentially hydrogen peroxide (H_2O_2)

cells have shown to accelerate glomerulosclerosis, mesangial expansion, and ECM accumulation by collagen IV and fibronectin overproduction (Jha et al. 2017). In accordance with human kidney biopsies, where NOX5 was found to be increased in patients with diabetes, silencing of *NOX5* in in vitro studies using human mesangial cells showed a reduction of high-glucose- and TGF-β-induced ROS (Jha et al. 2017; Holterman et al. 2014). Furthermore, podocyte-specific *Nox5* expression in transgenic mice led to an early onset of albuminuria even under nondiabetic conditions and resulted in even worse effects after diabetes was induced by STZ injections (Holterman et al. 2014). Further studies using animal models expressing NOX5 such as the rabbit, which expresses all NOX isoforms including NOX5 as in humans, are required (Serrander et al. 2007).

NOX Inhibitors

Based on the relevance of NOX in DKD, different NOX inhibitors have been developed in the past to be used as potential therapeutics in DKD (reviewed in Urner et al. 2020), and more specific inhibitors that target certain NOX isoforms, such as NOX5, are being currently developed. In line with preclinical genetic studies using knockout mouse models, the pharmacological inhibition of NOX by inhibitors has generally confirmed renoprotective effects when used in diabetic mice or rats. The NOX1/NOX4 inhibitor GKT137831 has already been widely tested in different diabetic mouse models. In STZ-injected diabetic *ApoE* knockout mice, treatment with GKT137831 led to attenuation of albuminuria, glomerulosclerosis as well as inflammation and oxidative stress in the kidney (Jha et al. 2014; Gray et al. 2017). In line with this, complementary in vitro experiments using human podocytes showed that GKT137831 treatment reduces ROS generation and the expression of fibrotic markers upon diabetic conditions (Jha et al. 2014). Importantly, the renoprotective effects of GKT137831 are very likely due to its inhibition of certainly NOX4, as genetic studies showed that deletion of *Nox4*, but not *Nox1*, leads to significant improvements of functional and structural renal characteristics in diabetic mice (Jha et al. 2014). Similar effects of GKT137831 were observed in OVE26 and Akita mice (Gorin et al. 2015; You et al. 2016), supporting its renoprotective properties in type 1 diabetes models. In line with this, another NOX1/NOX4 inhibitor, GKT136901, efficiently reduced albuminuria, glomerulosclerosis, and tubular damage when given to *db/db* mice (Sedeek et al. 2013), demonstrating that inhibition of NOX4 might be a promising target in both type 1 and type 2 diabetes. Based on the promising preclinical data, a short-term phase II clinical trial has been performed in which type 2 diabetes patients with DKD have been treated with the inhibitor GKT137831 for 12 weeks (NCT02010242). However, the inhibitor failed to meet the primary endpoint of reducing albuminuria, although several secondary efficacy endpoints were met. One reason why the inhibitor was not as efficient in patients could be that in this study only patients with very advanced kidney disease who also were on maximum blockade of RAAS were treated with GKT137831 for a short time of 12 weeks. In addition, particularly GKT137831 showed most promising effects in preclinical studies when applied to type 1 diabetes models, while DKD in type 2 diabetes patients is much more heterogenous. Another long-term phase II clinical

trial is currently running with type 1 diabetes patients who have a persistent albuminuria and a preserved renal function (ACTRN12617001187336). These patients are receiving GKT137831 treatment for a total of 48 weeks on top of stable RAAS blockade in higher doses. The primary endpoint of the study is the change in the albumin-creatinine-ratio, while the secondary endpoints include the eGFR and changes in inflammatory and fibrotic markers. This study will reveal whether inhibition of NOX1/NOX4 by GKT137831 has also beneficial effects on DKD in type 1 diabetes patients (Reutens et al. 2020). Other NOX inhibitors that are available are not specific to a certain NOX isoform. For example, apocynin downregulates intracellular ROS levels and inhibits the downstream signaling of all NOX isoforms (Altenhofer et al. 2015). However, it should be noted that its beneficial therapeutic effects in preclinical studies may not only be due to direct NOX inhibition (Heumuller et al. 2008). In STZ-induced diabetic Sprague-Dawley rats, treatment of apocynin attenuated diabetes-induced albuminuria and glomerulosclerosis (Thallas-Bonke et al. 2008). It particularly reduced the expression of fibronectin and collagen IV, and furthermore led to reduced PKC-α signaling, underlining anti-fibrotic as well as anti-inflammatory properties of apocynin in the diabetic kidney (Thallas-Bonke et al. 2008). Similar observations have been made in other studies with STZ-induced diabetic rats, which received apocynin and showed reduced ECM protein expression and thus less mesangial expansion, glomerulosclerosis, and interstitial fibrosis (Asaba et al. 2005; Xin et al. 2018). The novel pan-NOX inhibitor APX-115 also blocks all NOX isoforms, and several studies indicate that it has beneficial effects when used to treat DKD in preclinical animal models. Treatment of STZ-induced diabetic mice led to reduction in urinary albumin excretion, creatinine clearance, glomerular hypertrophy, glomerulosclerosis, tubular injury, podocyte injury as well as inflammation and oxidative stress, and furthermore improved mitochondrial and peroxisomal function in the kidney (Kwon et al. 2017). In *db/db* mice, similar observations have been made in terms of decreased albuminuria and preserved creatinine levels as well as reduced oxidative stress in diabetic mice treated with APX-115 in comparison with control-treated diabetic mice (Cha et al. 2017). In a recent study, it has been furthermore shown that APX-115 has renoprotective effects also through NOX5 inhibition. As NOX5 is not endogenously expressed in rodents, *Nox5* transgenic (podocyte-specific) high-fat diet fed diabetic mice were treated with APX-115, which blocked the diabetes-induced upregulation of NOX5 expression and led to reduced urinary albumin-creatinine-levels, renal fibrotic events, and inflammation (Lee et al. 2020). In summary, different inhibitors for NOX signaling have been widely studied in the context of DKD in animal models and turned out to have beneficial effects. Follow-up clinical trials with those inhibitors would reveal whether similar results can be observed in diabetes patients. Moreover, given the proven evidence from preclinical studies on the critical contribution of NOX5 to the development and progression of DKD, more specific inhibitors that target NOX5 should be developed and tested for a potential therapeutic use in DKD patients.

4.1.3 Xanthine Oxidoreductase

Xanthine oxidoreductases (XORs) have multiple activities, including xanthine dehydrogenase, xanthine oxidase, NADH oxidase, or nitrite reductase activity, responsible for uric acid formation as well as ROS and NO generation (reviewed in Bortolotti et al. 2021). Increased serum uric acid levels, driven by increased XOR activity, are considered as a risk factor for the development as well as progression of DKD in both type 1 and type 2 diabetes patients (Hovind et al. 2009; Zoppini et al. 2012). Furthermore, XOR activity likely is associated with ESRD, as particularly patients with CKD who require dialysis show upregulated circulating XORs (Boban et al. 2014). Several preclinical and clinical studies have investigated the effects of XOR inhibitors on DKD. Allopurinol is a specific inhibitor of xanthine oxidase and decreases serum uric acid formation (reviewed in Pacher et al. 2006). Treatment of diabetic db/db mice with allopurinol resulted in decreased uric acid levels, reduced albuminuria as well as ameliorated tubulointerstitial injury, but did not lead to changes in mesangial expansion or glomerulosclerosis (Kosugi et al. 2009). The observed beneficial renal effects are likely due to the reduction of serum uric acid by allopurinol instead of an inhibition of ROS generation by xanthine oxidase since the treatment did not reduce oxidative stress in the diabetic kidney (Kosugi et al. 2009). In two independent studies performed in diabetic Zucker obese rats or in STZ-induced diabetic Sprague-Dawley rats, treatment with another non-purine inhibitor of xanthine oxidase, febuxostat, also resulted in reduced albuminuria, respectively (Komers et al. 2016; Lee et al. 2014). Notably, both studies observed that febuxostat treatment also decreased the expression of the oxidative stress marker nitrotyrosine in the kidney of diabetic rats, suggesting that its mechanism of action in the kidney may differ from allopurinol (Komers et al. 2016; Lee et al. 2014). In patients with CKD, treatment with allopurinol reduced serum uric acid levels and delayed the progression of renal disease as shown by two small early clinical trials (Siu et al. 2006; Goicoechea et al. 2010). However, recently published high-profile long-term clinical trials in patients with CKD and a high risk of progression as in the CKD-FIX trial, or in type 1 diabetes patients with early-to-moderate DKD as in the PERL trial, did not show any benefits of serum uric acid reduction by allopurinol on renal outcomes (Doria et al. 2020; Badve et al. 2020). When patients with stage 3 and 4 CKD were treated with febuxostat for a short period of 6 months, their kidney function improved as defined by a delay of the eGFR decline (Sircar et al. 2015). However, when patients with stage 3 CKD underwent a long-term treatment with febuxostat for 18 months, no beneficial effects on the kidney function could be observed (Kimura et al. 2018). Furthermore, in patients with type 2 diabetes and DKD, febuxostat treatment for 6 months could not improve albuminuria or eGFR (Beddhu et al. 2016). However, the majority of studies suggest that XOR inhibitors represent a useful tool to reduce disease progression in CKD (Pisano et al. 2017).

4.1.4 Mitochondrial ROS

The kidney is one of the most metabolically active organs, and therefore also contains a high mitochondria content. Although mitochondria are well known as the powerhouses of the cell, they also provide an important site for ROS production

in the form of superoxide. During oxidative phosphorylation, NADH and $FADH_2$ donate their electrons into the mitochondrial inner membrane. The embedded protein complexes NADH ubiquinone oxidoreductase (complex I) and succinate dehydrogenase (complex II) accept the electrons and transfer them to ubiquinol. Coenzyme Q:cytochrome c reductase (complex III) receives the electrons from ubiquinol and uses them to reduce the electron carrier cytochrome c. The electrons are unloaded at cytochrome c oxidase (complex IV), the site where molecular oxygen is reduced to water. During this process, molecular oxygen can also be reduced with a single electron and produce superoxide anion radicals, which occurs predominantly at complex I, II, and III (Nolfi-Donegan et al. 2020). The superoxide molecules generated by the electron transport chain (ETC) are released to both the matrix and the intermembrane space (Galvan et al. 2017a). However, the ETC is not the exclusive mitochondrial source of superoxide as the mitochondrial matrix also accommodates other superoxide-producing enzymes, such as 2-oxoglutarate dehydrogenase, pyruvate dehydrogenase, and glycerol 3-phosphate dehydrogenase (Murphy 2009; Brand 2010; Coughlan and Sharma 2016). Either by spontaneous dismutation or by enzymatic activity of superoxide dismutases (SODs), superoxide is converted into hydrogen peroxide, which in contrast to superoxide is membrane-permeable. Hyperglycemia susceptible cells can experience excessive glucose influx, activating not only certain pathways in order to deal with the increased amounts of glucose, but also saturating glycolysis with ensued citric acid cycle, which produces large amounts of NADH and $FADH_2$ that feed the ETC and accelerate the superoxide leakage (Coughlan and Sharma 2016). The view of elevated glucose levels consequently leading to the overproduction of superoxide by mitochondria is still controversial and has been reviewed in Coughlan and Sharma (2016). Indeed, elevated superoxide levels have been reported in vitro in renal cells exposed to high glucose, as well as in vivo in diabetic kidney studies (Coughlan and Sharma 2016). Additionally, studies using diabetic *db/db* mice demonstrated increased mitochondrial matrix ROS (Galvan et al. 2017b). In contrast, another study involving STZ-induced diabetic mice reported a reduction of superoxide in the diabetic kidney (Dugan et al. 2013). Notably, experimental measurement of superoxide is a challenge due to its short half-life of seconds before dismutating to hydrogen peroxide (Dugan et al. 2013). The question arises whether the controversy is due to in fact low mitochondrial superoxide production or due to inadequate measurement methods. Furthermore, mitochondrial and cytoplasmic ROS may be formed in a time-dependent manner. Therefore, further studies are needed to define the dynamic changes of mitochondrial ROS in DKD. Nevertheless, there is solid evidence for the critical involvement of mitochondrial (dys)function in DKD (reviewed in Mise et al. 2020). A dysregulation of complex I, III, or IV has been previously detected in the diabetic kidney of both diabetic animal models and diabetic patients (reviewed in Mise et al. 2020). In this context, also changes in ATP production have been described for different stages of DKD (reviewed in Mise et al. 2020). Indeed, in a study with STZ-induced diabetic rats, changes in mitochondrial function and ATP synthesis appeared even before first renal tissue changes could be observed (Coughlan et al. 2016), suggesting mitochondrial dysfunction as an early

indicator for DKD. Therefore, restoring or improving mitochondrial function as well as balancing mitochondrial ROS production would likely be beneficial for slowing the pathological disease progression in the kidney. For example, the mitochondria-targeted peptide SS-31 localizes in the inner mitochondrial membrane, interacts with and stabilizes the mitochondrial phospholipid cardiolipin, and furthermore leads to reduced mitochondrial ROS levels (Mise et al. 2020). SS-31 has been shown to reduce proteinuria, glomerular hypertrophy as well as the expression of oxidative stress and fibrotic markers in the kidney of STZ-induced diabetic CD-1 mice (Hou et al. 2016), suggesting that improving mitochondrial function has renoprotective effects. Activation of the protein Sirtuin-1 (SIRT1), which is involved in regulating mitochondrial biogenesis and energetic homeostasis, by a newly developed selective SIRT1 agonist resulted also in marked reduction in albuminuria as well as glomerular injury associated with reduced podocyte loss and glomerular oxidative stress (Hong et al. 2018). Similar effects have been observed with coenzyme Q10 that plays an important role as an electron carrier during mitochondrial respiration as well as a mitochondrial endogenous ROS scavenger. Treatment of diabetic *db/db* mice with ubiquinone, the oxidized form of coenzyme Q10, resulted in reduced albuminuria and tubulointerstitial fibrosis, correlating with a normalized mitochondrial ATP synthesis and ameliorated mitochondrial hydrogen peroxide production (Sourris et al. 2012). A meta-analysis of 8 publications on clinical trials with coenzyme Q10 supplementation in patients also suggests that coenzyme Q10 can ameliorate DKD, at least when combined with other standardized therapy, such as blood pressure controlling agents (Zhang et al. 2019a). Another form of coenzyme Q10 is MitoQ that has been developed to enrich its uptake into mitochondria. In diabetic *db/db* mice, MitoQ treatment for 12 weeks also reduced albuminuria and improved the eGFR (Ward et al. 2017). Whether improvement of mitochondrial function by MitoQ acts also renoprotective in humans will hopefully be clarified by a phase 4 clinical trial with patients with CKD that is currently running (NCT02364648).

4.1.5 Antioxidants

The effects of supplemented direct antioxidants, such as vitamin A, vitamin C, vitamin E, selenium, zinc, magnesium, methionine, beta-carotene, glutathione, or ubiquinone alone or in combination, on DKD have been investigated in very heterogenous studies with different outcomes on the renal function (see (Bolignano et al. 2017)). For example, in one of the longest clinical trials of 4.5 years (HOPE), supplementation of vitamin E alone did not show any effects on the development of overt nephropathy in patients with diabetes and cardiovascular disease (Lonn et al. 2002). However, a recent meta-analysis of 15 publications revealed that antioxidant treatment significantly decreases albuminuria in patients with either type 1 or type 2 diabetes, and therefore may be preventive for early renal damage (Bolignano et al. 2017). Particularly the supplementation with vitamin C in combination with vitamin E as well as zinc or curcumin (turmeric) achieved a reduction in albuminuria in some studies including in diabetic patients (summarized in Bolignano et al. 2017).

Nrf2-KEAP

Another approach are indirect antioxidants, such as nuclear factor-2 erythroid related factor 2 (Nrf2), which is regulated by Kelch-like ECH associated protein 1 (KEAP). Nrf2 signaling activates the transcription of genes encoding antioxidant and detoxifying molecules and is an important regulator of ROS production by both NOXs and mitochondria (Kovac et al. 2015). In STZ-induced diabetic *Nrf2* knock-out mice, hyperglycemia-induced oxidative stress and renal damage is accelerated compared to diabetic wild-type controls, suggesting that Nrf2 is required for renal protection in DKD (Jiang et al. 2010). Treatment of STZ-induced diabetic mice with Nrf2-activating compounds, such as sulforaphane or cinnamic aldehyde, led to a reduction of oxidative stress and mesangial matrix expansion in renal cells and resulted in attenuated albuminuria (Zheng et al. 2011). Several other activators of Nrf2 signaling with different target mechanisms have since then been used in preclinical animal models of DKD to investigate their beneficial effects on the kidney and are summarized elsewhere (reviewed in Adelusi et al. 2020). The Nrf2 activator bardoxolone methyl has also been tested in phase 2 clinical trials with type 2 diabetes patients with CKD. While two studies with a duration of 8 weeks or 52 weeks of treatment (BEAM) showed that bardoxolone methyl improved the eGFR in diabetic patients with stage 3–4 CKD (Pergola et al. 2011a, b), a later study with type 2 diabetes patients and stage 4 CKD (BEACON) resulted in an increased rate of cardiovascular events in patients treated with bardoxolone methyl and had to be terminated after 9 months (de Zeeuw et al. 2013). Despite these safety concerns, another phase 2 clinical trial with bardoxolone methyl in type 2 diabetes patients and stage 3–4 CKD has been initiated afterwards, in which patients with a high risk for cardiovascular events have been excluded (TSUBAKI). The recently published outcome of this study revealed that treatment with bardoxolone methyl for 16 weeks resulted in significantly increased eGFR and did not lead to heart failure or death (Nangaku et al. 2020). Whether the clinical renal benefits of bardoxolone methyl treatment outweigh potential side effects in diabetic patients needs to be further evaluated in the future.

4.2 Inflammation

4.2.1 Inflammasome

The existence of a chronic low-grade renal inflammation is apparent in diabetes with its role in promoting the progression of DKD. Elements of the diabetic milieu including high-glucose and glyco/lipoxidation products stimulate immune cells and accelerate the production of pro-inflammatory cytokines, which in turn induce resident renal cells to produce a spectrum of chemokines (Fig. 7). The cytokines, particularly various interleukins such as interleukin-1β (IL-1β) and interleukin-18 (IL-18), tumor necrosis factor-α (TNF-α), and interferon-γ (INF-γ), direct the secretion of chemokines including MCP-1, intercellular adhesion molecule (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) which result into transmigration and infiltration of immune cells establishing an inflammatory cycle in the kidney

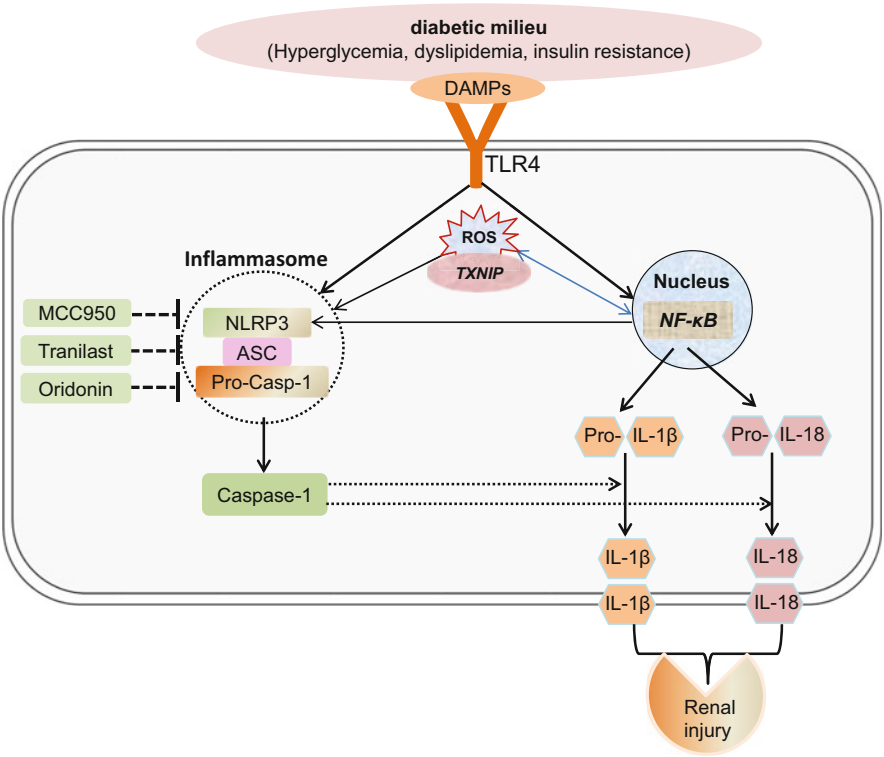

Fig. 7 Activation and inhibition of NLRP3 inflammasome in DKD. Diabetes-related DAMPs activate the NF-kB signaling pathway and the NLRP3 inflammasome via stimulation of TLR4, intracellular ROS, and the ROS-sensitive factor TXNIP. Activation of NF-kB leads to production of pro-IL-1β and pro-IL-18 cytokines. In parallel, activation of the NLRP3 inflammasome stimulates caspase-1 to form mature IL-1β and pro-IL-18 cytokines. The release of IL-1β and IL-18 cytokines creates an inflammatory environment resulting in renal cell injury in diabetes. Inhibition of NLRP3 inflammasome components (NLRP3, ASC, and pro-caspase-1) by MCC950, Tranilast, and Oridonin has been suggested to confer renoprotection in diabetes

(Lim and Tesch 2012). Moreover, the secretion of IL-1β and IL-18 in the kidney promotes the expression of adhesion molecules like ICAM-1, VCAM-1, and VEGF-A leading to systemic endothelial dysfunction, a process that promotes leukocyte adhesion and vascular leakage in the kidney (Chow et al. 2005). Among the complex network of pro-inflammatory cytokines shown to be implicated in DKD, IL-1β has been identified as a key player in initiating and promoting inflammation-induced organ dysfunction (Everett et al. 2018). A class of multi-protein complexes representing the critical components of innate immunity, known as inflammasomes, has been identified as a potential mediator in coordinating the inflammatory response in chronic diseases including DKD (Fig. 7). In response to the diabetic milieu, the assembly and thereby activation of inflammasomes direct the activation of caspase-1

leading to the secretion of the pro-inflammatory cytokines IL-1β and IL-18, subsequently resulting in pyroptosis, an inflammatory form of programmed cell death (Broz and Dixit 2016). A typical inflammasome consists of an upstream sensor protein of the NOD-like receptor (NLR) family, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), and the downstream effector cysteine protease pro-caspase-1. Activation of inflammasomes by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs, danger signal) direct the effector molecule caspase-1 to induce the conversion of pro-IL-1β and pro-IL-18 to their mature bioactive forms IL-1β and IL-18 causing the cell pyroptosis (Fig. 7) (Lamkanfi and Dixit 2014). Depending on their differences in structure and activation, several NLR proteins have been identified (NOD-, LRR-, and pyrin domain-containing 3 (NLRP3) and have been reported to play a role in regulating chronic renal inflammation and progression of DKD (Ram et al. 2020).

Activation of NLRP3 Inflammasome

Essential components of the NLRP3 inflammasome are found to be expressed in both resident renal cells (podocytes, mesangial cells, endothelial cells, and tubular epithelial cells) and immune cells, primarily macrophages and dendritic cells (Hutton et al. 2016). In contrast, the major cellular sources of IL-1β and IL-18 are the immune cells, particularly monocytes and macrophages, and certain renal cell populations, mainly tubular epithelial cells. Moreover, chronic injury to the kidney exposes renal cells to numerous inflammasome activators, such as defective autophagy, uric acid, extracellular ATP levels, and matrix degradation products shown to be implicated in DKD (Menini et al. 2020; Solini et al. 2013). In diabetic patients, higher levels of circulating and urinary IL-1β and IL-18 are linked with the NLRP3 inflammasome (Navarro-Gonzalez and Mora-Fernandez 2008). Moreover, inhibition of the NLRP3 inflammasome pathway appears to reduce inflammation and fibrosis in DKD (Yang et al. 2014). In experimental models of diabetes, activation of the NLRP3 inflammasome has been shown to promote renal injury by increased secretion of circulatory and renal IL-1β and IL-18 with enhanced albuminuria and renal fibrosis. Indeed, all these changes were significantly attenuated by genetic deletion and pharmacological inhibition of NLRP3 or caspase-1 in diabetic mice, suggesting the role for NLRP3 activation in DKD. Furthermore, not only in diabetes but also in models of acute kidney injury such as ureteral occlusion or renal ischemia/reperfusion-induced injury in mice, deficiency of NLRP3 demonstrated protection against renal tubular damage and interstitial inflammation (Vilaysane et al. 2010). In addition, activation of NLRP3 by high glucose in renal tubular cells (HK-2) was found to be associated with cleavage of caspase-1 and IL-1β leading to the release of the pro-inflammatory cytokines IL-1β and IL-18 (Garibotto et al. 2017).

TLR4-NFκB-ROS Signaling Pathway

The excessive activation of pro-inflammatory cytokines can promote the progression of renal fibrosis. It is evident from experimental studies that in DKD increased

expression of toll-like receptor 4 (TLR4) is associated with enhanced activation of NFκB and NLRP3 inflammasome leading to the release of pro-inflammatory cytokines (IL-1β and IL-18) and chemokines (MCP-1) in the kidney causing progression of DKD (Lin et al. 2012). Indeed, deficiency of *Tlr4* in mice showed attenuation of diabetes-induced increased albuminuria, renal fibrosis and interstitial macrophage infiltration via downregulation of renal NF-κB activation and MCP-1 expression (Lin et al. 2012). On the other hand, inhibition of TLR4/NF-κB signaling led to decreased expression of components of NLRP3 with less secretion of IL-1β and IL-18 in DKD, suggesting a role for the TLR4/NF-κB-inflammasome signaling pathway in DKD. The co-existence and interplay between inflammation and oxidative stress plays a critical role in creating an overwhelming inflammatory environment leading to progression of renal tissue damage and fibrosis in diabetes (Jha et al. 2018). Under diabetic conditions, enhanced renal ROS formation has been shown to modulate the activation of the NLRP3 inflammasome and subsequent kidney injury (Han et al. 2018). In addition, renal ROS in diabetes also regulates the function of the transcription factor NF-κB, which is responsible for the production of the immature pro-inflammatory cytokines pro-IL-1β and pro-IL-18, which are then cleaved by inflammasome complexes to their bioactive form causing kidney damage (Ram et al. 2020; Petrilli et al. 2007). An association between the ROS-sensitive factor thioredoxin-interacting protein (TXNIP) and NLRP3 inflammasome activation in response to high glucose has been demonstrated in renal cells with enhanced ROS production and IL-1β (Xiao et al. 2016). In addition, excessive production of mitochondrial ROS can activate the NLRP3 inflammasome through the TRX/TXNIP pathway. Moreover, inhibition of NF-κB in diabetic rats showed decreased levels of IL-1β and TNF-α in association with downregulation of TXNIP and NLRP3 in the kidney (Samra et al. 2016). Taken together, the ROS-TXNIP-NLRP3-NF-κB signaling pathway appears to be critical in promoting renal inflammation and subsequent kidney injury in diabetes (Fig. 7).

4.2.2 Novel Inflammation Inhibitors

MCC950, a diarylsulfonylurea-containing compound, is one of the most potent and highly specific small-molecule inhibitors of NLRP3 inflammasome. MCC950 prevents the NLRP3 conformational change and subsequent inflammasome formation by abrogating ASC oligomerization and thereby blocking the processing of IL-1β by caspase-1 (Coll et al. 2019). Anti-inflammatory and renoprotective effects of MCC950 have been demonstrated in various preclinical disease models including salt-sensitive hypertension, crystal-induced nephropathy (Krishnan et al. 2019) as well as in DKD (Zhang et al. 2019b). Indeed, MCC950 administration in *db/db* mice provided renoprotection as evidenced from attenuated renal fibrosis through suppression of pro-fibrotic markers such as TGF-β1, fibronectin, α-SMA, and collagen I as well as reduced thickening of the GBM, podocyte injury and albuminuria (Zhang et al. 2019b). The study reported that the renoprotective effects of MCC950 were achieved through downregulation of active caspase-1 and IL-1β by inhibiting the NLRP3/Caspase-1/IL-1β pathway in diabetic kidneys (Zhang et al. 2019b). More recently, anti-atherosclerotic effects of MCC950 have also been reported by our

group in a type 1 diabetic mouse model (Sharma et al. 2021). However, using the same dose and animal model, there was increased kidney injury with increased ROS formation and inflammation (Ostergaard et al. 2022). Thus, the renal effects of inflammasome inhibitors require further studies.

Tranilast, a tryptophan metabolite analog, appears to inhibit NLRP3 inflammasomes by impairing the assembly of endogenous NLRP3-ASC interaction. Use of tranilast has demonstrated significant preventive outcomes in gout arthritis and type 2 diabetic mouse models (Huang et al. 2018). Oral administration of this compound in diabetic mice showed improvement in reducing hyperglycemia and insulin resistance (Huang et al. 2018). In a clinical study, treatment of diabetic patients with tranilast was found to be associated with reduced urinary albumin and collagen IV excretion, suggesting the role of tranilast-driven inhibition of NLRP3 inflammasomes in early stage of DKD (Soma et al. 2006). Oridonin (Ori), a bioactive ent-kaurane diterpenoid, is reported to repress the release of inflammasome-dependent pro-inflammatory cytokines by inhibiting TLR4/NF-κB signaling pathways (Xu et al. 2009). Ori inhibits NLRP3 inflammasome activation by interacting with cysteine 279 of NLRP3 and thereby obliterating NLRP3-NEK7 interaction. Ori was found to be associated with reduced inflammation in experimental models of diabetes, peritonitis, and gout-related arthritis (He et al. 2018). In experimental diabetes, administration of Ori provided renoprotection by attenuating diabetes-induced renal injury and albuminuria via reduction in inflammation, including reduced infiltration of inflammatory cells in kidney tissues and decreased levels of pro-inflammatory cytokines, such as TNF-α, interleukin-6 (IL-6), IL-1β and MCP-1 through downregulation of TLR4 and inactivation of NF-κB pathways (Li et al. 2018). The experimental data suggest Ori as a potential therapeutic target in DKD and thus provides impetus for clinical studies in diabetic patients with nephropathy.

4.2.3 Chemokines and Cytokines

MCP-1, also known as monocyte chemoattractant C-C motif-ligand 2 (CCL2), is a pro-inflammatory chemokine and is upregulated in the kidneys as well as the urine of diabetic patients (Tashiro et al. 2002). *Mcp1* deletion in STZ-induced diabetic mice prevents the recruitment of macrophages to the glomeruli and reduces albuminuria (Chow et al. 2006). Pharmacological inhibition of MCP-1 signaling can be achieved with the so-called Spiegelmer emapticap pegol (NOX-E36), which is an anti-CCL2 L-enantiomeric RNA aptamer. Similar to the genetic studies, inhibition of MCP-1 by NOX-E36 in STZ-induced diabetic *ApoE* knockout mice resulted in attenuated albuminuria, and furthermore was shown to contribute to improvement of the glomerular filtration barrier by restoring the glomerular endothelial glycocalyx (Boels et al. 2017). Treatment of diabetic *db/db* mice with NOX-E36 resulted also in a reduced number of glomerular macrophages, associated with less distinct glomerulosclerosis and improved eGFR (Ninichuk et al. 2008). Furthermore, combination of NOX-E36 with the inhibitor NOX-A12 blocking another pro-inflammatory chemokine another pro-inflammatory chemokine, the stromal cell-derived factor 1 (SDF1), also known as C-X-C motif chemokine 12 (CXCL12),

even had additive protective effects on the kidney as it led to attenuated albuminuria and even higher eGFR levels (Darisipudi et al. 2011). Based on the promising findings of the preclinical studies, a phase 2 clinical study has been performed in type 2 diabetes patients with albuminuria who were treated with NOX-E36 for 12 weeks (NCT01547897). NOX-E36 treatment of diabetic patients resulted in reduced albuminuria, and thus confirmed its beneficial effects on the kidney, although no changes have been observed in the eGFR (Menne et al. 2017). The idea of targeting cytokines, such as IL-1β or IL-6, to inhibit their downstream signaling is based on their crucial role in the pathogenesis of DKD (reviewed in Donate-Correa et al. 2020). However, targeting of IL-1β by the specific inhibitor canakinumab resulted in less inflammation and lower rates of recurrent cardiovascular events in patients with previous infarction but also an increased number of fatal infections (CANTOS), and furthermore did not lead to clinically relevant improvement of the eGFR or albuminuria in patients with a previous myocardial infarction and CKD (Ridker et al. 2017; Ridker et al. 2018).

4.2.4 JAK-STAT

JAK signaling can be activated by ROS particularly under hyperglycemic conditions, and an increased JAK-STAT activity as well as expression has been observed in different mouse models of DKD (Brosius et al. 2016; Zhang et al. 2017). In diabetic 129S6 mice, overexpression of *Jak2* in podocytes significantly worsens DKD as these mice showed increased albuminuria, more glomerulosclerosis, and reduced podocyte density (Zhang et al. 2017). When these mice were treated with an inhibitor of both JAK-1 and JAK-2, LN3103801, this resulted in reduced JAK-STAT signaling as well as a reduction in albuminuria and mesangial expansion (Zhang et al. 2017). In *db/db* mice, inhibition of STAT3 acetylation, which is increased in mouse and human diabetic kidneys, was shown to reduce proteinuria and kidney injury (Brosius et al. 2016), suggesting that the JAK-STAT signaling is a promising target for the treatment of DKD in animal models. In humans, members of the JAK-STAT family have also been shown to be upregulated in glomerular and tubulointerstitial regions of the kidneys from patients with DKD (Berthier et al. 2009). Moreover, tubulointerstitial expression of JAK and STAT isoforms inversely correlates with the eGFR of these patients (Berthier et al. 2009). In a phase II clinical trial (NCT01683409), treatment with Baricitinib, a JAK-1/2 inhibitor, led to reduction of albuminuria and expression of renal inflammatory biomarkers in type 2 diabetes patients with DKD (Tuttle et al. 2018b). Thus, targeting the JAK-STAT pathway may be a potential novel therapeutic strategy for DKD.

4.2.5 Phosphodiesterase

Pentoxifylline is a phosphodiesterase inhibitor with anti-inflammatory and anti-fibrotic properties and has been shown to also have renoprotective properties in preclinical animal studies (Strutz et al. 2000; Davila-Esqueda and Martinez-Morales 2004; Lin et al. 2002). In STZ-induced diabetic Sprague-Dawley rats, pentoxifylline treatment reduced urinary albumin excretion and diminished oxidative stress in the kidney (Davila-Esqueda and Martinez-Morales 2004). In two smaller clinical trials with type 2 diabetes patients, administration of pentoxifylline for 4 months resulted

in decreased albuminuria (Navarro et al. 2003; Navarro et al. 2005). In another small study, pentoxifylline treatment of diabetic patients with advanced renal failure for 6 months also led to reduced urinary protein excretion (Navarro et al. 1999). This was confirmed in a longer clinical trial, in which type 2 diabetes patients with stage 3–4 CKD have received pentoxifylline for 2 years (PREDIAN) and showed a slowing of DKD progression as defined by attenuation of albuminuria and improvement of the eGFR decline (Navarro-Gonzalez et al. 2015). Notably, pentoxifylline treatment also results in a decrease of urinary TNF-α as well as MCP-1, which correlates with the urinary albumin excretion (Navarro et al. 2005; Navarro-Gonzalez et al. 2015; Lin et al. 2008), suggesting that its renoprotective effects are associated with its anti-inflammatory properties. This is supported by other studies, in which pentoxifylline treatment of patients with CKD resulted in a reduction of renal inflammatory markers (Goicoechea et al. 2010).

5 Outlook

For further identification of novel targets, the findings derived from single-cell sequencing in human and mouse diabetic and nondiabetic kidneys will help to identify gene patterns related to oxidative stress, inflammation, and fibrosis. This will form the basis for further evaluation of novel treatment targets in preclinical models of microvascular complications of diabetes, such as DKD, and ultimately for translation into the clinical context.

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