Mechanisms of Diabetic Nephropathy in Humans and Experimental Animals

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9.1 Introduction

Diabetic kidney disease (DKD) remains one of the main causes of end-stage kidney disease (ESKD) in the industrialized world and many developing countries and is likely to continue increasing given the pandemic of diabetes and obesity. While still considered a microvascular complication of diabetes, nephropathy involves more than just kidney capillaries, extending its damage across the various kidney cells and associated extracellular structures. This chapter will provide a comprehensive review of our current understanding of the pathophysiology of DKD especially focusing on lessons learned from experimental animal models.

9.2 Pathology

Histopathological changes of DKD in humans and in experimental animals involve all compartments of the kidney and correlate with functional and clinical manifestations of the disease. One of the earliest quantifiable changes in DKD is thickening of the glomerular basement membrane (GBM), a predictor of renal survival in patients with DKD [1]. Increased synthesis of extracellular matrix (ECM) components such as type IV collagen, laminins, and nidogen/entactin and decreased ECM degradation result in a near doubling of the GBM size [2]. More dramatic changes

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to the GBM are noted by ultrastructural studies, including denudation, abnormal folding, and the presence of shallow craterlike cavities and tunnels in fragmented segments of the GBM. Concurrently, there is a change in the composition of the GBM due to increased synthesis of the α1 chain of collagen IV and of perlecan by endothelial cells usually seen during embryonic development, along with overproduction of the mature GBM components (α3 and α5 chains of collagen IV and agrin) by podocytes [3, 4]. This transition, along with changes in nonenzymatic glycosylation, redistribution of GBM components, and nonspecific trapping of serum protein, likely affects the quality of the GBM and could explain, at least in part, the correlation of GBM thickness with its functional properties such as "leakiness" of serum macromolecules or the magnitude of proteinuria [5].

Aside from the altered GBM structure and function, the cellular components of the glomerular filtration barrier, namely, the podocytes and the endothelial cells, are both compromised in diabetes. The podocytes undergo cytoskeletal rearrangement, dedifferentiation, and autophagy manifested by effacement of their foot processes and decrease in slit diaphragm length with downregulation of its core components, such as nephrin [6]. Importantly, reduction in podocyte density secondary to detachment and dropout of the cells or apoptosis might be a useful predictor of DKD and its progression [7, 8]. On the other side of the GBM, the glomerular endothelium is a highly specialized, fenestrated layer coated by a negatively charged endothelial surface layer (ESL) with two components: the glycocalyx, which refers to membrane-bound proteoglycans (PG), and the endothelial cell coat that contains secreted PGs, negatively charged glycosaminoglycans (GAG), glycoproteins, and soluble proteins. Alteration of the composition and amount of PGs in the ESL leads to a reduced thickness of the ESL and decreased negative charge, but may also lead to disturbances in local signaling events [9, 10].

Another histopathologic hallmark of DKD is expansion of the mesangium. This is mostly due to increased deposition of extracellular mesangial matrix components and only minimally to mesangial cell hypertrophy and/or proliferation [11, 12]. Recent evidence suggests that mesangial expansion may also be due to, at least in part, overproduced GBM material that spreads into the mesangium. In general, mesangial expansion in DKD is diffusely uniform within the glomerulus [4]. As collagen deposition progresses with advanced nephropathy, diffuse diabetic glomerulosclerosis ensues and eventually leads to scarring of the glomeruli. Nodular glomerulosclerosis or the so-called Kimmelstiel–Wilson lesions may also be present in up to 50% of diabetic patients. Kimmelstiel–Wilson lesions are usually focal, segmental, and only occasionally diffuse. These develop due to continued local expansion of the mesangial matrix, or more likely as a result of mesangiolysis, with separation of the glomerular capillary from the mesangium and the formation of capillary aneurysms. The new capillary space is subsequently filled with mesangial matrix [13].

As for the renal vasculature in diabetes, a common finding is the accumulation of periodic acid–Schiff (PAS)-positive material around both the afferent and efferent arterioles, referred to as arteriolar hyalinosis. Hyalinosis of both arterioles is typical of DKD. The deposition of similar material in the subendothelial space of the

glomerular capillaries is referred to as a hyaline cap. These, together with capsular drops (hyaline material underneath the parietal epithelial cells of Bowman's capsule), constitute the exudative lesions of DKD.

Tubular basement membrane thickening develops in parallel with that of the GBM, and both correlate strongly with the degree of hyperglycemia in type 1 diabetes [6]. With progression of DKD, interstitial fibrosis and tubular atrophy develop and these changes correlate strongly with the progressive decline in kidney function as assessed by the glomerular filtration rate (GFR) [14–16]. This may be accompanied by chronic inflammatory infiltrates composed chiefly of T lymphocytes and macrophages.

To help with the staging of DKD, the Renal Pathology Society introduced a classification of the pathology of DKD, based on the degree of glomerular pathology, with a separate scoring system for tubular and vascular lesions [17] (Table 9.1). However, the classic description of DKD is mostly based on the glomerular pathology of kidneys in type 1 diabetes in humans (T1DKD). The pathognomonic glomerular changes are also identified in patients with type 2 diabetes and DKD (T2DKD) [18], but the overall pathological picture is more heterogeneous. Less than a third of T2DKD patients with microalbuminuria have the typical glomerular lesions expected in a similar stage of T1DKD [19, 20]. While there may be nuances in the pathogenesis of kidney disease in patients with type 1 compared with type 2 diabetes, these differences in pathology are more likely due to variability in the duration of DKD and the presence of comorbidities such as hypertension, obesity, and aging that have independent effects on the kidney.

Table 9.1 Glomerular classification of DKD

Class	Description	Inclusion criteria
I	Mild or nonspecific LM changes and EM-proven GBM thickening	Biopsy does not meet any of the criteria mentioned below for class II, III, or IV GBM >395 nm in female and >430 nm in male individuals 9 years of age and older (a)
IIa	Mild mesangial expansion	Biopsy does not meet criteria for class III or IV Mild mesangial expansion in >25% of the observed mesangium
IIb	Severe mesangial expansion	Biopsy does not meet criteria for class III or IV Severe mesangial expansion in >25% of the observed mesangium
III	Nodular sclerosis (Kimmelstiel–Wilson lesion)	Biopsy does not meet criteria for class IV At least one convincing Kimmelstiel–Wilson lesion
IV	Advanced diabetic glomerulosclerosis	Global glomerular sclerosis in >50% of glomeruli Lesions from classes I through III

Light microscopy (LM). (a) The basis of direct measurement of GBM width by EM, these individual cutoff levels may be considered indicative when other GBM measurements are used
From Tervaert TWC et al. [17]

9.3 Clinical Course

Tracking changes in GFR, urinary albumin excretion (UAE), and systemic arterial blood pressure, Mogensen and others classically described DKD to progress through distinct clinical stages (Fig. 9.1) [21]. In T1DKD these clinical stages correlate, in general, with the severity of renal pathology as described above. However, as with the kidney pathology, DKD in type 2 diabetes is a more heterogeneous disease, with variable degrees of glomerulosclerosis, tubulointerstitial fibrosis, and vasculopathy [22].

9.3.1 Normoalbuminuria

The initial stage of DKD is characterized by normoalbuminuria with a normal or high GFR and is overall clinically silent. However, in about a third or more of type 1 diabetes, a relatively large increase in GFR (greater than 150 mL/min/1.73 m^2) occurs and seems to be positively associated with glycemic control [23, 24]. This hyperfiltration is less common or much more attenuated in type 2 diabetic patients [25].

Hyperfiltration has been hypothesized to contribute to the initiation of nephron damage and progression of kidney disease [24]. The evidence to support that is mostly preclinical or based on observational studies. In a meta-analysis of cohort studies in type 1 diabetes, the pooled odds for the development of at least microalbuminuria was 2.71 (95% CI 1.20–6.11) in patients with hyperfiltration compared to those with normofiltration [26]. Similar findings were noted by the GFR study investigators [27]. In their longitudinal study of type 2 diabetic patients, the hazard

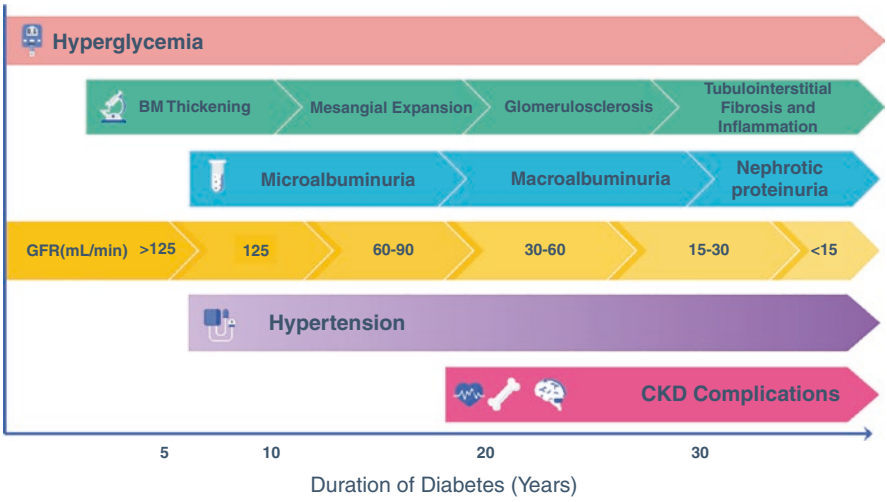

Fig. 9.1 Proposed model for clinicopathologic progression of diabetic kidney disease

ratio for progression to a minimum of microalbuminuria was 2.16 (95% CI 1.13–4.14). It was noted that 23.4% (11 of 47) of patients with persistent hyperfiltration progressed to micro- or macroalbuminuria compared to 10.6% (53 of 502) of patients who had hyperfiltration ameliorated at 6 months or who did not develop hyperfiltration since study inclusion. Dedicated prospective trials are needed to confirm whether targeting hyperfiltration improves clinically relevant end points (i.e., progressive GFR decline or incidence of ESKD). However, it remains the preferred mechanism proposed for the majority of the nephroprotective effects of drugs that intercept the renin–angiotensin–aldosterone (RAAS) system and the novel sodium–glucose cotransporter 2 inhibitors (SGLT2i).

9.3.2 Microalbuminuria

Traditionally, microalbuminuria is defined as a UAE of 30–300 mg/d or 20–200 μg/min, and it develops five years after the onset of type 1 diabetes in 20–40% of patients and can be present at the time of diagnosis of type 2 diabetes in 20–40% of patients. Hyperglycemia, hypertension, and elevated body mass index (BMI) are all independent risk factors for the development of microalbuminuria in type 1 and type 2 diabetic patients [28]. Onset of albuminuria tends to correlate pathologically with continued thickening of the glomerular and tubular basement membranes and some degree of podocyte loss. Mesangial matrix expansion and diffuse glomerulosclerosis may also be noted.

Longitudinal studies had previously suggested that approximately 80% of type 1 diabetic patients progress from microalbuminuria to proteinuria over a period of 6–14 years [29]. More recent studies suggest this could be closer to 40% [30]. While improved control of glycemia and hypertension over the years and the widespread use of RAAS blockers in microalbuminuric patients could explain these findings, it is also conceivable that microalbuminuria is not uniformly a predictor of macroalbuminuria in all diabetic patients [31]. On the other hand, UAE has been repeatedly and strongly validated as a risk factor for cardiovascular disease, peripheral vascular disease, stroke, and mortality from coronary heart disease [32–35].

Within 1 or 2 years of the onset of microalbuminuria in type 1 diabetes, patients may develop hypertension. GFR remains normal or is slightly elevated in type 1 diabetic patients with microalbuminuria [36]. On the other hand, GFR begins to normalize and then decline at rates approximating 3 to 4 mL/min/year in microalbuminuric type 2 diabetic patients [37].

9.3.3 Overt Nephropathy

With progressive podocyte loss and the onset of diffuse and/or nodular glomerulosclerosis, overt proteinuria (total urinary protein excretion exceeding 500 mg/d) or macroalbuminuria (UAE exceeding 300 mg/d) develops (Fig. 9.1). This occurs after an average of 15 years of the diabetic state in type 1 diabetes. In parallel,

progressive mesangial expansion leads to a reduction in the glomerular surface area available for filtration and has been shown to inversely correlate with declining GFR [38]. Hypertension is almost always present at this stage, and its poor control starts contributing to disease progression. Proteinuria by itself is another independent risk factor for further worsening of renal damage [39].

Untreated patients may progress to nephrotic-range proteinuria, which could signal the onset of rapid decline in GFR at a mean rate of 1 mL/min/month (stage IV) until ESKD ensues (stage V). The average time from the initial diagnosis of type 1 diabetes to ESKD is around 20–25 years. However, this time course is extremely variable among individual patients.

While this proposed staging system helps align the structure and function of the kidney in diabetes, growing evidence suggests that not all patients progress in a linear manner. Regression from micro- to normoalbuminuria and direct progression to ESKD have been reported in type 1 and type 2 diabetes [31, 40]. While the more frequent use of RAAS inhibitors may contribute to this trend, some studies have failed to confirm this correlation [41].

9.4 Metabolic Dysregulation of Diabetic Nephropathy

Hyperglycemia is the main driver for the pathophysiology and progression of DKD. In fact, glycemic control can slow the advancement of nephropathy and, at times, may reverse the original pathology [42–46]. As glucose accumulates intracellularly to excess, there is increased flux through glycolysis and possibly through the tricarboxylic acid (TCA) cycle, with less efficient oxidative phosphorylation. Indeed, diabetic kidneys upregulate glucose transporters GLUT-1 and GLUT-4 in the glomeruli, as well as the glycolytic enzymes hexokinase and phosphofructokinase, thus promoting flux into anaerobic glycolysis, in a manner reminiscent of the Warburg effect [47–51]. Growing evidence has implicated mitochondria in the metabolic dysregulation of diabetes. Increased mitochondrial fission and fragmentation as well as reduced levels of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) levels in the tubules, abnormalities in electron transport chain complex assembly/activity, and increased expression of uncoupling protein UCP1 have been reported [51–53]. It remains unclear whether the altered glucose metabolism is the cause or a result of diseased mitochondria in diabetic kidneys and whether the mitochondria will make a meaningful target for disease control.

Evidence is emerging that lipid metabolism may also play a role in the progression of DKD. Kimmelstiel and Wilson noted significant intratubular lipid accumulation in their seminal work on diabetic pathology [54]. Defective lipid metabolism likely contributes to lipid accumulation and may be associated with impaired mitochondrial function and the development of tubulointerstitial fibrosis [55]. Lipotoxicity can also manifest in the podocyte with intracellular accumulation of lipid droplets, abnormal glucose metabolism, inflammation, oxidative stress, endoplasmic reticulum stress, and actin cytoskeleton rearrangements [56].

The change in glucose metabolism is also manifested as an increased flux into alternative pathways: the pentose phosphate pathway, sorbitol/polyol pathway, advanced glycation end-product pathway, protein kinase C (PKC) pathway, and hexosamine pathway. These metabolic pathways had long been thought to contribute to glucotoxicity in the kidney through various mechanisms. However, research from the Joslin Medalist Study suggests that increased glycolytic flux and sorbitol/polyol pathway may protect from diabetic nephropathy by reducing the accumulation of glucose toxic metabolites and improving mitochondrial function [57, 58].

9.4.1 Advanced Glycation Reactions

Advanced glycation end products (AGEs) are proteins, lipids, or nucleic acids that are irreversibly cross-linked with reducing sugars. AGEs accumulate in both glomerular and tubular cells in experimental and human DKD [59, 60]. As renal function declines, higher concentrations of these products are retained in the plasma [61]. Experimental evidence shows that infusion of AGEs into normal rodents leads to the increased glomerular volume, accumulation of PAS-positive deposits, basement membrane widening, mesangial matrix expansion, and glomerulosclerosis [62]. Concurrently, inhibition of AGEs in experimental animal models of diabetes ameliorates albuminuria and glomerulosclerosis [63].

AGEs contribute to DKD injury by altering the function of the glycated proteins. ECM proteins, like in collagen, may become less susceptible to enzymatic hydrolysis by matrix metalloproteinases (MMPs), facilitating their accumulation in the extracellular space [64]. Glycation of sulfated proteoglycans modifies the charge-selective properties of the basement membrane and contributes to the development of microalbuminuria [65]. Concomitantly, AGEs act as signaling molecules either by acting intracellularly or by interacting with their receptor for advanced glycation end products (RAGE) that is expressed on the surfaces of podocytes and tubular epithelia. AGEs induce intracellular oxidant stress and activate NF-κB by redox-sensitive signaling pathways. They also activate PKC and regulate the expression of diverse growth factors and cytokines such as angiotensin II (Ang II) and transforming growth factor-beta1 (TGF-β1) [66, 67].

9.4.2 Protein Kinase C Signaling

As glycolytic metabolites react with glycerol phosphate, diacylglycerol (DAG), the major endogenous activator of PKC, is formed [68]. Other by-products of glucotoxicity such the polyol metabolites, AGE accumulation, RAGE activation, production of reactive oxygen species (ROS), and Ang II further activate PKC [69]. On the other hand, altered lipid metabolism and particularly the imbalance between lipid delivery and intracellular oxidation of fatty acids could lead to the accumulation of DAG [70].

PKC isoforms cooperate in the pathogenesis of DKD. While PKC-beta can lead to renal hypertrophy and glomerulosclerosis, PKC-alpha appears to contribute primarily to diabetic albuminuria by acting through vascular endothelial growth factor (VEGF) and by affecting nephrin expression [71]. Animal experiments with double knockouts of PKC-alpha and PKC-beta or the administration of an inhibitor of both PKC isoforms confirmed this hypothesis [71]. However, the PKC-beta inhibitor, ruboxistaurin, did not show a significant reduction in albumin/creatinine ratios when evaluated in a randomized clinical trial in patients [72].

9.4.3 Oxidative Stress

Oxidative stress has long been considered an integral pathogenic mechanism in the metabolic dysregulations of hyperglycemia [73]. Superoxide, hydroxyl radicals, hydrogen peroxide, and peroxynitrite, all commonly referred to as ROS, are increased in a diabetic kidney. These species, along with the oxidized proteins, lipids, nucleic acids, and the carbohydrates they produce, contribute to glomerular hypertrophy, cause injury to the podocyte, and promote fibrogenesis in the glomeruli and tubules [74, 75].

The notable sources of ROS production in the diabetic kidney are the mitochondria, the cytosolic NADPH oxidase (NOX), nitric oxide synthases, xanthine oxidase, and lipoxygenase [70, 76]. The prevailing hypothesis was that altered glucose metabolism increased mitochondrial electron transport chain activity, resulting in a high proton gradient, and high electrochemical potential differences led to the enhanced generation of mitochondrial superoxide [73]. However, measuring mitochondrial superoxide is difficult and has yielded inconsistent conclusions, with some groups finding a decrease in mitochondrial ROS [53, 70, 76, 77]. In fact, some level of mitochondrial superoxide may be beneficial and may retard organ dysfunction [76, 77]. With improved tools and real-time imaging, more sensitive spatiotemporal ROS measurements are being pursued to elucidate the role of mitochondrial ROS in DKD.

Meanwhile, NOX4, another notable source of ROS, has been consistently shown to be upregulated in animal models of diabetic kidney disease [68]. Its activity or expression appears to be influenced by various mediators of the diabetic milieu, including hyperglycemia, Ang II, TGF-β, AGEs, VEGF, endothelin, and aldosterone [74]. NOX4-mediated stimulation of PKC-alpha may contribute to many of the NOX4-dependent effects in DKD [78]. Moreover, NOX4 can inhibit fumarate hydratase, leading to the accumulation of fumarate, a TCA cycle metabolite with oncogenic properties that has been linked to the stimulation of hypoxia-inducible factor 1-alpha (HIF1α), TGF-β, and other matrix genes promoting fibrosis [79].

9.5 Glomerular Hemodynamics

As hyperfiltration is one of the earliest pathophysiologic features of DKD, it has been the target of many therapeutic interventions. Physiologically, four factors determine the GFR: (a) the glomerular plasma flow, (b) the systemic oncotic

pressure, (c) the glomerular transcapillary hydraulic pressure difference, and (d) the glomerular ultrafiltration (permeability) coefficient, K_f. These factors are affected in diabetes, resulting in hyperfiltration. First, diabetic glomeruli become hypertrophied and then filtration surface area increases, leading to an increased ultrafiltration coefficient [80]. Second, and more importantly, abnormal vascular control in diabetic nephropathy leads to differential reduction in afferent glomerular arteriolar resistance and a net increase in efferent arteriolar resistance. This results in increased renal blood flow and glomerular capillary hypertension, all resulting in an elevated single-nephron GFR [81]. This change in intraglomerular hemodynamics occurs in response to an imbalance of a variety of vasoactive substances and growth factors including the RAAS, atrial natriuretic peptide, insulin-like growth factor-1, endothelin, prostanoids, eicosanoids, and the nitric oxide (NO) system secondary to endothelial dysfunction [82, 83]. The rise in glomerular capillary pressure promotes the production of various mediators of DKD [84]. Angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) lower glomerular pressure and limit hyperfiltration by blocking the effect of Ang II on the efferent arteriole [85–88].

The impressive results from recent SGLT2i studies have shed light on the prior proposed mechanisms of glomerular hyperfiltration involving increased proximal tubular reabsorption of glucose and sodium (Na) [89]. In diabetes, hyperglycemia, tubular hypertrophy, and augmented SGLT2 expression in the proximal tubule contribute to increased Na/glucose reabsorption via SGLT2 and SGLT1, as well as increased Na reabsorption via NHE3 [90]. As a result, less sodium is delivered to the macula densa, thus attenuating tubuloglomerular feedback. This results in facilitated dilation of the afferent arteriole. Indeed, glomerular hyperfiltration is blunted in diabetic mice deficient in the adenosine receptor A1, which lack the tubuloglomerular feedback mechanism [91]. However, there have been conflicting results using this mouse model [92]. In addition, the decreased distal delivery lowers the tubular back pressure in Bowman space, which increases the effective glomerular filtration pressure and may explain a significant portion of diabetic hyperfiltration [93, 94]. Gene-targeted SGLT2 knockout and pharmacologic inhibition of SGLT2 prevent glomerular hyperfiltration in animal models of diabetes [95]. Treatment of type 1 and type 2 diabetic patients with the SGLT2i empagliflozin has been shown to attenuate renal hyperfiltration, as reflected by the estimated GFR (eGFR) [96, 97]. This effect appears to be independent of lowering blood glucose [98, 99].

After an SGLT2i was consistently observed to have excellent secondary kidney outcomes in a cardiovascular trial in patients with type 2 diabetes (as in the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients—EMPA-REG OUTCOME), other dedicated kidney outcome trials have been completed with other SGLT2i agents such as canagliflozin and dapagliflozin, all demonstrating robust benefits on primary kidney outcomes. CREDENCE (Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation) was a randomized, double-blind, placebo-controlled, multicenter clinical trial of patients with type 2 diabetes and albuminuric chronic kidney disease [100]. It showed that the SGLT2i was able to prevent ESKD (dialysis, transplantation, or sustained eGFR <15 mL/min/1.73m^2), doubling of serum creatinine, or

death from renal causes, with a hazard ratio (HR) of 0.70 (0.59–0.82). Similarly, the DAPA-CKD (Dapagliflozin and Prevention of Adverse Outcomes in Chronic Kidney Disease) trial studied dapagliflozin in patients with chronic kidney disease, with or without type 2 diabetes. It showed significant prevention of renal outcomes (≥50% decrease in eGFR, ESKD, or death from renal or cardiovascular causes) with an HR of 0.61 (0.51–0.72). This effect appears to be additive to ACEi and ARBs. It appears that renoprotection is a consistent feature across the class of SGLT2i.

9.6 Cellular and Molecular Mechanisms of Glomerulopathy

Hyperfiltration and intraglomerular hypertension are transduced as a biomechanical stress on the endothelial cells, the mesangial cells, and the podocytes, resulting in activation of molecular signaling pathways. As such, endothelial cells have increased nitric oxide synthase (eNOS) dysfunction initiated by hyperglycemia and metabolic dysregulation [101]. Moreover, mesangial cells respond to increased mechanical stretch by upregulating GLUT-1, ECM protein accumulation, and TGF-β1 activity [102, 103].

Regarding the podocyte, complex interactions between their intricate actin-based cytoskeleton, cell–cell, and cell–matrix contact proteins allow them to maintain the glomerular filtration barrier in the face of mechanical challenges resulting from the filtration of a pulsatile blood flow [104, 105]. The morphologic and functional changes of diabetes such as glomerular hypertrophy, thickening and stiffening of the GBM, and glomerular hyperfiltration and hypertension all result in shear and tensile stresses on the podocyte that challenge the cell's attachment to the GBM [104]. Meanwhile, the metabolic dysregulation in diabetes further compromises the cytoskeletal architecture of the podocytes. Glucotoxicity, Ang II, TGF-β, VEGF, and other signaling pathways result in the downregulation of the expression of nephrin, a key protein of the slit diaphragm and of cytoskeletal function in podocytes [6]. Furthermore, hyperglycemia, AGEs, ROS, and others result in dysregulation of the Rho family of GTPases, key regulators of the actin cytoskeleton [6]. Lastly, studies have shown that podocyte integrin expression is decreased in diabetes, compromising cell–matrix interactions [6]. Altogether, these stressors result in effacement of the foot processes, detachment, and the loss of a number of podocytes and their shedding into the urinary space. Other podocytes succumb to apoptosis under the effect of hyperglycemia, ROS, and activation of the TGF-β pathway [106]. The remaining podocytes attempt to cover the newly denuded GBM by hypertrophy, with activation of the mammalian target of rapamycin (mTOR) [107]. However, once podocyte loss reaches 20%, glomerulosclerosis develops [108].

In sum, the metabolic and hemodynamic dysregulations in DKD converge and activate second messenger signaling pathways, transcription factors, and cytokines, including the RAAS, TGF-β, VEGF, and others, all of which contribute to the development of albuminuria and glomerulosclerosis, characteristic features of established diabetic nephropathy.

The RAAS is one of the most important pathways in DKD pathophysiology. Along with the systemic RAAS activation, renal cells such as mesangial cells, podocytes, and even tubular cells synthesize Ang II and express its receptors, which may contribute to the regional activation of RAAS [102, 109]. Indeed, hyperglycemia directly, and via ROS and AGEs, upregulates the expression of renin and angiotensinogen [81, 110, 111]. The RAAS drives the hemodynamic changes of DKD but also independently activates a multitude of cytokines such as TGF-β, connective tissue growth factor (CTGF), interleukin-6, monocyte chemoattractant protein-1 (MCP-1), and VEGF-A. Accordingly, high levels of Ang II can contribute to the early hyperplasia and hypertrophy of the renal cells observed in diabetes and can modulate glomerular ECM deposition in the later stages of diabetes [112]. In addition to the classical ACE/Ang II/AT1R axis, the RAAS comprises another important axis, the ACE2/Ang-(1–7)/Mas receptor, considered the counterregulatory axis of ACE/Ang II/AT1R. Indeed, an imbalance between the Ang II and Ang-(1–7) systems is associated with vascular dysfunction, inflammation, and fibrosis, making the ACE2/Ang-(1–7)/Mas receptor a potential ameliorating and therapeutic target in DKD [113].

RAAS blockers in clinical use may not be sufficient to fully arrest the activation of this system due to "aldosterone breakthrough," the increase of plasma aldosterone to basal levels after several weeks of ACEi or ARB administration. The mineralocorticoid receptor is also expressed in kidney cells outside of the aldosterone-sensitive distal nephron, such as vascular cells, podocytes, fibroblasts, and inflammatory cells. Activation of the mineralocorticoid receptor in those cells has been associated with activation of inflammatory and fibrotic pathways in the kidney, and this has deleterious effects on podocytes and mesangial cells [114]. Clinical studies show that steroidal mineralocorticoid receptor antagonists (MRAs) have an anti-albuminuric effect in diabetic kidney disease. Finerenone is a novel, nonsteroidal MRA with a better therapeutic index than the steroidal MRAs such as spironolactone and eplerenone. In the FIDELIO-DKD trial, finerenone reduced CKD progression and improved cardiovascular outcomes compared with placebo when added to an optimized regimen of renin–angiotensin–aldosterone system inhibitors. Plus, the incidence of hyperkalemia was manageably low [115].

VEGF is one of the key signaling pathways of the crosstalk between glomerular endothelium and podocytes. Healthy podocytes produce VEGF-A which helps maintain the endothelial cell's structure and function upon binding to vascular endothelial growth factor receptor 2 (VEGFR2) [116]. Targeted genetic deletion of all VEGF-A isoforms from podocytes leads to glomerular disease in healthy mice [117]. The role of VEGF signaling in diabetes was difficult to decipher initially. Some studies reported increased VEGF-A activity in diabetic glomeruli, with improvement of DKD upon inhibition of VEGF-A or VEGFR2 [118–121]. Other research showed that total glomerular VEGF-A levels decreased as diabetic nephropathy progressed and that targeted genetic deletion of all VEGF-A isoforms from podocytes accelerated nephropathy in diabetic animals [119]. More likely, the glomerular cells tightly control a state of delicate VEGF balance, and too much or too little can be pathogenic [122]. More recent evidence has also shown that the

different isoforms of VEGF-A may confer additional nuances of signaling. VEGF-A_{165a} is a potent vasoactive agent, increasing vasodilation, vascular permeability, and angiogenesis [123]. Meanwhile, VEGF-A_{165b} is a protective factor in diabetic nephropathy[124]. In diabetic mice, podocyte-specific $VEGF_{165b}$ overexpression or $VEGF_{165b}$ administration maintained the glycocalyx and prevented endothelial and podocyte cell death, resulting in reduced albuminuria [124].

Other paracrine signals such as NO and angiopoietins can also feed into this crosstalk and tip the balance toward pathogenesis. New insights have revealed that endothelin-1 (ET-1), an endothelial-derived vasoconstrictor, can signal to the podocyte and then back to the endothelial cell [125]. Atrasentan, an ET-1 receptor antagonist, has been shown clinically to ameliorate early microalbuminuric diabetic kidney disease [126].

TGF-β appears to be a common pathway that leads to hypertrophic changes early on and then promotes fibrosis and sclerosis in the later stages of diabetic kidney disease [127, 128]. Under the impact of metabolic and hemodynamic forces in DKD, multiple mediators converge upon the activation of the TGF-β system. These include high glucose concentration [129], AGE-modified proteins [130], ROS [73], cyclical stretch/relaxation of mesangial cells in culture [131], PKC activation [132], and Ang II [133]. TGF-β has been shown to stimulate the synthesis of type I collagen, type IV collagen, fibronectin, and laminin. Further, TGF-β inhibits matrix metalloproteinases and can also stimulate the inhibitors of proteases, thus preventing the degradation of ECM proteins and leading to their deposition and accumulation [134]. Blocking TGF-β upstream of its receptor or downstream in the intracellular signaling cascade results in marked improvement in glomerulosclerosis, ECM deposition, GBM thickening, and other histological and molecular parameters of diabetic renal disease [82, 121, 135, 136]. This provides proof of the cytokine's central role in DKD pathophysiology.

9.7 Tubulopathy in Diabetes

Along with glomerulopathy, tubular damage plays a significant role in the pathogenesis of DKD [137]. Growing clinical and pathological data confirm that elevated baseline plasma biomarkers of tubular injury such as KIM-1 have been significantly associated with the risk of early decline of kidney function, independent of albuminuria [138]. Tubular dysfunction as well as tubulointerstitial fibrosis are known to correlate significantly with the decline in GFR and the progression of kidney disease.

Various mechanisms come into play in diabetic tubulopathy [139]. First, the increased metabolic stress in diabetes promotes a hypoxic environment for the proximal tubule. As SGLT2 and NHE3 increase their reabsorptive capacity, there is a commensurate increase in the demand for ATP to maintain the crucial activity of Na^+/K^+-ATPase to support ion transport [139]. Moreover, proximal tubular epithelial cells increase gluconeogenesis in the setting of diabetes [139]. However, because of mitochondrial injury and metabolic dysfunction, the proximal tubular cells consume more O_2 for each molecule of ATP generated. This increased demand and

inefficient utilization of O_2 are met with reduced blood supply due to concomitant endothelial injury, intrinsic capillary loss within the affected tubulointerstitium, and glomerular capillary occlusion, resulting in significant hypoxia [139].

Hypoxic proximal tubular epithelial cells undergo apoptosis but also promote tubulointerstitial fibrosis via TGF-β and other mechanisms [139]. The expansion of the extracellular matrix further exacerbates hypoxia and microvascular rarefaction, starting the spiral of fibrosis and chronic kidney injury.

Several other pathomechanisms target the proximal tubule in DKD. These include the RAAS as well as the toxic effects of leaked albumin and albumin-bound fatty acids into the tubular lumen due to albuminuria, among others [139, 140].

With their advent, the new single-cell modalities such as transcriptomics, epigenetics, metabolomics, and proteomics are starting to show the effect of diabetes on various tubular segments. For instance, a recent single-nucleus RNA sequencing (snRNA-seq) on cryopreserved human diabetic kidney samples showed that the diabetic thick ascending limb, late distal convoluted tubule, and principal cells of the collecting ducts all adopt a gene expression signature consistent with increased potassium secretion, including alterations in Na^+/K^+-ATPase, WNK1, mineralocorticoid receptor, and NEDD4L expression, as well as decreased paracellular calcium and magnesium reabsorption [141].

Furthermore, there is evidence of retrograde crosstalk between the proximal tubules and the podocytes. Indeed, recent animal studies have shown that selective proximal tubular injury can lead to podocytopathy and extensive glomerular injury reminiscent of diabetes [142]. Tubular epithelial cells can protect against albuminuria in diabetes by maintaining nicotinamide mononucleotide concentrations around glomeruli and by influencing podocyte function [143].

9.8 Inflammation

Metabolic and hemodynamic abnormalities, including hyperglycemia, AGEs, ROS, Ang II, and TGF-β, have been shown to promote a proinflammatory state [144]. The immune system is involved in the pathophysiology of DKD at multiple levels [145]. First, from an innate immunity standpoint, mononuclear phagocytic cells that reside in the kidney are activated in diabetes and are joined by renal cells in the release of proinflammatory cytokines and paracrine signals [146]. Subsequently, additional monocytes and macrophages are recruited into the kidney, further amplifying cytokine and chemokine release from the kidney [147, 148]. The mast cell is another innate immune cell that infiltrates the tubulointerstitium in DKD. Its degranulation releases inflammatory mediators such as TGF-β and proteolytic enzymes, the most notable of which is chymase [145]. Mast cell chymase is 40 times more potent than ACE at converting Ang I to Ang II [149, 150].

Pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NOD-like receptors or NLRs) are essential to the proper function of the innate immune system. PRRs are upregulated in mononuclear phagocytic cells as well as in endothelial cells and

podocytes [145]. They recognize pathogen-associated molecular patterns (PAMPs) and endogenous stress signals or damage-associated molecular patterns (DAMPs) that indicate cellular stress and injury, including uric acid, extracellular ATP, as well as glucose and ROS. Upon sensitization of PRRs, there is activation of the inflammasome and, among other effects, release of inflammatory cytokines.

Numerous interleukin cytokines have been implicated in the pathogenesis of DKD. For instance, IL-1, IL-6, and IL-18 have been linked with morphological changes of DKD, such as GBM thickening, as well as functional changes, such as albuminuria and loss of GFR [145]. Early in diabetes, both glomerular and tubular cells increase expression of TNF-α [151]. This cytokine is cytotoxic to glomerular mesangial and epithelial cells and has been demonstrated to increase vascular endothelial permeability, induce oxidative stress, and affect glomerular hemodynamics and GFR [152, 153]. Its receptors, TNFR1 and TNFR2, are candidate biomarkers of DKD. The serum level of TNFR1 was a predictor of ESKD, even after adjustment for clinical covariates in a cohort of type 1 diabetes [154].

Chemokines mediate the migration of monocytes and macrophages into kidney tissue and are also upregulated in DKD. Of particular interest is the CC chemokine ligand 2 (CCL2, also known as MCP-1). Its expression is upregulated in response to the metabolic and hemodynamic features of the diabetic milieu, including Ang II [155]. In the kidney, its receptor CCR2 is also expressed on podocytes, extending its role beyond the recruitment of macrophages to the tubulointerstitium [156]. Several studies have implicated CCR2 in the effacement of foot processes, podocytopenia, and damage to the slit diaphragm, leading to albuminuria [157]. CCR2 inhibitors are being evaluated for the management of DKD [158].

Another therapeutic target in DKD is the Janus kinase–signal transducer and activator of transcription (JAK-STAT) pathway. This pathway transduces inflammatory signals from cytokines and chemokines as well as AGEs and growth factors/hormones [159]. The JAK-STAT pathway has been shown to be upregulated in DKD, including in intrinsic renal cells. Baricitinib, an oral, reversible, selective inhibitor of JAK1 and JAK2, has shown promise as an intervention to slow the progression of DKD [159].

Overall, resident immune cells, infiltrating cells, and resident renal cells converge to activate the innate immune system in DKD. Renal cells produce cytokines and chemokines and increase the expression of adhesion molecules that facilitate adhesion of the inflammatory cells [144, 145]. Eventually, the adaptive immune system is also involved in diabetes, as T cells infiltrate the kidney in DKD, albeit not as prominently as macrophages [145]. The T helper phenotype in DKD appears to be shifted toward Th1/Th17 cells rather than regulatory T cell, Tregs [145, 160]. This promotes further macrophage-induced injury rather than repair of the kidney. There is limited evidence for the involvement of B cells in DKD.

9.9 Conclusion

The pathophysiology of DKD is complex (Fig. 9.2) and most of these pathways were the fruit of deploying various experimental animal models to elucidate mechanisms of injury at the cellular and molecular level and to inform clinical and

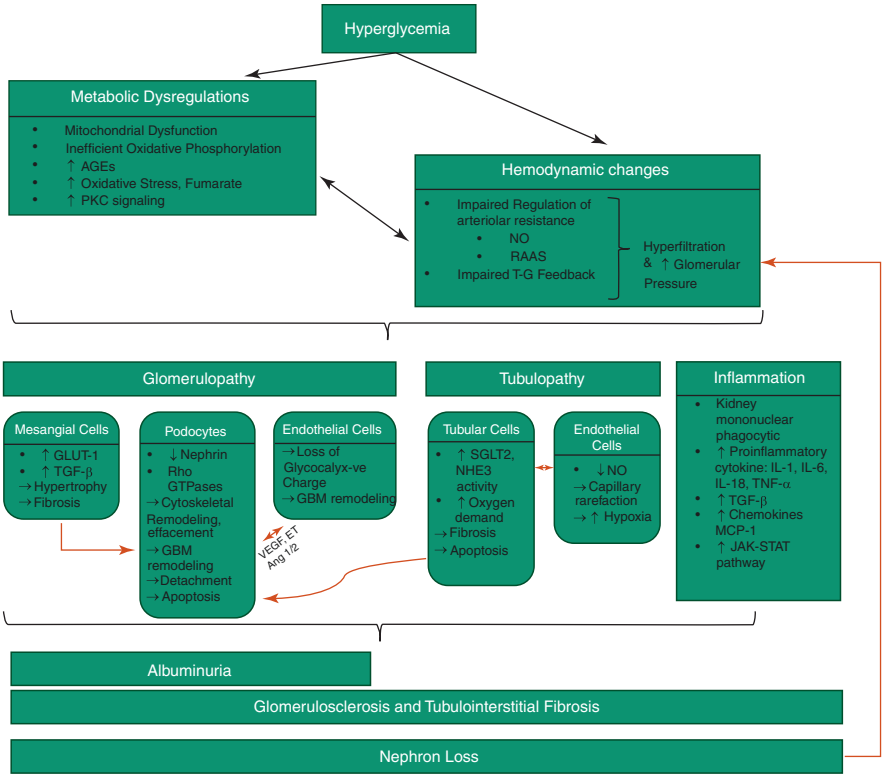

Fig. 9.2 Conceptual model of the pathogenesis of diabetic kidney disease

pathological studies in humans. Glucotoxicity and glomerular hypertension plus deleterious combinations of toxic metabolites, growth factors, and cytokines promote injury in the various compartments of the kidney, leading to albuminuria and progressive fibrosis and loss of renal function. While the treatment and prevention of DKD in clinical practice had long been dependent on ACEi and ARBs as well as the control of systemic hypertension and hyperglycemia, recent clinical studies have brought new options for the management of this disease. The SGLT2i and MRAs currently offer hope for additional nephroprotective effects. By further elucidating the pathophysiology of DKD, we expect that newer and more effective therapies will be on the horizon.

References

1. Stefan G, Stancu S, Zugravu A, Petre N, Mandache E, Mircescu G. Histologic predictors of renal outcome in diabetic nephropathy: Beyond renal pathology society classification. Medicine (Baltimore). 2019;98:e16333.
2. Fioretto P, Mauer M. Histopathology of diabetic nephropathy. Semin Nephrol. 2007;27:195–207.

3. Zhu D, Kim Y, Steffes MW, Groppoli TJ, Butkowski RJ, Mauer SM. Glomerular distribution of type IV collagen in diabetes by high resolution quantitative immunochemistry. Kidney Int. 1994;45:425–33.
4. Kriz W, Lowen J, Federico G, van den Born J, Grone E, Grone HJ. Accumulation of worn-out GBM material substantially contributes to mesangial matrix expansion in diabetic nephropathy. Am J Physiol Renal Physiol. 2017;312:F1101–11.
5. Caramori ML, Kim Y, Huang C, Fish AJ, Rich SS, Miller ME, et al. Cellular basis of diabetic nephropathy: 1. Study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. Diabetes. 2002;51:506–13.
6. Lin JS, Susztak K. Podocytes: the weakest link in diabetic kidney disease? Curr Diab Rep. 2016;16:45.
7. Toyoda M, Najafian B, Kim Y, Caramori ML, Mauer M. Podocyte detachment and reduced glomerular capillary endothelial fenestration in human type 1 diabetic nephropathy. Diabetes. 2007;56:2155–60.
8. Meyer TW, Bennett PH, Nelson RG. Podocyte number predicts long-term urinary albumin excretion in Pima Indians with Type II diabetes and microalbuminuria. Diabetologia. 1999;42:1341–4.
9. Kashihara N, Watanabe Y, Makino H, Wallner EI, Kanwar YS. Selective decreased de novo synthesis of glomerular proteoglycans under the influence of reactive oxygen species. Proc Natl Acad Sci U S A. 1992;89:6309–13.
10. Khramova A, Boi R, Friden V, Granqvist AB, Nilsson U, Tenstad O, et al. Proteoglycans contribute to the functional integrity of the glomerular endothelial cell surface layer and are regulated in diabetic kidney disease. Sci Rep. 2021;11:8487.
11. Østerby R. Morphometric studies of the peripheral glomerular basement membrane in early juvenile diabetes. I. Development of initial basement membrane thickening. Diabetologia. 1972;8:84–92.
12. Drummond K, Mauer M. The early natural history of nephropathy in type 1 diabetes: II. Early renal structural changes in type 1 diabetes. Diabetes. 2002;51:1580–7.
13. Saito Y, Kida H, Takeda S, Yoshimura M, Yokoyama H, Koshino Y, et al. Mesangiolysis in diabetic glomeruli: its role in the formation of nodular lesions. Kidney Int. 1988;34:389–96.
14. Bader R, Bader H, Grund KE, Markensen-Haen S, Christ H, Bohle A. Structure and function of the kidney in diabetic glomerulosclerosis: Correlations between morphologic and functional parameters. Pathol Res Practice. 1980;167:204–16.
15. Lane PH, Steffes MW, Fioretto P, Mauer SM. Renal interstitial expansion in insulin-dependent diabetes mellitus. Kidney Int. 1993;43:661–7.
16. Ziyadeh FN, Goldfarb S. The renal tubulointerstitium in diabetes mellitus. Kidney Int. 1991;39:464–75.
17. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol. 2010;21:556–63.
18. Ponchiardi C, Mauer M, Najafian B. Temporal profile of diabetic nephropathy pathologic changes. Curr Diab Rep. 2013;13:592–9.
19. Fioretto P, Mauer M, Brocco E, Velussi M, Frigato F, Muollo B, et al. Patterns of renal injury in NIDDM patients with microalbuminuria. Diabetologia. 1996;39:1569–76.
20. Gambara V, Mecca G, Remuzzi G, Bertani T. Heterogeneous nature of renal lesions in type II diabetes. J Am Soc Nephrol. 1993;3:1458–66.
21. Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. Diabetes. 1983;32(Suppl 2):64–78.
22. Olivarius Nde F, Andreasen AH, Keiding N, Mogensen CE. Epidemiology of renal involvement in newly-diagnosed middle-aged and elderly diabetic patients. Cross-sectional data from the population-based study “Diabetes Care in General Practice”, Denmark. Diabetologia. 1993;36:1007–16.
23. Mogensen CE, Steffes MW, Deckert T, Christiansen JS. Functional and morphological renal manifestations in diabetes mellitus. Diabetologia. 1981;21:89–93.

24. Ficociello LH, Perkins BA, Roshan B, Weinberg JM, Aschengrau A, Warram JH, et al. Renal hyperfiltration and the development of microalbuminuria in type 1 diabetes. Diabetes Care. 2009;32:889–93.
25. Myers BD, Nelson RG, Williams GW, Bennett PH, Hardy SA, Berg RL, et al. Glomerular function in Pima Indians with noninsulin-dependent diabetes mellitus of recent onset. J Clin Invest. 1991;88:524–30.
26. Magee GM, Bilous RW, Cardwell CR, Hunter SJ, Kee F, Fogarty DG. Is hyperfiltration associated with the future risk of developing diabetic nephropathy? A meta-analysis. Diabetologia. 2009;52:691–7.
27. Ruggenenti P, Porrini EL, Gaspari F, van Bommel EJ, Heerspink HJL, van Raalte DH, et al. Glomerular hyperfiltration and renal disease progression in type 2 diabetes. Diabetes Care. 2012;35:2061–8.
28. Cederholm J, Eliasson B, Nilsson PM, Weiss L, Gudbjörnsdottir S, Steering Committee of the Swedish National Diabetes Register. Microalbuminuria and risk factors in type 1 and type 2 diabetic patients. Diabetes Res Clin Pract. 2005;67:258–66.
29. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Engl J Med. 1984;310:356–60.
30. Caramori ML, Fioretto P, Mauer M. The need for early predictors of diabetic nephropathy risk: is albumin excretion rate sufficient? Diabetes. 2000;49:1399–408.
31. Perkins BA, Ficociello LH, Silva KH, Finkelstein DM, Warram JH, Krolewski AS. Regression of microalbuminuria in type 1 diabetes. New Engl J Med. 2003;348:2285–93.
32. Ninomiya T, Perkovic V, Verdon C, Barzi F, Cass A, Gallagher M, et al. Proteinuria and stroke: a meta-analysis of cohort studies. Am J Kidney Dis. 2009;53:417–25.
33. Perkovic V, Verdon C, Ninomiya T, Barzi F, Cass A, Patel A, et al. The relationship between proteinuria and coronary risk: a systematic review and meta-analysis. PLoS Med. 2008;5:1486–95.
34. Deckert T, Yokoyama H, Mathiesen E, Rønn B, Jensen T, Feldt-Rasmussen B, et al. Cohort study of predictive value of urinary albumin excretion for atherosclerotic vascular disease in patients with insulin dependent diabetes. BMJ. 1996;312:871–4.
35. Damsgaard EM, Froland A, Jorgensen OD, Mogensen CE. Microalbuminuria as predictor of increased mortality in elderly people. BMJ. 1990;300:297–300.
36. Mathiesen ER, Feldt-Rasmussen B, Hommel E, Deckert T, Parving HH. Stable glomerular filtration rate in normotensive IDDM patients with stable microalbuminuria. A 5-year prospective study. Diabetes Care. 1997;20:286–9.
37. Gaede P, Tarnow L, Vedel P, Parving H-H, Pedersen O. Remission to normoalbuminuria during multifactorial treatment preserves kidney function in patients with type 2 diabetes and microalbuminuria. Nephrol Dial Transplant. 2004;19:2784–8.
38. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. J Clin Invest. 1984;74:1143–55.
39. Remuzzi G, Bertani T. Is glomerulosclerosis a consequence of altered glomerular permeability to macromolecules? Kidney Int. 1990;38:384–94.
40. Yamada T, Komatsu M, Komiya I, Miyahara Y, Shima Y, Matsuzaki M, et al. Development, progression, and regression of microalbuminuria in Japanese patients with type 2 diabetes under tight glycemic and blood pressure control: the Kashiwa study. Diabetes Care. 2005;28:2733–8.
41. Macisaac RJ, Jerums G. Diabetic kidney disease with and without albuminuria. Curr Opin Nephrol Hypertens. 2011;20:246–57.
42. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. JAMA. 2003;290:2159–67.

43. United Kingdom Prospective Diabetes Study Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352:837–53.
44. Fioretto P, Steffes MW, Sutherland DER, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. New Engl J Med. 1998;339:69–75.
45. Barbosa J, Steffes MW, Sutherland DE, Connett JE, Rao KV, Mauer SM. Effect of glycemic control on early diabetic renal lesions. A 5-year randomized controlled clinical trial of insulin-dependent diabetic kidney transplant recipients. JAMA. 1994;272:600–6.
46. Diabetes Control and Complications Trial Research Group, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329:977–86.
47. Heilig CW, Brosius FC, Henry DN. Glucose transporters of the glomerulus and the implications for diabetic nephropathy. Kidney Int Suppl. 1997;60:S91–9.
48. Zhang G, Darshi M, Sharma K. The Warburg effect in diabetic kidney disease. Semin Nephrol. 2018;38:111–20.
49. Tanner LB, Goglia AG, Wei MH, Sehgal T, Parsons LR, Park JO, et al. Four key steps control glycolytic flux in mammalian cells. Cell Syst. 2018;7:49–62.e8.
50. Sas KM, Kayampilly P, Byun J, Nair V, Hinder LM, Hur J, et al. Tissue-specific metabolic reprogramming drives nutrient flux in diabetic complications. JCI Insight. 2016;1:e86976.
51. Sharma K, Karl B, Mathew AV, Gangoiti JA, Wassel CL, Saito R, et al. Metabolomics reveals signature of mitochondrial dysfunction in diabetic kidney disease. J Am Soc Nephrol. 2013;24:1901–12.
52. Bhargava P, Schnellmann RG. Mitochondrial energetics in the kidney. Nat Rev Nephrol. 2017;13:629–46.
53. Galvan DL, Green NH, Danesh FR. The hallmarks of mitochondrial dysfunction in chronic kidney disease. Kidney Int. 2017;92:1051–7.
54. Kimmelstiel P, Wilson C. Intercapillary lesions in the glomeruli of the kidney. Am J Pathol. 1936;12:83–98.7.
55. Kang HM, Ahn SH, Choi P, Ko Y, Han SH, Chinga F, et al. Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. Nat Med. 2015;21:37–46.
56. Martinez-Garcia C, Izquierdo-Lahuerta A, Vivas Y, Velasco I, Yeo T, Chen S, et al. Renal lipotoxicity-associated inflammation and insulin resistance affects actin cytoskeleton organization in podocytes. PLoS One. 2015;10:e0142291.
57. Qi W, Li Q, Gordin D, King GL. Preservation of renal function in chronic diabetes by enhancing glomerular glucose metabolism. J Mol Med (Berl). 2018;96:373–81.
58. Qi W, Keenan HA, Li Q, Ishikado A, Kannt A, Sadowski T, et al. Pyruvate kinase M2 activation may protect against the progression of diabetic glomerular pathology and mitochondrial dysfunction. Nat Med. 2017;23:753–62.
59. Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, et al. Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic nephropathy. J Clin Invest. 1997;100:2995–3004.
60. Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. J Clin Invest. 1997;99:457–68.
61. Cooper ME. Interaction of metabolic and haemodynamic factors in mediating experimental diabetic nephropathy. Diabetologia. 2001;44:1957–72.
62. Vlassara H, Striker LJ, Teichberg S, Fuh H, Li YM, Steffes M. Advanced glycation end products induce glomerular sclerosis and albuminuria in normal rats. Proc Natl Acad Sci U S A. 1994;91:11704–8.
63. Busch M, Franke S, Rüster C, Wolf G. Advanced glycation end-products and the kidney. Eur J Clin Invest. 2010;40:742–55.

64. Catania JM, Chen G, Parrish AR. Role of matrix metalloproteinases in renal pathophysiologies. Am J Physiol Renal Physiol. 2007;292:F905–11.
65. Wautier JL, Guillausseau PJ. Advanced glycation end products, their receptors and diabetic angiopathy. Diabetes Metab. 2001;27:535–42.
66. Yamagishi S, Inagaki Y, Okamoto T, Amano S, Koga K, Takeuchi M, et al. Advanced glycation end product-induced apoptosis and overexpression of vascular endothelial growth factor and monocyte chemoattractant protein-1 in human-cultured mesangial cells. J Biol Chem. 2002;277:20309–15.
67. Fukami K, Ueda S, Yamagishi S, Kato S, Inagaki Y, Takeuchi M, et al. AGEs activate mesangial TGF-beta-Smad signaling via an angiotensin II type I receptor interaction. Kidney Int. 2004;66:2137–47.
68. Studer RK, Craven PA, DeRubertis FR. Role for protein kinase C in the mediation of increased fibronectin accumulation by mesangial cells grown in high glucose medium. Diabetes. 1993;42:118–26.
69. Thallas-Bonke V, Thorpe SR, Coughlan MT, Fukami K, Yap FY, Sourris KC, et al. Inhibition of NADPH oxidase prevents advanced glycation end product-mediated damage in diabetic nephropathy through a protein kinase C-alpha-dependent pathway. Diabetes. 2008;57:460–9.
70. Hallan S, Sharma K. The role of mitochondria in diabetic kidney disease. Curr Diab Rep. 2016;16:61.
71. Menne J, Shushakova N, Bartels J, Kiyan Y, Laudeley R, Haller H, et al. Dual inhibition of classical protein kinase C-α and protein kinase C-β isoforms protects against experimental murine diabetic nephropathy. Diabetes. 2013;62:1167–74.
72. Tuttle KR, Bakris GL, Toto RD, McGill JB, Hu K, Anderson PW. The effect of ruboxistaurin on nephropathy in type 2 diabetes. Diabetes Care. 2005;28:2686–90.
73. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414:813–20.
74. Gorin Y, Block K. Nox4 and diabetic nephropathy: with a friend like this, who needs enemies? Free Radic Biol Med. 2013;61:130–42.
75. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes. 1991;40:405–12.
76. Coughlan MT, Sharma K. Challenging the dogma of mitochondrial reactive oxygen species overproduction in diabetic kidney disease. Kidney Int. 2016;90:272–9.
77. Sharma K. Mitochondrial dysfunction in the diabetic kidney. In: Santulli G, editor. Mitochondrial dynamics in cardiovascular medicine. Cham: Springer International Publishing; 2017. p. 553–62.
78. Thallas-Bonke V, Jha JC, Gray SP, Barit D, Haller H, Schmidt HHHW, et al. Nox-4 deletion reduces oxidative stress and injury by PKC-α-associated mechanisms in diabetic nephropathy. Physiol Rep. 2014;2:e12192.
79. You YH, Quach T, Saito R, Pham J, Sharma K. Metabolomics reveals a key role for fumarate in mediating the effects of NADPH oxidase 4 in diabetic kidney disease. J Am Soc Nephrol. 2016;27:466–81.
80. Christiansen JS, Gammelgaard J, Frandsen M, Parving HH. Increased kidney size, glomerular filtration rate and renal plasma flow in short-term insulin-dependent diabetics. Diabetologia. 1981;20:451–6.
81. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJL, van Raalte DH, et al. Glomerular hyperfiltration in diabetes: mechanisms, clinical significance, and treatment. J Am Soc Nephrol. 2017;28:1023–39.
82. De Vriese AS, Stoenoiu MS, Elger M, Devuyst O, Vanholder R, Kriz W, et al. Diabetes-induced microvascular dysfunction in the hydronephrotic kidney: role of nitric oxide. Kidney Int. 2001;60:202–10.
83. Goligorsky MS. Endothelial cell dysfunction and nitric oxide synthase. Kidney Int. 2000;58:1360–76.
84. Hostetter TH, Rennke HG, Brenner BM. The case for intrarenal hypertension in the initiation and progression of diabetic and other gomerulopathies. Am J Med. 1982;72:375–80.

85. Anderson S, Rennke HG, Brenner BM. Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. J Clin Invest. 1986;77:1993–2000.
86. Lambers Heerspink HJ, de Borst MH, Bakker SJL, Navis GJ. Improving the efficacy of RAAS blockade in patients with chronic kidney disease. Nature Rev Nephrol. 2012;9:112–21.
87. Holtkamp FA, de Zeeuw D, Thomas MC, Cooper ME, de Graeff PA, Hillege HJL, et al. An acute fall in estimated glomerular filtration rate during treatment with losartan predicts a slower decrease in long-term renal function. Kidney Int. 2011;80:282–7.
88. Bakris GL, Weir MR. Angiotensin-converting enzyme inhibitor–associated elevations in serum creatinine: is this a cause for concern? Arch Int Med. 2000;160:685–93.
89. Vallon V, Thomson SC. Renal function in diabetic disease models: the tubular system in the pathophysiology of the diabetic kidney. Annu Rev Physiol. 2012;74:351–75.
90. Wang XX, Levi J, Luo Y, Myakala K, Herman-Edelstein M, Qiu L, et al. SGLT2 protein expression is increased in human diabetic nephropathy: SGLT2 protein inhibition decreases renal lipid accumulation, inflammation, and the development of nephropathy in diabetic mice. J Biol Chem. 2017;292:5335–48.
91. Vallon V, Schroth J, Satriano J, Blantz RC, Thomson SC, Rieg T. Adenosine A(1) receptors determine glomerular hyperfiltration and the salt paradox in early streptozotocin diabetes mellitus. Nephron Physiol. 2009;111:30–8.
92. Carmines PK. The renal vascular response to diabetes. Curr Opin Nephrol Hypertens. 2010;19:85–90.
93. Vallon V, Richter K, Blantz RC, Thomson S, Osswald H. Glomerular hyperfiltration in experimental diabetes mellitus: potential role of tubular reabsorption. J Am Soc Nephrol. 1999;10:2569–76.
94. Hallow KM, Gebremichael Y, Helmlinger G, Vallon V. Primary proximal tubule hyperreabsorption and impaired tubular transport counterregulation determine glomerular hyperfiltration in diabetes: a modeling analysis. Am J Physiol Renal Physiol. 2017;312:F819–35.
95. Vallon V, Rose M, Gerasimova M, Satriano J, Platt KA, Koepsell H, et al. Knockout of Na-glucose transporter SGLT2 attenuates hyperglycemia and glomerular hyperfiltration but not kidney growth or injury in diabetes mellitus. Am J Physiol Renal Physiol. 2013;304:F156–67.
96. Cherney DZ, Perkins BA, Soleymanlou N, Maione M, Lai V, Lee A, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. Circulation. 2014;129:587–97.
97. Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, et al. Empagliflozin and progression of kidney disease in type 2 diabetes. N Engl J Med. 2016;375:323–34.
98. Thomson SC, Rieg T, Miracle C, Mansoury H, Whaley J, Vallon V, et al. Acute and chronic effects of SGLT2 blockade on glomerular and tubular function in the early diabetic rat. Am J Physiol Regul Integr Comp Physiol. 2012;302:R75–83.
99. Skrtic M, Yang GK, Perkins BA, Soleymanlou N, Lytvyn Y, von Eynatten M, et al. Characterisation of glomerular haemodynamic responses to SGLT2 inhibition in patients with type 1 diabetes and renal hyperfiltration. Diabetologia. 2014;57:2599–602.
100. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. N Engl J Med. 2019;380:2295–306.
101. Chade AR. Renal vascular structure and rarefaction. Compr Physiol. 2013;3:817–31.
102. Gnudi L, Thomas SM, Viberti G. Mechanical forces in diabetic kidney disease: a trigger for impaired glucose metabolism. J Am Soc Nephrol. 2007;18:2226–32.
103. Gruden G, Zonca S, Hayward A, Thomas S, Maestrini S, Gnudiet L, et al. Mechanical stretch-induced fibronectin and transforming growth factor-beta1 production in human mesangial cells is p38 mitogen-activated protein kinase-dependent. Diabetes. 2000;49:655–61.
104. Endlich K, Kliewe F, Endlich N. Stressed podocytes-mechanical forces, sensors, signaling and response. Pflugers Arch. 2017;469:937–49.

105. Kriz W, Lemley KV. A potential role for mechanical forces in the detachment of podocytes and the progression of CKD. J Am Soc Nephrol. 2015;26:258–69.
106. Schiffer M, Bitzer M, Roberts IS, Kopp JB, ten Dijke P, Mundel P, et al. Apoptosis in podocytes induced by TGF-beta and Smad7. J Clin Invest. 2001;108:807–16.
107. Inoki K, Mori H, Wang J, Suzuki T, Hong S, Yoshida S, et al. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. J Clin Invest. 2011;121:2181–96.
108. Wharram BL, Goyal M, Wiggins JE, Sanden SK, Hussain S, Filipiak WE, et al. Podocyte depletion causes glomerulosclerosis: diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. J Am Soc Nephrol. 2005;16:2941–52.
109. Durvasula RV, Petermann AT, Hiromura K, Blonski M, Pippin J, Mundel P, et al. Activation of a local tissue angiotensin system in podocytes by mechanical strain. Kidney Int. 2004;65:30–9.
110. Thomas MC, Tikellis C, Burns WM, Bialkowski K, Cao Z, Coughlan MT, et al. Interactions between renin angiotensin system and advanced glycation in the kidney. J Am Soc Nephrol. 2005;16:2976–84.
111. Tan ALY, Forbes JM, Cooper ME. AGE, RAGE, and ROS in diabetic nephropathy. Semin Nephrol. 2007;27:130–43.
112. Wolf G. New insights into the pathophysiology of diabetic nephropathy: from haemodynamics to molecular pathology. Eur J Clin Invest. 2004;34:785–96.
113. Zoja C, Xinaris C, Macconi D. Diabetic nephropathy: novel molecular mechanisms and therapeutic targets. Front Pharmacol. 2020;11:586892.
114. Barrera-Chimal J, Lima-Posada I, Bakris GL, Jaisser F. Mineralocorticoid receptor antagonists in diabetic kidney disease—mechanistic and therapeutic effects. Nat Rev Nephrol. 2022;18:56–70.
115. Bakris GL, Agarwal R, Anker SD, Pitt B, Ruilope LM, Rossing P, et al. Effect of finerenone on chronic kidney disease outcomes in type 2 diabetes. N Engl J Med. 2020;383:2219–29.
116. Tufro A, Veron D. VEGF and podocytes in diabetic nephropathy. Semin Nephrol. 2012;32:385–93.
117. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, et al. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest. 2003;111:707–16.
118. Sung SH, Chen S, Laping NJ, Ziyadeh FN. Albuminuria is ameliorated by an inhibitor of VEGF receptors, SU5416, in diabetic db/db mice but is unaffected in Smad3-knockout mice rendered diabetic with streptozotocin. J Am Soc Nephrol. 2004:(Abstract).
119. Sivaskandarajah GA, Jeansson M, Maezawa Y, Eremina V, Baelde HJ, Quaggin SE. VEGFA protects the glomerular microvasculature in diabetes. Diabetes. 2012;61:2958–66.
120. De Vriese AS, Tilton RG, Elger M, Stephan CC, Kriz W, Lameire NH. Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. J Am Soc Nephrol. 2001;12:993–1000.
121. Flyvbjerg A, Dagnaes-Hansen F, De Vriese AS, Schrijvers BF, Tilton RG, Rasch R. Amelioration of long-term renal changes in obese type 2 diabetic mice by a neutralizing vascular endothelial growth factor antibody. Diabetes. 2002;51:3090–4.
122. Majumder S, Advani A. VEGF and the diabetic kidney: more than too much of a good thing. J Diabetes Complications. 2017;31:273–9.
123. Bates DO. Vascular endothelial growth factors and vascular permeability. Cardiovasc Res. 2010;87:262–71.
124. Oltean S, Qiu Y, Ferguson JK, Stevens M, Neal C, Russell A, et al. Vascular endothelial growth factor-A165b is protective and restores endothelial glycocalyx in diabetic nephropathy. J Am Soc Nephrol. 2015;26:1889–904.
125. Garsen M, Lenoir O, Rops AL, Dijkman HB, Willemsen B, van Kuppevelt TH, et al. Endothelin-1 Induces proteinuria by heparanase-mediated disruption of the glomerular glycocalyx. J Am Soc Nephrol. 2016;27:3545–51.
126. de Zeeuw D, Coll B, Andress D, Brennan JJ, Tang H, Houser M, et al. The endothelin antagonist atrasentan lowers residual albuminuria in patients with type 2 diabetic nephropathy. J Am Soc Nephrol. 2014;25:1083–93.

127. Reeves WB, Andreoli TE. Transforming growth factor beta contributes to progressive diabetic nephropathy. Proc Natl Acad Sci USA. 2000;97:7667–9.
128. Ziyadeh FN. Evidence for the involvement of transforming growth factor-beta in the pathogenesis of diabetic kidney disease: are Koch's postulates fulfilled? Curr Pract Med. 1998;1:87–9.
129. Ziyadeh FN, Sharma K, Ericksen M, Wolf G. Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by autocrine activation of transforming growth factor-beta. J Clin Invest. 1994;93:536–42.
130. Yang CW, Vlassara H, Peten EP, He C-J, Striker GE, Striker LJ. Advanced glycation end products up-regulate gene expression found in diabetic glomerular disease. Proc Natl Acad Sci USA. 1994;91:9436–40.
131. Riser BL, Cortes P, Yee J, Sharba AK, Asano K, Rodriguez-Barbero A, et al. Mechanical strain- and high glucose-induced alterations in mesangial cell collagen metabolism: role of TGF-beta. J Am Soc Nephrol. 1998;9:827–36.
132. Fumo P, Kuncio GS, Ziyadeh FN. PKC and high glucose stimulate collagen alpha1(IV)transcriptional activity in a reporter mesangial cell line. Am J Physiol. 1994;267:F632–8.
133. Wolf G, Neilson EG. Angiotensin II as a hypertrophogenic cytokine for proximal tubular cells. Kidney Int Suppl. 1993;39:S100–7.
134. Sharma K, Ziyadeh FN, Alzahabi B, McGowan TA, Kapoor S, Kurnik BR, et al. Increased renal production of transforming growth factor-beta1 in patients with type II diabetes. Diabetes. 1997;46:854–9.
135. Ziyadeh FN, Hoffman BB, Han DC, Iglesias-De La Cruz MC, Hong SW, Isono M, et al. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal anti-TGF-β antibody in db/db diabetic mice. Proc Natl Acad Sci USA. 2000:8015–20.
136. Chen S, Kasama Y, Lee JS, Jim B, Marin M, Ziyadeh FN. Podocyte-derived vascular endothelial growth factor mediates the stimulation of alpha3(IV) collagen production by transforming growth factor-beta1 in mouse podocytes. Diabetes. 2004;53:2939–49.
137. Yu SM, Bonventre JV. Acute kidney injury and progression of diabetic kidney disease. Adv Chronic Kidney Dis. 2018;25:166–80.
138. Sabbisetti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A, et al. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. J Am Soc Nephrol. 2014;25:2177–86.
139. Gilbert RE. Proximal tubulopathy: prime mover and key therapeutic target in diabetic kidney disease. Diabetes. 2017;66:791–800.
140. Ziyadeh FN, Snipes ER, Watanabe M, Alvarez RJ, Goldfarb S, Haverty TP. High glucose induces cell hypertrophy and stimulates collagen gene transcription in proximal tubule. Am J Physiol. 1990;259:F704–14.
141. Wilson PC, Wu H, Kirita Y, Uchimura K, Ledru N, Rennke HG, et al. The single-cell transcriptomic landscape of early human diabetic nephropathy. Proc Natl Acad Sci USA. 2019;116:19619–25.
142. Grgic I, Campanholle G, Bijol V, Wang C, Sabbisetti VS, Ichimura T, et al. Targeted proximal tubule injury triggers interstitial fibrosis and glomerulosclerosis. Kidney Int. 2012;82:172–83.
143. Hasegawa K, Wakino S, Simic P, Sakamaki Y, Minakuchi H, Fujimura K, et al. Renal tubular Sirt1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes. Nat Med. 2013;19:1496–504.
144. Alicic RZ, Johnson EJ, Tuttle KR. Inflammatory mechanisms as new biomarkers and therapeutic targets for diabetic kidney disease. Adv Chronic Kidney Dis. 2018;25:181–91.
145. Pichler R, Afkarian M, Dieter BP, Tuttle KR. Immunity and inflammation in diabetic kidney disease: translating mechanisms to biomarkers and treatment targets. Am J Physiol Renal Physiol. 2017;312:F716–31.
146. Nelson PJ, Rees AJ, Griffin MD, Hughes J, Kurts C, Duffield J. The renal mononuclear phagocytic system. J Am Soc Nephrol. 2012;23:194–203.

147. Nguyen D, Ping F, Mu W, Hill P, Atkins RC, Chadban SJ. Macrophage accumulation in human progressive diabetic nephropathy. Nephrology (Carlton). 2006;11:226–31.
148. Furuta T, Saito T, Ootaka T, Soma J, Obara K, Abe K, et al. The role of macrophages in diabetic glomerulosclerosis. Am J Kidney Dis. 1993;21:480–5.
149. Wintroub BU, Schechter NB, Lazarus GS, Kaempfer CE, Schwartz LB. Angiotensin I conversion by human and rat chymotryptic proteinases. J Invest Dermatol. 1984;83:336–9.
150. Reilly CF, Tewksbury DA, Schechter NM, Travis J. Rapid conversion of angiotensin I to angiotensin II by neutrophil and mast cell proteinases. J Biol Chem. 1982;257:8619–22.
151. Navarro-Gonzalez JF, Jarque A, Muros M, Mora C, Garcia J. Tumor necrosis factor-alpha as a therapeutic target for diabetic nephropathy. Cytokine Growth Factor Rev. 2009;20:165–73.
152. Awad AS, You H, Gao T, Cooper TK, Nedospasov SA, Vacher J, et al. Macrophage-derived tumor necrosis factor-alpha mediates diabetic renal injury. Kidney Int. 2015;88:722–33.
153. McCarthy ET, Sharma R, Sharma M, Li JZ, Ge XL, Dileepan KN, et al. TNF-alpha increases albumin permeability of isolated rat glomeruli through the generation of superoxide. J Am Soc Nephrol. 1998;9:433–8.
154. Gohda T, Niewczas MA, Ficociello LH, Walker WH, Skupien J, Rosetti F, et al. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. J Am Soc Nephrol. 2012;23:516–24.
155. Brosius FC, Khoury CC, Buller CL, Chen S. Abnormalities in signaling pathways in diabetic nephropathy. Expert Rev Endocrinol Metab. 2010;5:51–64.
156. Lee EY, Chung CH, Khoury CC, Yeo TK, Pyagay PE, Wang A, et al. The monocyte chemoattractant protein-1/CCR2 loop, inducible by TGF-beta, increases podocyte motility and albumin permeability. Am J Physiol Renal Physiol. 2009;297:F85–94.
157. You H, Gao T, Raup-Konsavage WM, Cooper TK, Bronson SK, Reeves WB, et al. Podocyte-specific chemokine (C-C motif) receptor 2 overexpression mediates diabetic renal injury in mice. Kidney Int. 2017;91:671–82.
158. de Zeeuw D, Bekker P, Henkel E, Hasslacher C, Gouni-Berthold I, Mehling H, et al. The effect of CCR2 inhibitor CCX140-B on residual albuminuria in patients with type 2 diabetes and nephropathy: a randomised trial. Lancet Diabetes Endocrinol. 2015;3:687–96.
159. Tuttle KR, Brosius FC 3rd, Adler SG, Kretzler M, Mehta RL, Tumlin JA, et al. JAK1/JAK2 inhibition by baricitinib in diabetic kidney disease: results from a Phase 2 randomized controlled clinical trial. Nephrol Dial Transplant. 2018;33:1950–9.
160. Zeng C, Shi X, Zhang B, Liu H, Zhang L, Ding W, et al. The imbalance of Th17/Th1/Tregs in patients with type 2 diabetes: relationship with metabolic factors and complications. J Mol Med (Berl). 2012;90:175–86.