Chapter 1
Diabetic Kidney Disease

Mai Sugahara, Tetsuhiro Tanaka, Reiko Inagi, and Masaomi Nangaku

Abstract Renal senescence is accompanied by a gradual decrease in its function. Although it rarely causes clinical problems per se, superimposition of various diseases, such as diabetes, may accelerate this functional decline. Recent research has revealed some of the complex mechanisms of how diabetes promotes the aging process in the kidney, including the pathogenic roles of hemodynamic changes, tubulointerstitial hypoxia, oxidative stress, advanced glycation end-products, and impaired autophagy. Diabetes also modulates aging-related signaling pathways, such as sirtuins and mammalian target of rapamycin. Current therapeutic strategy for diabetic kidney disease consists of glycemic control and antihypertensive treatment with renin-angiotensin system inhibitors. However, they fail to fully prevent the progression of diabetic kidney disease, raising an urgent need for novel therapeutic methods. Some pharmacological agents are being developed based on the knowledge of hemodynamic and molecular basis of diabetes- and aging-related kidney function decline.

Keywords Diabetic kidney disease • Hypoxia • Oxidative stress • Advanced glycation end-products • Autophagy

1.1 Introduction

Aging is a universal process that affects all organs including the kidney. Even in healthy individuals, glomerular filtration rate (GFR) starts to decline at 30 years of age and proceeds at the rate of approximately 8 ml/min/1.73 m^2 per decade [1]. This loss in renal function is largely attributed to hemodynamic changes within the kidney and age-related vulnerability to physiological stress, such as hypoxia and oxidative stress.

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The rate of kidney function decline may increase with superimposition of various diseases. Diabetes is one of the strong drivers of this decline, and it accelerates the process of aging through several different mechanisms, such as hemodynamic changes, endothelial dysfunction, tubulointerstitial hypoxia, oxidative stress, accumulation of advanced glycation end-products (AGEs), and impaired autophagy. Diabetes also modulates some aging-related signaling pathways including sirtuins and mammalian target of rapamycin (mTOR). This chapter discusses the pathogenesis of diabetic kidney disease (DKD) in relation to aging process and introduces some potential therapeutic methods to cope with diabetes-related kidney function decline.

1.2 Hemodynamic Changes and Chronic Hypoxia at the Center of Aging Kidney and DKD

1.2.1 Vasculature of the Kidney (Fig. 1.1) [2]

The kidney has a unique spatial arrangement of vasculature; it has two capillary networks that run in series. The renal artery bifurcates into interlobar arteries, arcuate arteries, interlobular arteries, and afferent arterioles. The afferent arterioles give

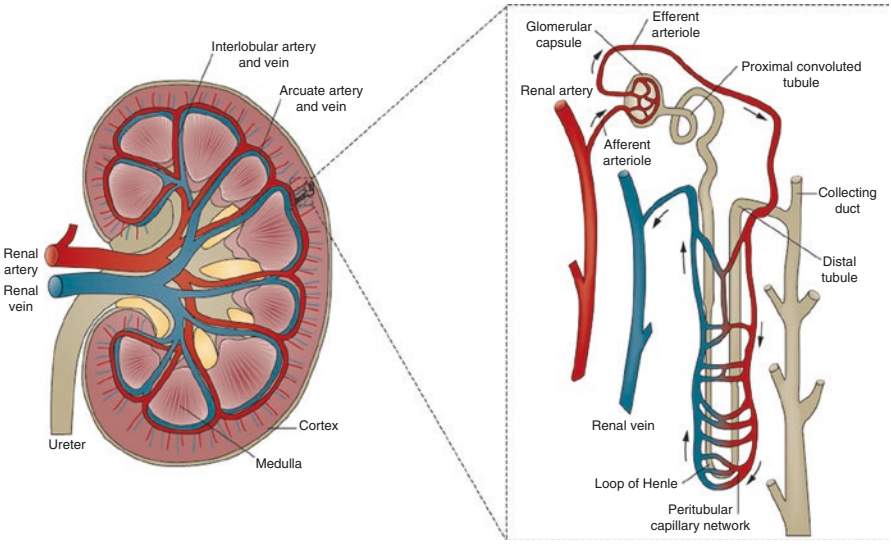

Fig. 1.1 Vasculature of the kidney. The renal artery bifurcates into interlobar arteries, arcuate arteries, interlobular arteries, and afferent arterioles. The afferent arterioles give rise to the first capillary network, the glomerulus, and they merge again at the vascular pole to form efferent arterioles. The efferent arterioles enter the second capillary network, the peritubular capillaries, which surround tubules and offer oxygen and nutrients to tubular and interstitial cells. The enlargement shows the architecture of a nephron with arrows indicating the direction of blood flow. Reprinted by permission from Macmillan Publishers Ltd.: Nat Rev. Nephrol 6: 667–78, © 2010

rise to the first capillary network, the glomerulus, and they merge again at the vascular pole to form efferent arterioles. The efferent arterioles enter the second capillary network, the peritubular capillaries (PTCs), which surround tubules and offer oxygen and nutrients to tubular and interstitial cells.

Although this anatomy is crucial for the regulation of renal blood flow (RBF), GFR, urine concentration, and other specialized kidney functions, arterial and venous vessels running in close parallel to each other gives rise to a diffusional oxygen shunt, which results in comparatively low oxygen tensions in the renal tissue. The renal medulla is especially prone to hypoxia, because its blood supply depends on vasa recta which emerge from juxtamedullary glomeruli and travel along the long loop of Henle.

1.2.2 Morphological and Hemodynamic Changes of Aging Kidney

Histological observations of kidneys from autopsies and nephrectomies have revealed some universal changes associated with aging (Table 1.1), not only in the glomerulus and tubules but also in the vessels. After 40 years of age, atherosclerotic changes become evident in the pre-glomerular arteries, i.e., interlobar arteries, arcuate arteries, and interlobular arteries, causing RBF to decrease at a rate of approximately 10% per decade [3]. This decrease is most profound in the renal cortex, resulting in cortical atrophy. The degree of atherosclerosis correlates with the degree of glomerulosclerosis [3], which is a common finding in the aging kidney, suggesting a hemodynamic role in the aging process.

In the arterioles, hyaline deposition within the vascular wall leads to obliteration of the lumen and is also related to glomerulosclerosis. There are two types of structural changes associated with arteriolopathy; it results in complete atrophy of the cortical glomeruli, whereas it forms a shunt between the afferent and efferent arterioles in the juxtamedullary nephrons (Fig. 1.2) [4]. The latter change increases the

Table 1.1 Histological changes in DKD and aging kidney

	DKD	Aging kidney
Glomerulus	Mesangial matrix expansion Uniform thickening of capillary wall Nodular lesion Diffuse lesion Exudative lesion (hyalinosis)	Focal and segmental glomerulosclerosis Hyaline deposition Thickening of glomerular basement membrane
Tubulointerstitium	Thickening of tubular basement membrane Tubular atrophy Fibrosis	Tubular atrophy Fibrosis
Vessels	Hyaline deposition in arterioles Atherosclerosis Loss of PTCs New vessel formation near the hilus	Hyaline deposition in arterioles Atherosclerosis Loss of PTCs Aglomerular arterioles

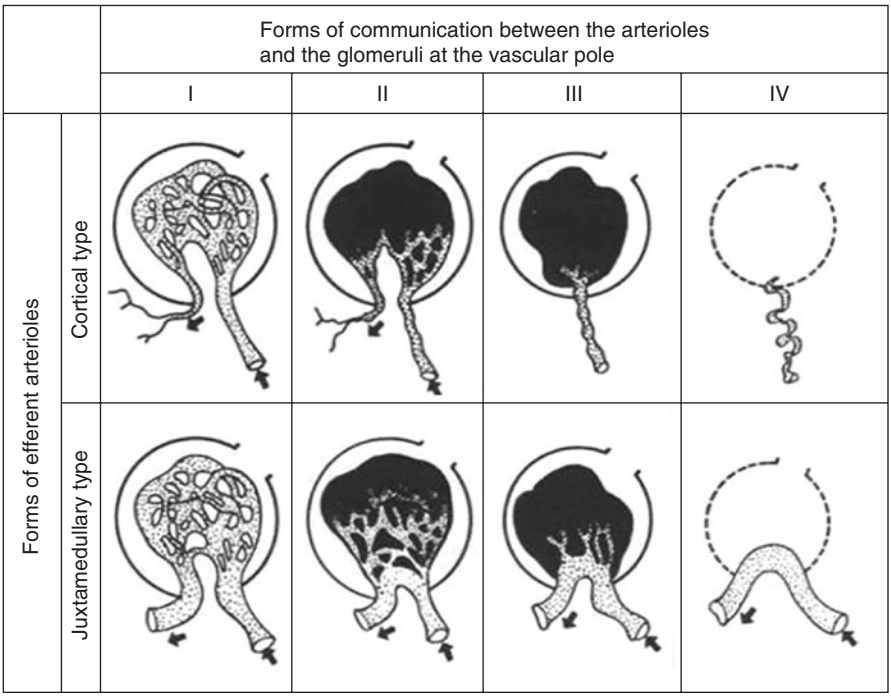

Fig. 1.2 Two types of structural changes associated with arteriolopathy. There are two types of structural changes associated with arteriolopathy; it results in complete atrophy of the cortical glomeruli, whereas it forms a shunt between the afferent and efferent arterioles in the juxtamedullary nephrons, resulting in aglomerular arterioles. Reprinted by permission from Elsevier: Kidney Int 2: 224–30, © 1972

blood flow to the medulla, resulting in a redistribution of blood flow from the cortex to the medulla.

As a consequence of glomerulosclerosis, the aging kidney fails to maintain PTCs, causing hypoxia in the tubulointerstitial area. Hypoxia has now been recognized as a common mediator to progressive kidney diseases [2, 5], and the same scenario also applies to the process of physiological aging. Experimentally, the kidneys from old rats were indeed exposed to extensive degrees of hypoxia [6].

1.2.3 Hemodynamic Changes of DKD

Diabetes is the leading cause of end-stage kidney disease (ESKD) and is associated with increased cardiovascular mortality. The earliest sign of DKD is persistent albuminuria, and even with appropriate glycemic control and antihypertensive treatment, a certain number of patients progress to overt proteinuria and eventually to renal failure. Histological changes typically observed in DKD are listed in Table 1.1.

As DKD is often referred to as one of the "microvascular" complications of diabetes, vascular dysfunction plays an important role in the pathogenesis of DKD.

Both arteries and arterioles invariably show the typical changes of arteriosclerosis and arteriolosclerosis, respectively. The arteries exhibit intimal thickening accompanied by reduplication of elastic lamina [7], the finding also observed in the aging kidney. Hyaline arteriolosclerosis is also a frequent and early manifestation of DKD. Arteriolopathy results in a maladaptive autoregulation that permits the transmission of systemic hypertension to glomerular capillaries. Moreover, diabetes disturbs vasoactive humoral systems which control the glomerular circulation. The balance between factors influencing the afferent arteriolar tone is shifted toward vasodilation, whereas opposite changes may occur on the efferent arterioles, resulting in glomerular hypertension and subsequent hyperfiltration [8].

Glomerular hypertension causes mechanical damage to the capillary wall, eventually leading to glomerulosclerosis and loss of PTCs. Elevated glomerular pressure also increases filtration of proteins to tubular lumen. Consequently, enhanced tubular reabsorption induces synthesis of pro-inflammatory and profibrotic factors, further resulting in tubulointerstitial inflammation and fibrosis. Fibrosis and loss of PTCs generate hypoxic state in associated tubulointerstitial areas, which is known as the common final pathway of progressive kidney diseases [2, 5]. These insights indicate that arterio- and arteriolosclerosis underlie the pathogenesis of both diabetic and aging kidney. This would further imply that diabetes accelerates the process of aging through hemodynamic changes and associated tubulointerstitial injury in the kidney.

1.3 Cellular and Molecular Biology of DKD in Relation to Aging

Hyperglycemia alters metabolic pathways and cellular environments; many of these changes are involved not only in the pathogenesis of DKD but also in renal senescence. The altered metabolism further contributes to the progression of hemodynamic changes and tubulointerstitial injury described above. In these regards, understanding the molecular basis of DKD may also help to unveil the aging process in the kidney.

1.3.1 Reactive Oxygen Species (ROS)

It has been generally accepted that the imbalance between antioxidant defense mechanism and ROS production leads to oxidative stress and subsequent pathological conditions including diabetic and aging kidney. ROS are chemically reactive molecules containing oxygen. Several ROS with unpaired electrons, such as superoxide, hydroxyl radicals, and lipid radicals, are considered as free radicals. Hydrogen

peroxide (H_2O_2), peroxynitrite ($ONOO^-$), and hydrochlorous acid (HOCl) are not free radicals but possess an oxidizing effect. ROS are usually considered cytotoxic, because they oxidize important macromolecules including proteins, lipids, carbohydrates, and nucleic acids, disrupting their physiological functions.

One pathogenic mechanism of ROS in relation to DKD and aging kidney is through inducing endothelial dysfunction and subsequent atherosclerosis [9]. For example, superoxide anions inactivate endothelium-derived NO by converting it to peroxynitrite. NO has important anti-atherosclerotic properties which include regulation of vascular tone and vessel wall permeability, suppression of leukocyte adhesion to the endothelial surface, inhibition of vascular smooth muscle cell migration, and proliferation; its inactivation inevitably results in atherosclerosis. In the kidney, reduced levels of NO leads to vasoconstriction and a subsequent decrease in PTC blood flow [2, 5]. Furthermore, ROS elicit inflammatory pathway, another important contributor in the pathogenesis of atherosclerosis.

Within the mammalian cell, there are mainly three sources of ROS; mitochondrion, endoplasmic reticulum (ER), and the enzyme systems in the cytosol, such as xanthine oxidase, lipoxygenase, and nicotinamide adenine dinucleotide phosphatase oxidase (NOX) [10]. The traditional view has been that production of ROS would generate harmful effects, regardless of the cell types or their source. However, recent findings revealed that some ROS are essential in specific intracellular signaling pathways and actually function in a beneficial manner.

The ROS molecules whose functions are extensively reevaluated include superoxide produced in mitochondria. It has been assumed that excess glucose uptake would lead to an increase in pyruvate entry into mitochondria and an increased flux of substrates to the electron transport chain, resulting in hyperpolarization of the mitochondrial membrane and accumulation of electrons at complex III and coenzyme Q, which donate electrons to molecular oxygen and generate superoxide anions. This pathway has been considered as the central source of ROS in the pathogenesis of DKD. However, a new *in vivo* method of measuring superoxide via *in vivo* administration of dihydroethidium (DHE) to mice revealed that superoxide was actually reduced in the kidney in both streptozotocin (STZ)-induced diabetic mice and Ins2-Akita mice [11]. Mice with reduced superoxide dismutase (SOD) 2, an antioxidant enzyme, did show increased renal superoxide production, but the degrees of mesangial expansion or albuminuria were comparable to the wild-type mice when they were made diabetic. Furthermore, a stimulation of mitochondrial biogenesis with adenosine monophosphate kinase (AMPK) activation resulted in an increased superoxide production, accompanied by reduced mesangial matrix and albuminuria in STZ-induced diabetic mice [11].

These observations led to the proposal of a new theory, "mitochondrial hormesis," whereby a "physiological" increase in mitochondrial superoxide is actually beneficial, whereas excessively high levels of superoxide and/or decreased superoxide may contribute to the disease progression or be a permissive factor for inflammation and fibrosis [10]. The same applies to the process of aging. It has been proposed that ROS in general contributed to chronic organ damage in the elderly,

but some recent studies provided compelling results. For example, exposure of *Caenorhabditis elegans* to 2-deoxyglucose (DOG), an inhibitor of glycolysis, increased mitochondrial ROS and increased life span. In the same study, co-administration of N-acetylcysteine with 2-DOG reduced ROS production and the benefit in life span extension [12].

Overall, the way oxidative stress contributes to the pathogenesis of DKD and aging kidney might be more complex than initially thought. A further investigation is required to unveil these two sides of ROS.

1.3.2 AGEs

AGEs are a heterogeneous group of bioactive molecules that are formed by the nonenzymatic glycation of proteins, lipids, and nucleic acids. In diabetes, hyperglycemia and oxidative stress promote the reversible glycation of target substrates, forming Schiff bases. The following chemical rearrangement forms the more stable early glycated product, Amadori product. Then, the slow complex rearrangement finally proceeds to the formation of AGEs. This final process is irreversible, resulting in the accumulation of AGEs in diabetes [7, 8] (Fig. 1.3a).

AGEs contribute to the pathogenesis of DKD in both receptor-independent and dependent manners [8] (Fig. 1.3b). The former includes modification of extracellular matrix (ECM) structure by cross-linking of proteins such as collagen and elastin. This process increases ECM rigidity and its resistance to proteolytic degradation, leading to mesangial matrix accumulation and interstitial fibrosis. Cross-linking of matrix proteins also increases permeability of glomerular basement membranes.

In addition to altering the structure of extracellular proteins, AGEs exert their effects through various specific cell surface receptors, collectively referred to as "RAGE," receptor for AGEs. The AGE-RAGE interaction stimulates multiple intracellular signaling pathways which contribute to the pathophysiology of DKD; for example, it activates NOX and increases cytosolic production of ROS. It also activates several nuclear transcription factors, such as nuclear factor kappa-B (NF-κB). The subsequent cellular responses include production of cytokines, growth factors, and cell adhesion molecules, promoting inflammation and fibrosis [8].

Recent evidence suggests that AGEs are also involved in renal senescence. A cross-sectional study of older community-dwelling women showed that higher levels of serum carboxymethyl-lysine (CML), a common AGE, correlated with reduced GFR [13]. Experimentally, overexpression of glyoxalase I (GLO1), an enzyme which detoxifies precursors of AGEs, was shown to ameliorate age-related interstitial thickening as well as serum creatinine increase in aged rats [14]. A part of this anti-aging effect arises from preserved endothelial function. In GLO1 transgenic rats, NO production in endothelial cells was well maintained even at 53 weeks of age [15]. Therefore, AGEs may be a novel therapeutic target of both DKD and aging kidney.

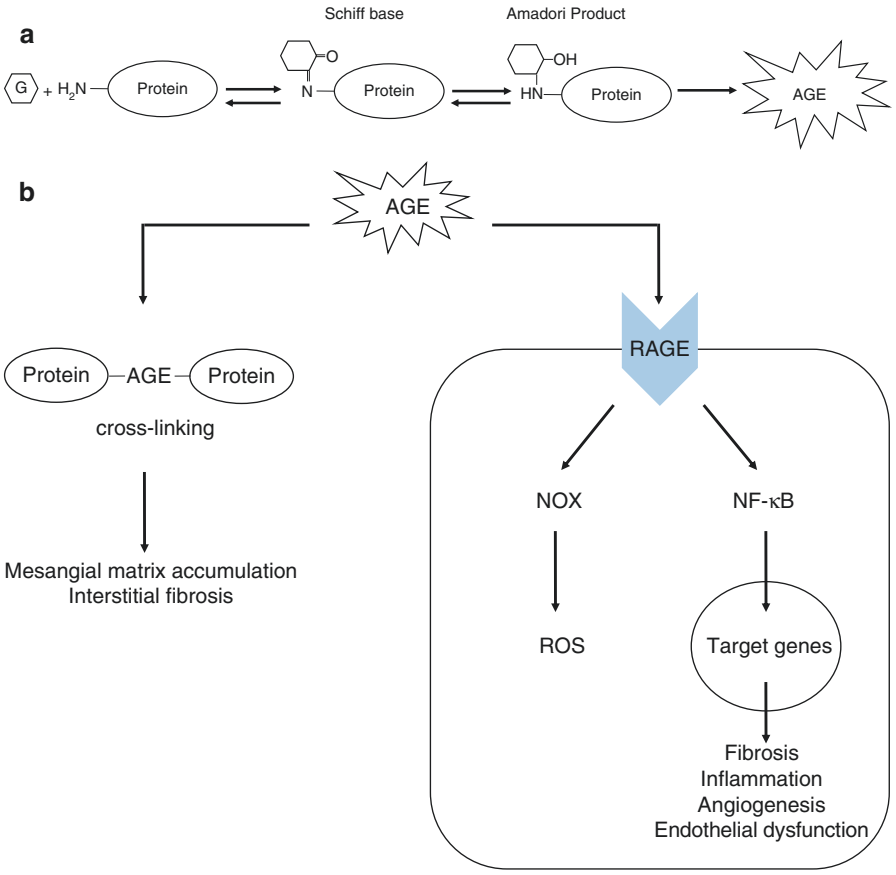

Fig. 1.3 (**a**) AGE formation. AGEs are formed by nonenzymatic Maillard reaction between carbonyl groups of reducing sugars and amino groups on proteins, lipids, or nucleic acids. The rates of these reactions are slow under physiological conditions, but are accelerated under persistent hyperglycemia, dyslipidemia, and oxidative stress. (**b**) Effects of AGEs. AGEs contribute to the pathogenesis of DKD in both receptor-independent and dependent manners. AGEs form cross-links between ECM proteins, which increase ECM rigidity and its resistance to proteolytic degradation, leading to mesangial matrix accumulation and interstitial fibrosis. AGEs also modulate cellular activities through RAGE. The AGE-RAGE interaction activates NOX and increases cytosolic production of ROS. It also activates several nuclear transcription factors, including NF-κB. However, this drawing shows only a part of the complex mechanism of how AGEs contribute to the pathogenesis of DKD

1.3.3 Impaired Autophagy

Autophagy is a "self-eating" pathway by which cells degrade macromolecules and organelles to maintain intracellular homeostasis. It has two major physiological roles; one is to recycle intracellular resources in response to conditions of nutrient

deficiency, and the other is to remove damaged proteins and organelles under various stress conditions [16]. Accumulating evidence has shown that autophagy is impaired in both diabetic and aging kidney, making renal cells more vulnerable to pathological stresses such as ROS, AGEs, and hypoxia.

Transgenic mice systemically expressing green fluorescent protein (GFP)-labeled LC-3, a marker protein for autophagosomes, revealed a high basal level of autophagy in podocytes [17], and its inhibition was detrimental to podocytes' cytoskeleton [18]. Hyperglycemia was shown to reduce autophagic activity [18], indicating a pathogenic role of impaired autophagy in DKD. Indeed, mice with podocyte-specific deletion of autophagy-related 5 (*Atg5*) developed podocyte loss and massive proteinuria in a high-fat diet-induced diabetic model [19].

Autophagy is also essential in maintaining homeostasis in renal tubular cells. Unlike podocytes, the basal autophagic activity in proximal tubular cells is low [16]. However, insults to the kidney, such as ischemia-reperfusion and cisplatin, resulted in increased autophagy [20, 21]. In these studies, mice lacking *Atg5* specifically in proximal tubular cells exhibited severer acute kidney injury (AKI), suggesting renoprotective effects of autophagy. Proteinuria is also known to exert nephrotoxic stress on tubular cells, stimulating autophagy. However, in animal models of diabetes, autophagy was suppressed in proximal tubules, which was accompanied by exacerbation of proteinuria-induced tubulointerstitial damage [22]. The investigation of human kidney biopsy also revealed lower autophagic activity in type 2 diabetic patients [22]. These findings may indicate that insufficient upregulation of autophagy underlies the tubulointerstitial injury in DKD.

The association of autophagy with aging has also been extensively studied; it is generally considered that autophagic activity declines with aging, leading to accumulation of damaged proteins and organelle within cells, which subsequently results in functional deterioration in the elderly. In the field of nephrology, mice with podocyte-specific deletion of *Atg5* were shown to develop a higher level of albuminuria and a significant increase in glomerulosclerosis at the age of 20–24 months compared with control littermates [23]. Moreover, accumulating body of evidence has revealed that some life span-related signaling pathways, including mTOR and sirtuins, regulate autophagy in the kidney, casting an influence on the progression of DKD and renal senescence (discussed in the next section).

1.4 Signaling Pathways Related to DKD and Aging Kidney

Research into the aging process has revealed some key signaling pathways associated with life span, such as mTOR-, sirtuin-, insulin-like growth factor (IGF)-, and Klotho-related pathways. Diabetes may shorten patients' lives by modulating these pathways [24]. This section describes some of the proposed relationships between mTOR or sirtuin signaling and kidney aging in diabetic patients.

1.4.1 mTOR Signaling Pathways

mTOR is an evolutionarily conserved serine-threonine kinase that regulates cell growth, proliferation, and metabolism. mTOR forms at least two distinct functional complexes, called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [25]. Inhibition of mTOR increases life span of animals, and calorie restriction (CR) is considered to induce longevity by downregulating mTOR [24].

Although both mTORC1 and mTORC2 regulate a number of different downstream pathways, the most extensively studied in relation to DKD is mTORC1's effect on autophagy. mTORC1 negatively regulates autophagy by inhibiting the activity of the Unc-51-like kinase 1 (ULK1), one of the autophagy initiating kinases, through direct phosphorylation [26]. Enhanced mTORC1 activity is observed in human patients and animal models of diabetes [26], which may be one of the causes of impaired autophagy discussed above. Interestingly, nondiabetic mice with podocyte-specific activation of mTORC1 also manifested mesangial expansion, glomerular basement membrane thickening, and proteinuria, all of which resemble DKD [27]. Moreover, treatment with rapamycin, an inhibitor of mTORC1, suppressed the development of DKD in STZ-induced diabetic rats and db/db mice, suggesting the important pathogenic role of mTORC1 in DKD [26].

1.4.2 Sirtuins

Sirtuins are another family of molecules associated with life span of organisms. One of the members, silent information regulator 2 (SIR2), was originally found to promote longevity in yeast. There are seven mammalian homologues of SIR2, SIRT1–7, of which SIRT1 most closely resembles SIR2. SIRT1 was initially recognized as an NAD-dependent histone deacetylase, which induces chromatin silencing and transcriptional repression. However, recent studies have revealed that its targets are not confined to histones; SIRT1 regulates a wide variety of cellular processes, including glucose and lipid metabolism, mitochondrial biogenesis, inflammation, and autophagy, which are largely organ protective [28].

SIRT1 is also considered to have an anti-aging effect in the kidney. Kume et al. reported a decreased expression of SIRT1 in 24-month-old mice, which was recovered by long-term CR, attenuating hypoxia-associated mitochondrial damage [29]. SIRT1 deacetylases forkhead box O3 (FOXO3), a transcription factor regulating the expression of BCL2/adenovirus E1B 19-kDa interacting protein 3 (Bnip3), which is an essential component of autophagy. Taken together, SIRT1 deficiency underlies the impaired autophagic activity in the aging kidney, resulting in decreased cellular resistance to stress conditions, such as hypoxia. Furthermore, an accumulating body of evidence has shown anti-apoptotic, anti-inflammatory, and anti-fibrotic effects of SIRT1, suggesting multiple pathways by which SIRT1 deficiency promotes the process of kidney aging [28].

A decrease in SIRT1 protein expression has also been observed in both type 1 and type 2 diabetic animal models [26]. In addition to its effect on renal cells, SIRT1 modulates glucose and lipid metabolism through various mechanisms; it promotes insulin secretion, improves insulin resistance, and increases adiponectin excretion [30]. Therefore, activation or upregulation of SIRT1 may serve as an effective therapeutic strategy against DKD.

1.5 Potential Renoprotective Treatments for DKD

Current therapeutic strategy for DKD consists of glycemic control and antihypertensive treatment with renin-angiotensin system (RAS) inhibitors. However, they fail to fully prevent the progression of kidney function decline, emphasizing the need for novel therapies. This section explains how RAS inhibitors slow the progression of DKD and introduces some potential therapeutic methods that are being developed based on new knowledge of the pathogenesis of DKD.

1.5.1 RAS Inhibitors

RAS blockade induces vasodilation of glomerular arterioles; the degree of dilatation is greater in the efferent arterioles, resulting in reduced intraglomerular pressure and, thus, less damage to the capillary walls. RAS inhibitors also restore PTC blood flow and subsequently improve oxygenation of the tubulointerstitium. In addition, RAS inhibitors have an important role as antioxidants, ameliorating oxidative stress induced by hyperglycemia [2, 5]. Furthermore, RAS inhibitors contribute to reorganization of functional molecules composing the slit diaphragm, which maintains the barrier function of glomerular capillary walls and reduces proteinuria [31].

1.5.2 Sodium-Glucose Cotransporter-2 (SGLT2) Inhibitors

SGLT2 inhibitors are another family of drugs that may slow the progression of DKD by restoring intrarenal hemodynamics. SGLT2 is located in the early proximal tubule and couples glucose reabsorption to sodium reabsorption. Since it reabsorbs 80–90% of filtered glucose, SGLT2 blockade results in glucose excretion into urine and a subsequent decrease in blood glucose levels.

Although developed as a new class of antidiabetic agent, SGLT2 inhibitors slow the progression of DKD possibly by a mechanism independent of their blood glucose lowering effects. In a randomized controlled trial, EMPA-REG OUTCOME trial, patients treated with empagliflozin revealed a significantly smaller decrease in estimated GFR (eGFR) during the 192-week interventional period compared to the

placebo-treated group [32]. It has been postulated that SGLT2 inhibition results in increased sodium delivery to the juxtaglomerular apparatus, which subsequently leads to vasoconstriction of afferent arterioles and lowering of intraglomerular pressure through the mechanism of tubuloglomerular feedback. Other effects, such as those on arterial stiffness, vascular resistance, serum uric acid levels, and the systemic and renal neurohormonal systems may also contribute to the renoprotective effects of SGLT2 inhibitors [32]. Additionally, some studies point to an increasing trend in serum erythropoietin (EPO) levels by SGLT2 inhibitors [33], which may also contribute to the protective effect. At cellular levels, hyperglycemia accelerates tubular senescence through upregulation of p21 expression; this upregulation was also attenuated by SGLT2 knockdown in an *in vitro* experiment [34].

1.5.3 Prolyl Hydroxylase Domain (PHD) Inhibitors

As mentioned earlier, tubulointerstitial hypoxia underlies the pathogenesis of progressive kidney diseases, including DKD. On the other hand, cells possess several mechanisms for adapting themselves to hypoxic conditions. Facilitating these responses may mitigate detrimental consequences of hypoxia and possibly induce renoprotective effects.

Hypoxia-inducible factor (HIF) lies at the center of these adaptive responses to hypoxia. HIF is a heterodimeric transcription factor composed of an oxygen-dependent α-subunit and a constitutively expressed β-subunit. In hypoxic conditions, HIF upregulates its target genes, which are involved in angiogenesis, erythropoiesis, glycolysis, and cell survival. Cells continuously synthesize HIF-α subunits, and their transcriptional activity is primarily controlled by the rate of degradation. Under normoxic conditions, oxygen-dependent HIF-α degradation is initiated by PHD enzymes. They use molecular oxygen to hydroxylate HIF-α at specific proline residues, and these proline-hydroxylated HIF-α is recognized by the von Hippel-Lindau (VHL)-E3 ubiquitin ligase complex, resulting in ubiquitination of HIF-α. Ubiquitinated HIF-α is then destroyed by the proteasome. Under hypoxic conditions, hydroxylation of HIF-α is inhibited, allowing it to translocate to the nucleus where it dimerizes with HIF-β and enhances transcription of target genes (Fig. 1.4) [2, 5].

Inhibition of PHD enzymes, therefore, would increase the expression of HIF-target genes including EPO. Based on these findings, small-molecule inhibitors of PHDs have been developed as a new therapeutic method for anemia in chronic kidney disease (CKD) patients. At least six compounds are currently tested in clinical trials, most of them showing promising results [35]. There are some expectations that these PHD inhibitors would also ameliorate CKD; however, preclinical studies present inconclusive results. HIF activation by cobalt reduced proteinuria and tubulointerstitial damage in STZ-induced diabetic rats [36], while proximal tubular deletion of HIF-1α ameliorated fibrosis in the unilateral ureteral obstruction model, suggesting a profibrotic role of HIF [37]. Along similar lines, overexpression of

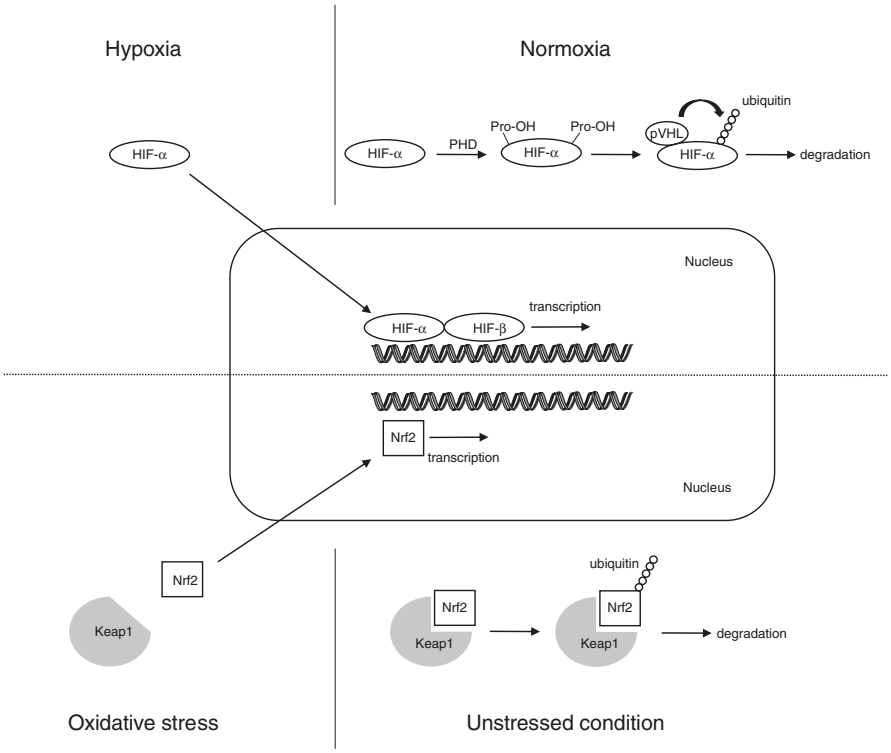

Fig. 1.4 Cellular defense mechanisms against hypoxia and oxidative stress. (*Upper panel*) Under normoxic conditions, HIF-α is hydroxylated at specific proline residues by PHD enzymes. Proline-hydroxylated HIF-α is recognized by VHL proteins, resulting in ubiquitination of HIF-α. Ubiquitinated HIF-α is then destroyed by the proteasome. Under hypoxic conditions, hydroxylation of HIF-α is inhibited, allowing them to translocate to the nucleus where they dimerize with HIF-β and induces transcription of target genes. (*Lower panel*) Under unstressed conditions, Nrf2 is continuously ubiquitinated by Keap1 and degraded within the proteasome. Under oxidative stress, ubiquitination of Nrf2 is inhibited, allowing it to translocate to the nucleus where it enhances transcription of target genes

HIF-2α in distal tubules using an ectopic promoter resulted in fibrogenesis [38]. Further studies are required to clarify the optimal timing and cell types of HIF activation as well as the specific PHD subtypes to inhibit, which would generate desirable renoprotective effects in CKD including DKD.

1.5.4 Bardoxolone Methyl

Oxidative stress, another key player in the pathogenesis of DKD, is also a target of new therapeutic strategy. Similar to the PHD-HIF axis against hypoxic stress, cells have antioxidant mechanism called the Keap1-Nrf2 pathway (Nrf2: nuclear factor-erythroid

2-related factor 2). Nrf2 is a transcription factor which regulates the expression of several antioxidant and cytoprotective genes. Its level is primarily regulated by the rate of degradation by Keap1. Under unstressed conditions, Nrf2 is continuously ubiquitinated by Keap1 and degraded within the proteasome. Under oxidative stress, on the other hand, ubiquitination of Nrf2 is inhibited, allowing it to translocate to the nucleus where it enhances transcription of target genes (Fig. 1.4) [39].

Bardoxolone methyl is a potent inducer of Nrf2 originally developed as an anticancer drug. In two phase I clinical trials with 81 oncology patients, however, it showed unexpected improvement in eGFR and was further developed as a renal drug [39, 40]. In a phase II clinical trial with 20 patients whose eGFR were between 15 and 45 ml/min/1.73 m^2, bardoxolone methyl did improve renal function with only mild side effects [40]. Unfortunately, the subsequent phase III BEACON trial enrolling patients with stage 4 CKD and type 2 diabetes had to be terminated prematurely because of a higher rate of cardiovascular events in the drug-treated group [41]. However, secondary analysis revealed that the elevated baseline B-type natriuretic peptide and previous hospitalization for heart failure were the risk factors for cardiovascular events related to bardoxolone methyl; for patients without these baseline characteristics, the risk was in fact similar between the bardoxolone methyl- and placebo-treated patients [42]. In addition, the prevalence of cardiovascular diseases in the Japanese population tends to be lower than that in the American and other Western populations, suggesting that it is relatively safe to continue a clinical trial in Japan, provided that patients at risk are excluded from the study. A phase II clinical trial involving patients with stage 3 CKD and type 2 diabetes is currently underway (TSUBAKI study).

1.6 Conclusion

Although numerous efforts have been made to find a new therapeutic method to treat DKD, it continues to be the leading cause of ESKD worldwide. Recent research into pathogenesis of DKD has revealed that much of its molecular mechanism is shared with that of renal senescence. In addition, diabetes induces atherosclerosis and subsequent hemodynamic changes, which also accelerate the aging process within the kidney. This highlights the importance of unveiling the influence of both diabetes and aging in the field of nephrology, especially in a highly aging society.

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