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REVIEW ARTICLE

Mechanisms of metabolic memory and renal hypoxia as a therapeutic target in diabetic kidney disease

Yosuke Hirakawa, Tetsuhiro Tanaka, Masaomi Nangaku*

Division of Nephrology and Endocrinology, the University of Tokyo School of Medicine, Tokyo, Japan

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***Correspondence**
Masaomi Nangaku
Tel.: +81-3-3815-5411
Fax: +81-3-5800-8806
E-mail address:
mnangaku-tky@umin.ac.jp

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ABSTRACT

Diabetic kidney disease (DKD) is a worldwide public health problem. The definition of DKD is under discussion. Although the term DKD was originally defined as 'kidney disease specific to diabetes,' DKD frequently means chronic kidney disease with diabetes mellitus and includes not only classical diabetic nephropathy, but also kidney dysfunction as a result of nephrosclerosis and other causes. Metabolic memory plays a crucial role in the progression of various complications of diabetes, including DKD. The mechanisms of metabolic memory in DKD are supposed to include advanced glycation end-products, deoxyribonucleic acid methylation, histone modifications and non-coding ribonucleic acid including micro ribonucleic acid. Regardless of the presence of diabetes mellitus, the final common pathway in chronic kidney disease is chronic kidney hypoxia, which influences epigenetic processes, including deoxyribonucleic acid methylation, histone modification, and conformational changes in micro ribonucleic acid and chromatin. Therefore, hypoxia and oxidative stress are appropriate targets of therapies against DKD. Prolyl hydroxylase domain inhibitor enhances the defensive mechanisms against hypoxia. Bardoxolone methyl protects against oxidative stress, and can even reverse impaired renal function; a phase 2 trial with considerable attention to heart complications is currently ongoing in Japan.

INTRODUCTION

Diabetic kidney disease (DKD) is one of the complications of diabetes mellitus, and is the major cause of end-stage kidney disease in developed countries[1,2]. In past years, diabetic patients suffered from classical diabetic nephropathy (DN), which starts with glomerular hyperfiltration, followed by microalbuminuria, macroalbuminuria and nephrotic-range proteinuria with a decrease in glomerular filtration and eventual development of end-stage kidney disease. However, improvements of blood glucose control, utilization of renin–angiotensin blockers and an increase in the aging population have changed the phenotypes of DKD. A recent comparison of type 1 diabetes patients with a control population in Finland revealed the absence of 'glomerular hyperfiltration' these days[3]. Among adults with diabetes in the USA, the overall prevalence of kidney disease did not change significantly, but that of albuminuria declined and that of reduced estimated glomerular filtration rate (eGFR) increased[4]. These show that the role of classical DN in the pathogenesis of DKD is decreasing, whereas that of glomerulosclerosis as a result of aging, atherosclerosis and so on is increasing. The definition of DKD is still under discussion. The term DKD was originally used as a reference to 'kidney disease specific to diabetes'[5]. Although DKD is still used as its original meaning,[2] and sometimes clearly separated from glomerulosclerosis[6], nowadays the term DKD frequently covers classical DN and other types of kidney dysfunction in diabetic patients, and is currently the term being used to reflect the changes in kidney disease phenotypes of diabetes patients. DN was originally a pathological definition that was characterized by mesangial expansion, nodular glomerular sclerosis and tubulointerstitial fibrosis[7,8]. Now, DN is frequently diagnosed without renal biopsy, usually in patients with long-standing diabetes mellitus, absence of hematuria and existence of diabetic retinopathy. Therefore, the definition of DN is a part of DKD, which has a wide heterogeneity (Figure 1).

Recent large clinical trials on DKD included patients with kidney diseases other than the classical DN; the inclusion

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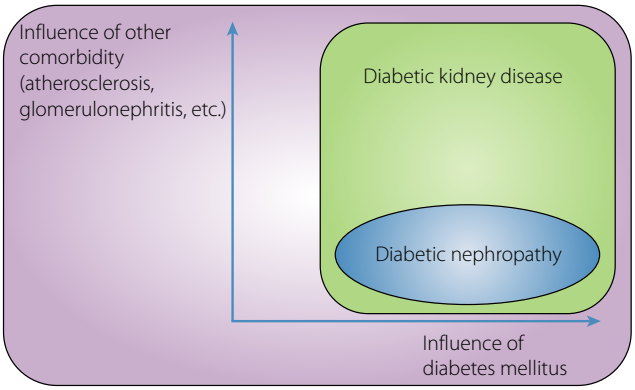

Figure 1 | A Venn diagram of the notions of diabetic kidney disease and diabetic nephropathy. Diabetic nephropathy, which was originally a pathological diagnosis, includes only patients or animals whose kidney damage was obviously from diabetes mellitus. In contrast, diabetic kidney disease means chronic kidney disease with diabetes, regardless of other comorbidities, such as atherosclerosis and glomerulonephritis. Therefore, diabetic kidney disease encompasses diabetic nephropathy.

criteria in these trials did not require proof of DN by renal biopsy, absence of hematuria, duration of diabetes mellitus or existence of diabetic retinopathy in type 2 diabetes mellitus patients[9–13]. Diabetic animal models have been utilized, but these have rarely shown the classic pathological changes in DN[14–18]. These differences must be taken into consideration when comparing clinical studies and animal experiments.

METABOLIC MEMORY

Accumulating evidence clarifies the involvement of various factors in the pathogenesis of DKD; these factors include high blood glucose, activation of the renin–angiotensin system, oxidative stress, increase in advanced glycation end-products (AGEs) and glomerular hyperfiltration[19,20]. In the clinical course of DKD, there is a famous phenomenon called 'metabolic memory'[21,22], which indicates the long-term effect of glycemic control on diabetic complications including DKD. Further understanding and clarification of this mechanism have drawn increasing attention. AGEs and epigenetic changes are candidates for the underlying mechanism of metabolic memory[23,24], as both AGE accumulation and epigenetic changes are prolonged phenomena.

ADVANCED GLYCATION END-PRODUCTS AND THEIR DETOXIFIER, GLYOXALASE 1

AGE is a collective term for compounds formed through non-enzymatic glycation of proteins and nucleic acids[25]. The rate of AGE formation is affected by inflammation and oxidative stress, in addition to plasma glucose level[26]. AGEs are chemically stable and are toxic to living organs, either by direct means or through receptors for AGEs (RAGE). AGEs can directly bind and produce degenerative changes in extracellular matrix protein, such as collagen. AGEs can also bind to RAGE to increase oxidative stress and activate nuclear factor-κB and the subsequent inflammatory pathways[27,28]. Therefore, accumulation of AGE is considered to be the cause of diabetic complications.

Indeed, skin collagen glycation, which is correlated with complications of type 1 diabetes mellitus[29], is reduced under strict glycemic control. Surprisingly, this difference in skin AGEs affects the long-term risk for diabetic complications for as long as 10 years[30]. Although an invasive procedure, AGEs on skin biopsy were used in past research to estimate whole-body AGEs. Later on, non-invasive techniques to assess skin AGE accumulation were developed, an example of which is the measurement of skin autofluorescence that is unique to AGEs, which emit strong fluorescence at a wavelength of 440 nm when excited at 370 nm[31,32]. Recently, the measurement of skin autofluorescence has attracted major concern, and has been shown to be related to the risk of developing complications in diabetes patients[33,34]. Skin autofluorescence was reported to correlate with all-cause mortality and vascular diseases in CKD patients[35–37], suggesting that whole-body AGE accumulation is closely related to the prognosis of DKD.

In rats, AGEs were shown to cause direct kidney injury, and induce albuminuria, glomerular sclerosis, basement membrane widening and mesangial matrix expansion[38]. Furthermore, as the kidney is the major site of AGE clearance, renal dysfunction results in AGE accumulation[39]. Therefore, renal dysfunction and AGE accumulation potentiate each other, and reduction of AGEs is a promising strategy against renal dysfunction, including DKD.

Glyoxalase 1 (GLO-1) is a detoxifier of AGEs. Overexpression of GLO-1 gene was shown to ameliorate diabetic kidney changes in streptozotocin-induced type 1 diabetes mellitus in rats[40]. In contrast, *in vivo* knockdown of the *GLO-1* gene in non-diabetic mice resulted in DN-like phenotypes, such as albuminuria, glomerular enlargement, mesangial matrix expansion and basement membrane thickening without diabetes[41]. Taken together, changes in GLO-1 activity and subsequent changes in AGE content might be closely related with the development of DN.

EPIGENETICS IN DIABETIC KIDNEY DISEASE

Epigenetics indicates persistent changes in gene expression and the resulting phenotypic changes, which occur without changes in deoxyribonucleic acid (DNA) sequence. Epigenetic changes include DNA methylation, histone modification, non-coding ribonucleic acids (ncRNAs) and chromosome conformational changes[42,43]. As these epigenetic changes have long-term effects, they are likely to play a critical role in metabolic memory.

DNA methylation

DNA methylation occurs mainly at the 5′ cytosines of CpG dinucleotides. In general, DNA methylation at the promoter region results in gene repression[44]. Although the status can

differ among various organs, DNA methylation in whole blood or circulating monocytes was estimated to reflect whole-body DNA methylation status in diabetes patients, although DNA methylation status can differ among various organs[45–47]. Therefore, investigation of DNA methylation, at least in circulating cells, can be done in DKD patients. Recently, Chen *et al.*[46] have shown that changes in DNA methylation status in circulating cells of diabetic patients persisted for over 15 years. They investigated whole blood and blood monocytes, from which 12 annotated and differentially methylated loci, including hypomethylation of thioredoxin-interacting protein (T*XNIP*), were extracted. As systemic knockout of the *TXNIP* gene is known to ameliorate streptozotocin-induced DN[48], persistent hypomethylation in this gene could render the patients vulnerable to DKD. In contrast, Ko *et al.*[49] examined the DNA methylation status in the tubules of surgically resected human kidneys. As DNA methylation, like other epigenomes, is cell-specific, they microdissected tubules from an entire kidney of DKD patients, and discovered the presence of hypomethylation of the *RUNX1* gene and upregulation of *RUNX1*.

Several interesting changes in DNA methylation have been shown in DKD model mice. Hasegawa *et al.*[50] reported that DN phenotypes of podocytopathy were aggravated in tubular-specific Sirtuin 1 (Sirt1) knockout diabetic mice, and ameliorated in SIRT1 transgenic diabetic mice. One interesting point of this research was the proof that proximal tubules affect glomeruli. Another point was that the SIRT1 transgene might protect the kidney, especially the podocytes, from diabetic insult through claudin-1 hypermethylation. They showed that the SIRT1 transgene suppressed claudin-1 expression and increased methylated CpG, and that claudin-1 aggravated diabetic podocytopathy in microdissected tubules of diabetic mice. The SIRT1 is a histone deacetylase that is well known to have a role against histones, not DNA. Although there was a report showing that SIRT1 activated DNA methyltransferase 1, which binds to hemi-methylated CpG sites and methylates CpG[51], the mechanism of DNA hypomethylation resulting from SIRT1 overexpression remains obscure. Another report focused on the temporal profile of DNA methylation changes in purified proximal tubules of diabetic mice kidneys, which were prepared using a cell sorter[52]. They identified several aberrant hypomethylation and hypermethylation sites, and highlighted the hypomethylation of the *Agt* gene, which is known to persist after pioglitazone antidiabetic therapy. As the Agt protein, or angiotensinogen, in proximal tubular cells is closely related to the progression of DN[53], changes in its methylation status must have a role in metabolic memory, at least in mice.

Histone modifications

DNA is packaged in the chromatin, which is composed of the nucleosome as its fundamental unit. The nucleosome comprises an octamer of four core histones (H3, H4, H2A and H2B), which is wrapped by DNA. The histones can undergo modifications, such as acetylation and methylation on specific lysines[54]. In general, histone acetylation at gene promoters is linked to active transcription. H3K9ac, H3K14ac and H4K5ac are representative histone acetylations. In contrast, whether histone methylation results in gene activation or gene suppression depends on the amino acid residue and extent of methylation (i.e., mono-, di- or tri-methylation). H3K4me1/2/3 and H3K36me2/3 are representative transcriptionally active marks, whereas H3K9me3 and H3K27me3 are repressive ones[24]. Miao *et al.*[55] have elucidated the histone modifications of peripheral blood lymphocytes and monocytes in type 1 diabetes patients. They checked H3K9ac, H3K4me3 and H3K9me2, and found that monocytes from the high glycated hemoglobin group displayed greater enrichment of H3K9ac at promoter regions than those from the low glycated hemoglobin group. Interestingly, many genes with high concentrations of H3K9ac in the promoter region are related to the nuclear factor-κB pathway, which is closely related to the development of diabetic complications[19,56]. This result might partly explain systemic metabolic memory.

How about kidney-specific histone modification? From this viewpoint, several experimental studies have been carried out on diabetic animal models. Chen *et al.*[57] focused on monocyte chemotactic protein-1 (*MCP-1*), a chemokine that plays an important role in DKD progression[58], and investigated the histone modifications in the promoter region of its gene. They clarified that SET7/9, a histone lysine methyltransferase that monomethylates H3K4, accumulated in the promoter region of the *MCP-1* gene, and that the H3K4me1 level was higher in the same region in diabetic mice than in control mice. Cai *et al.*[59] focused on osteopontin, which is recognized as an important gene for mesangial matrix expansion in DN[60]. They showed that the kidneys of diabetic mice upregulated the osteopontin gene; increased H3K9ac, H3K4me1 and H3K4me3 levels; and decreased H3K27me3 levels in the promoter region of the osteopontin gene[59]. However, these results need to be carefully interpreted, because they analyzed whole kidney lysate; epigenetics are dependent on cell types, and solation of single or low-cell types must yield more interpretable results.

To overcome this limitation, glomerular fractions have been used to determine more specific and reliable changes in histone modification, although it is important to note that glomeruli contain various types of cells, such as podocytes, endothelial cells and mesangial cells. De Marinis *et al.*[61] examined histone modifications on the promoter region of the *Txnip* gene in a diabetic mouse model and showed increased Txnip expression; increased levels of H3K9ac, H3K4me1 and H3K4me3; and decreased level of H3K27me3. They also showed that an increase in glomerular *Txnip* gene expression depended on fasting blood glucose, which is consistent with hypomethylation of the *TXNIP* gene in the peripheral blood cells of diabetic patients[46]. Reddy *et al.*[62] examined the effect of losartan, an angiotensin II type 1 receptor blocker, on epigenetics in diabetic mice. They found that the elevated glomerular fractions of the *Mcp-1*, plasminogen activator inhibitor-1 (*Pai-1*) and *Rage*

genes in diabetic mice decreased after losartan administration. Similar to *Mcp-1* and *Rage*, *Pai-1* upregulation is supposed to worsen DN, as *Pai-1* knockout alleviates DN in mice[63,64]. They also analyzed glomerular fractions from diabetic kidneys, with and without losartan, and showed an increase of H3K9/14Ac in the promoter regions of *Pai-1* and *Rage* genes; after losartan treatment, the elevated H3K36me3 in the promoter region of the *Rage* gene was diminished. They showed that several histone acetyltransferases, histone deacetylases and histone methyltransferases were upregulated in the glomerular fraction of diabetic mice, and were diminished by angiotensin receptor blocker administration. Although it is still obscure whether these epigenetic changes were directly induced or indirectly caused, such as by blood pressure lowering, these results gave new insights into the renoprotective effect of angiotensin receptor blockers. Furthermore, the epigenetic changes in the promoter region of the *RAGE* gene implied a cross-talk between epigenetics and AGEs, which are two candidate factors related to metabolic memory.

These results showed that histone modifications play a role in metabolic memory, and that targeting histone modification seems a promising strategy against DKD. Indeed, two inhibitors of EZH2, which trimethylates H3K27, were shown to ameliorate renal fibrosis induced by unilateral ureteral obstruction[65]. One limitation of these studies on histone modification in the context of DKD was the lack of data obtained from diseased kidneys, as histone modification must depend on differences between species, as well between clinical DKD and experimental DN.

Non-coding RNA and MicroRNA

NcRNAs are ubiquitous and endogenous RNAs that are transcribed from DNA, but do not code protein; small ncRNAs that are approximately 22-nucleotide long are called microRNA (miR)[24,66]. These ncRNAs, including miRs, can work as epigenetic factors, because they can regulate gene expressions through transcriptional and post-transcriptional mechanisms. Among numerous ncRNAs, miRs are eagerly researched partly because detection of miR is generally easier than that of long ncRNA.

One milestone of miRNA research in DN is about miR-192 and its relationship with transforming growth factor-β1 (TGF-β), which is an important mediator of DN[67]. Kato *et al.*[68,69] showed that miR-192, miR-200b and miR-200c were upregulated in the glomeruli of two independent diabetic model mice. The miR-192 targets smad-interacting protein 1, and is upregulated by TGF-β in mouse mesangial cells. Silencing of miR-192 has been shown to increase *Col1a2* promoter activation, probably through smad-interacting protein 1[68]. The expression of miR-200b and miR-200c were downregulated by *in vivo* inhibition of miR-192, showing that miR-200b and miR-200c are downstream genes of miR-192 in mouse mesangial cells, although miR-192 does not directly bind to either of them. They also showed that both miR-192 and miR-200b increased the expression of TGF-β and Col1a2 in mouse mesangial cells[69]. Given that mesangial matrix expansion is a hallmark of DN, they concluded that miR-192 and miR-200b/c must play important roles in the pathogenesis of DN. Indeed, *in vivo* silencing of miR-192 reversed mesangial matrix expansion, and decreased urinary protein in streptozotocin-induced diabetic mice[70].

How about microRNAs in human DKD? From this viewpoint, Krupa *et al.*[71] examined miR-array of renal biopsy samples of DKD, and found that miR-192 expression positively correlated with eGFR and negatively correlated with fibrosis score. Although this research had a limitation of using whole lysate only, it provided evidence of changes in miR concentration in human DKD. Another approach to miR changes in DKD is examination of plasma or urine where RNAse is abundant, because carrier proteins, such as argonaute2, protect these from degradation[72,73]. Plasma miR-126 concentration was shown to be relatively low in type 2 diabetes patients, and was speculated to reflect the loss of systemic endothelial miR-126, not from a single organ[74]. In contrast, urinary miR profile must reflect that in the kidney, especially in podocytes and tubular cells. Using urine miR microarray, Delic *et al.* showed significant changes in 16 miR concentrations in type 2 diabetes patients with reduced eGFR[75]. Among these 16 miRs, they focused on miR-320c, which positively correlates with the urinary albumin-to-creatinine ratio and negatively correlates with eGFR, meaning that miR-320c increases with the progression of DKD. Evidence of the relationship between miR320c and DKD progression was based on the fact that thrompospondine 1 was a predicted target of miR320c, and that antagonizing thrompospondine 1-mediated latent TGF-β activation resulted in amelioration of DN in mice[76]. Another group also showed that urinary miR profiles differed according to the different stages of DKD in type 1 diabetes patients, including downregulation of miR-221 in several conditions[77]. Interestingly, miR-221 is known to be downregulated by AGEs in human endothelial cells[78]. Taken together, urinary miRs must be useful biomarkers, but their additional pathogenic role has not been elucidated.

RENAL HYPOXIA AND ITS LINK TO METABOLIC MEMORY

The progression of renal damage in CKD becomes consistent and irreversible above a certain threshold, independent of the cause of CKD. Renal hypoxia, especially tubulointerstitial hypoxia, is the 'final common pathway' in CKD progression[79,80]. Although the kidneys receive 20% of the cardiac output, oxygen tension in the kidney is innately low compared with that in other organs. This innately low oxygen tension in the kidney has been proven by needle electrodes and visualization of hypoxia-inducible factor (HIF) activity using luciferase in mice[81,82]. One reason is the existence of an oxygen shunt between the intrarenal arteries and veins[83]. With the progression of CKD, the kidneys suffer from much lower oxygen

tension or hypoxia. This pathophysiology of renal hypoxia in CKD is multifactorial, and includes loss of peritubular capillaries[84,85], renal anemia[86] and increased oxygen demand in the renal tubules[87,88]. Of these, increased oxygen demand in the renal tubules has been shown in a mouse model of DN. Several clinical studies implicated the effect of renal hypoxia on renal prognosis. Increased hemoglobin level, which must alleviate renal hypoxia, correlates with better renal prognosis. In contrast, increased CKD risk has been proven with the existence of intermittent or persistent hypoxemia in patients with sleep apnea syndrome or chronic obstructive pulmonary disease, respectively[89,90].

Another factor is oxidative stress. Generally, hypoxia is linked to increased oxidative stress, which is closely involved in the development of diabetic complications[56,91]. Therefore, tubulointerstitial hypoxia is supposed to be an additional aggravating factor in DKD. However, determination of renal oxygenation on blood oxygen level-dependent magnetic resonance imaging in DKD patients yielded conflicting results on the correlation between renal hypoxia and renal function in DKD patients[92–95]. These findings are considered the basis for the heterogeneity of DKD.

A master regulator of cellular response against hypoxia is HIF, which comprises two subunits, HIF-α and HIF-β[96]. HIF-α is an oxygen-dependent subunit, and is hydroxylated through prolyl hydroxylase domain (PHD). Hydroxylated HIF-α is recognized by von Hippel–Lindau tumor suppressor, resulting in proteasomal degradation. Because the rate-limiting step of HIF degradation is hydroxylation of HIF by PHD, the concentration of oxygen determines the HIF-α concentration. Under hypoxic conditions or pharmaceutical PHD inhibition, HIF-α is stabilized in the cytosol and forms a heterodimer with HIF-1β, which is an oxygen-insensitive heterodimer that can translocate into the nucleus and act as a transcriptional factor by binding

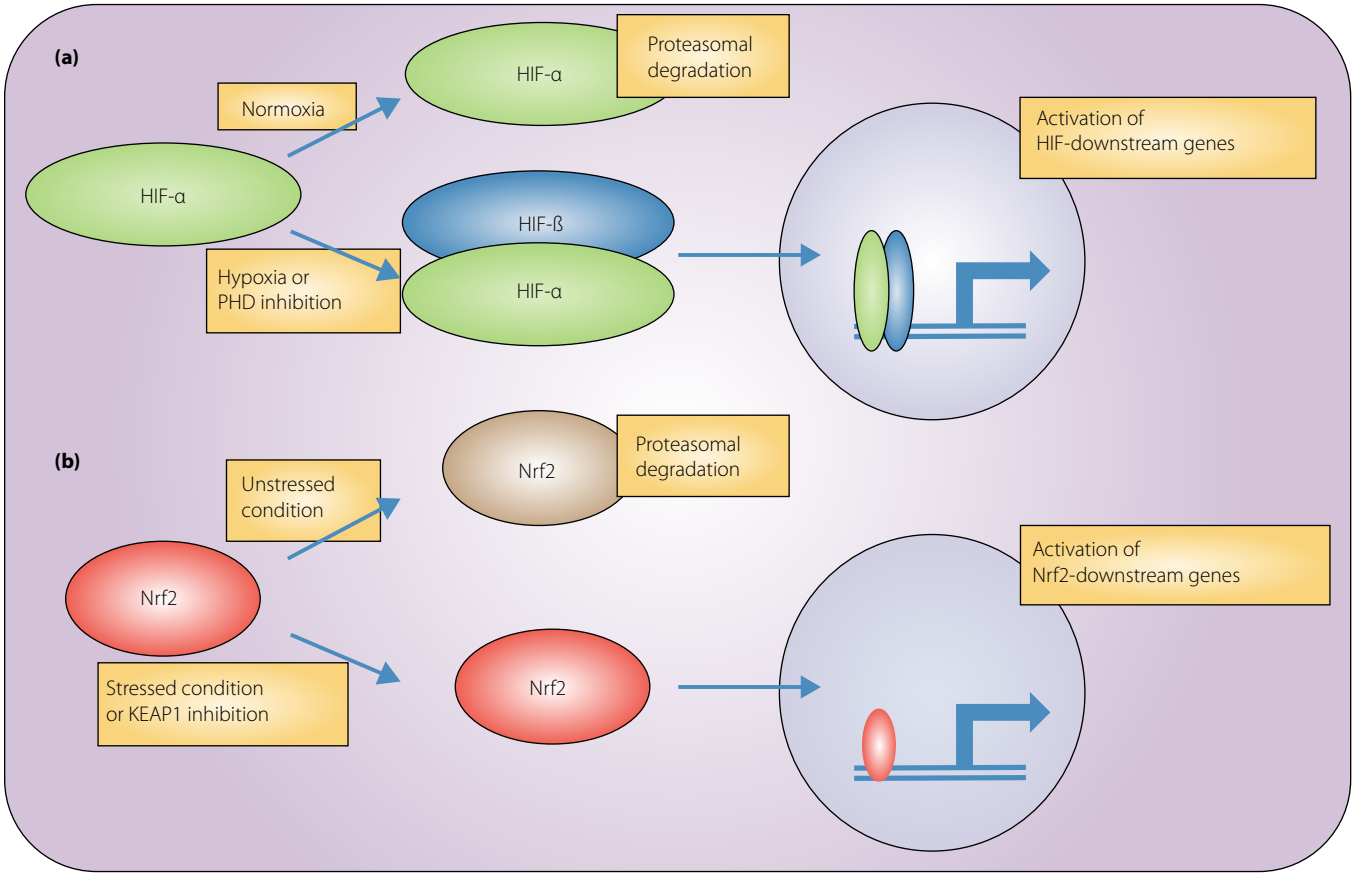

Figure 2 | Regulation of hypoxia-inducible factor (HIF) and NF-E2-related factor 2 (Nrf2)**.** (a) Regulation of HIF. HIF-α undergoes hydroxylation by prolyl hydroxylase domain (PHD) in a normoxic condition, resulting in proteasomal degradation. Under hypoxia or PHD inhibition, HIF-α is not hydroxylated, but is stabilized in cytosol and forms a heterodimer with HIF-β. This heterodimer translocates into the nucleus, binds to the consensus enhancer through hypoxia-responsive elements and activates downstream genes. (b) Regulation of Nrf2. Nrf2 is recognized by Kelch-like ECH-associated protein 1 (KEAP1) under non-stressful conditions, followed by proteasomal degradation. Under a stressful condition of pharmaceutical KEAP1 inhibition, Nrf2 cannot be recognized by KEAP1, and is stabilized in the cytosol. Increased concentration of Nrf2 results in nuclear translocation, binding to the consensus enhancer through anti-oxidant-responsive elements, and activation of downstream genes.

with the consensus enhancer through hypoxia-responsive elements (Figure 2a). Erythropoietin and vascular endothelial growth factor are well-known HIF downstream genes that counteract hypoxia through erythropoiesis and neovascularization, respectively.

Hypoxia also induces epigenetic changes. The oxygen molecule is required for the activity of the ten-eleven translocation enzyme, an oxidation enzyme against 5-methylcytosine that is similar to PHDs with regard to being dependent on Fe^{2+} and α-ketoglutarate[97]. Oxidized 5-methylcytosine results in replacement to unmodified cytosine; therefore, the ten-eleven translocation enzyme has power to inhibit methylation[98,99]. This confirms the importance of hypoxia in DNA methylation and that the promoters of tumor suppressor genes are more methylated in hypoxic tumor tissues[100]. Another mechanism of hypoxia-induced changes in epigenetics is through Dicer, which is a key endoribonuclease that processes precursor miR to mature miR, and whose expression and activity are impaired in hypoxia[101]. This downregulation of Dicer expression is through hypoxia-induced increase in the H3K27me3 level in the Dicer promoter region in tumor cells[102]. The expression of miRs in renal tubular cells also changes after hypoxia–reoxygenation in human cultured tubular cells and in human kidney 2 cells[103]. Among these, the expression of miR-205, which binds to the 3′-untranslated region of the PHD1, was most decreased in hypoxia–reoxygenation. MiR-205 inhibition leads to decreased expression of anti-oxidant enzymes, such as hemeoxygenase 1, copper/zinc superoxide dismutase and manganese superoxide dismutase, all of which are downstream genes of HIF.

Chromatin conformational change, which is another mechanism of epigenetics, has been reported to occur in hypoxia. Mimura *et al.*[104] showed that chromatin conformational change occurred in the promoter region of the *SLC2A3* (GLUT3) gene in human umbilical vein endothelial cells. In normoxic conditions, HIF binds to the transcriptional starting sites (TSS) and enhancer 1 (−35 kbp from TSS), resulting in conformational proximity between these two regions. Under hypoxic conditions, the levels of H3K27ac increase in the TSS, enhancer 1 and enhancer 2 (−24 kbp from TSS), resulting in HIF binding with enhancer 2. This process changes the chromatin conformation and brings the TSS, enhancer 1 and enhancer 2 closer to form lysine (K)-specific demethylase 3A, an H3K9me2 demethylase; therefore, H3K9me2 is recruited and is subsequently demethylated in these three regions. Removal of H3K9me2, a repressive histone mark, activates *SLC2A3* gene transcription; this chromatin conformational change is the mechanism of robust upregulation of the *SLC2A3* gene in hypoxic conditions. Inoue *et al.*[105] reported a similar chromatin conformational change in the angiopoietin-like 4 (*ANGPTL4*) gene. They elucidated that hypoxia and peroxisome proliferator-activated receptors-β/δ agonists synergistically activated ANGPLT4. When peroxisome proliferator-activated receptor-β/δ agonists and HIF1α coexist, the peroxisome proliferator-activated receptor-response elements come closer to and result in increased H3K27ac levels in the HIF1α binding site.

THERAPEUTICS

Existing therapeutics against DKD

Accumulating clinical evidence showed that strict glycemic control[106–108] and blood pressure control[109,110] improves the prognosis of diabetic patients, including those with DKD. Pharmaceutical inhibition of the renin–angiotensin–aldosterone system by angiotensin-converting enzyme inhibitors or angiotensin receptor blockers were also shown to protect the DKD kidneys of both type 1[111–113] and type 2[11–13] diabetes patients; these agents are now used as first-line therapy against hypertension in DKD. The effectiveness of direct renin inhibitors, another renin–angiotensin–aldosterone system blocker, is still controversial[10,114]. Dietary protein restriction was found to be ineffective based on the results of a meta-analysis. According to the standards of medical care in diabetes, reducing the amount of dietary protein below the recommended daily allowance of 0.8 g/kg ideal bodyweight was not advisable for people with DKD, because it does not alter glycemic measures, cardiovascular risk measures or the course of GFR decline (grade A evidence)[115,116].

Sodium glucose co-transporter 2 (SGLT2) inhibitors improve glycemic control by enhancing excretion of glucose into urine. The Randomized, Placebo-Controlled Cardiovascular Outcome Trial of Empagliflozin (EMPA-REG OUTCOME trial) showed that the use of empagliflozin, an SLGT2 inhibitor, in addition to standard care prevented composite cardiovascular outcomes and death from any cause[117]. A subanalysis of this trial showed that empagliflozin slowed the progression of DKD and lowered the rates of clinically relevant renal events compared with a placebo[118]. The mechanism of renoprotection by an SGLT2 inhibitor is believed to be mediated by amelioration of glomerular hyperfiltration and, possibly, renal hypoxia. An SGLT2 inhibitor improves anemia in CKD by enhancing the expression of erythropoietin[119]. Reabsorption of glucose from the glomerular filtrate also requires simultaneous reabsorption of sodium, which is driven by the concentration gradient between the glomerular filtrate and tubular cells. Maintenance of this gradient requires adenosine triphosphate; therefore, glucose reabsorption through SGLT increases energy demand and oxygen consumption[88]. A recent report showed that the low cortical oxygen tension in diabetic rats was normalized by administration of phlorizin, another SGLT2 inhibitor[120]. However, phlorizin reduces medullary oxygen tension independent of the presence of diabetes mellitus. Therefore, it remains to be fully elucidated whether SGLT2 inhibitor counteracts renal hypoxia.

Hypoxia-oriented therapies against DKD

As hypoxia serves as the final common pathway, it is expected to be an effective target of treatment strategies against CKD, including DKD. Cobalt chloride, a classic PHD inhibitor, has

been shown to have protective effects on experimental CKD models without diabetes, and to reduce proteinuria and interstitial fibrosis in DN in mice after amelioration of renal hypoxia[121–123]. However, cobalt chloride cannot be used for human patients because of its side-effects. Therefore, synthesis of new and safe PHD inhibitors has been encouraged; at least six PHD inhibitors of renal anemia are now under clinical trials[124]. FG-4497, which is one of these PHD inhibitors, has been shown to ameliorate lipid metabolism and insulin resistance in mice that were fed a high-fat diet[125]. Taken together, PHD inhibitors might protect the kidneys from DKD by targeting the kidney itself and/or by metabolic dysfunction; therefore, these are promising drugs not only for renal anemia, but also for DKD progression.

Another approach is targeting oxidative stress. Activation of NF-E2-related factor 2 (Nrf2) results in powerful anti-oxidative effects. Clinical trials on bardoxolone methyl, an Nrf2 activator, showed encouraging results in DKD patients, although there is a problem that requires attention. Nrf2 is a transcriptional factor that activates the expression of anti-oxidant genes[126]. Interestingly, Nrf2 also activates *GLO-1* gene expression and reduces glycative stress; therefore, Nrf2 activation might intervene with metabolic memory through AGEs[127]. Nrf2 is recognized by Kelch-like ECH-associated protein 1 and is subsequently ubiquitinated, resulting in proteasomal degradation under non-stressful conditions. Once cellular stress, such as oxidative stress, is induced, Nrf2 recognition by Kelch-like ECH-associated protein 1 is inhibited and Nrf2 is stabilized in cytosol, followed by nuclear translocation (Figure 2b). Bardoxolone methyl is a Kelch-like ECH-associated protein 1 inhibitor that was found to have a renoprotective effect in CKD patients when used as an anticancer drug[128]. This renoprotective effect of bardoxolone methyl against DKD has attracted attention, as oxidative stress was the main pathogenic factor of DKD[56]. Two phase 2 trials showed that bardoxolone methyl increased the eGFR of patients with type 2 diabetes mellitus and those in stage 3b–4 CKD[9,128]. However, a subsequent phase 3 trial (Bardoxolone Methyl in Type 2 Diabetes and Stage 4 Chronic Kidney Disease [BEACON] trial) was terminated because of a relatively high rate of cardiovascular events, especially heart failure, in the first month[6]. In that phase 3 trial, an increase in eGFR was also documented in the bardoxolone methyl group. Secondary analysis of the BEACON study showed that elevated B-type natriuretic peptide and a history of hospitalization for heart failure were risk factors for cardiovascular events in the bardoxolone group[129]. After the first month, the risks for cardiovascular events were similar between the bardoxolone group and the placebo groups. Considering that bardoxolone methyl might be a revolutionary drug that can reverse eGFR decline in DKD, a the Phase II Study of Bardoxolone Methyl in Patients with Chronic Kidney Disease and Type 2 Diabetes (TSUBAKI study) on bardoxolone methyl for type 2 diabetes patients with CKD is currently underway in Japan, with considerable caution regarding the occurrence of cardiovascular events.

CONCLUSION

The present review focused on the mechanism of metabolic memory, its relationship with renal hypoxia and on treatment, especially hypoxia-oriented pharmaceutical therapies, of DKD. It is now widely recognized that experimental animal DN and human DKD differ widely; therefore, careful analysis of results is required to overcome the worldwide public health problem of DKD. Hypoxia-oriented therapies seem to be promising for this disease.

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DISCLOSURE

The authors declare no conflict of interest.

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