The research progress of epigenetics and metabolic memory in diabetic kidney disease

Han-Zhou Li1,2, Zi-Ang Ma1, Ming-Yue Cui3, Huan-Tian Cui4, Shu-Quan Lu2*

1Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China. 2Cangzhou Hospital of Integrated Traditional Chinese Medicine and Western Medicine of Hebei Province Affiliated to Hebei University of Chinese Medicine, Cangzhou 061013, China. 3Chengde Medical University, Chengde 067000, China. 4Yunnan University of Chinese Medicine, Yunnan 650500, China.

*These authors contributed equally to this work and are co-first authors for this paper.

Corresponding to: Huan-Tian Cui, Yunnan University of Chinese Medicine, No. 1076, Yuhua Road, Yunnan 650500, China. E-mail: 1762316411@qq.com.
Shu-Quan Lu, Cangzhou Hospital of Integrated Traditional Chinese Medicine and Western Medicine of Hebei Province Affiliated to Hebei University of Chinese Medicine, No. 31, Huanghe Road, Cangzhou 061013, China. E-mail: czlvshuquan@163.com.

Author contributions
Han-Zhou Li and Zi-Ang Ma wrote the manuscript with the help of Ming-Yue Cui, Huan-Tian Cui, and Shu-Quan Lu. All authors contributed to this manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare no conflicts of interest.

Acknowledgments
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Peer review information
Life Research thanks Xue-Chao Lu and other all anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations
DKD, diabetic kidney disease; ESRD, end-stage renal disease; DCCT, Diabetes Control and Complications Trial; EDIC, Epidemiology of Diabetes Interventions and Complications; UKPDS, United Kingdom Prospective Diabetes Study; STZ, streptozotocin; AGES, advanced glycation end products; TGF-β1, transforming growth factor-β1; AngII, angiotensin II; PDGF, platelet-derived growth factor; HPTM, histone post-translational modifications; ncRNAs, non-coding RNAs; DNMT, DNA methyltransferase; SAM, S-adenosyl-methionine; CPG, cytosine-guanine; PTMs, Post-translational modifications; HDACs, histone deacetylases; miRNAs, microRNAs; Spry1, sprouty homolog 1.

Citation

Abstract
Diabetic kidney disease (DKD) is a clinical syndrome that is one of the major causes of end-stage renal disease (ESRD). The pathogenesis of DKD is complex and multifaceted, with most studies indicating its association with genetics, advanced glycosylation end-product deposition, polyol pathway and protein C activation, lipid metabolism abnormalities, microcirculatory dysfunction, oxidative stress, inflammatory factors, and the kallikrein-kinin system. Epigenetics is the science studying gene expression regulation without changes in the DNA sequence. In recent years, increasing evidence has shown that epigenetic mechanisms play a crucial role in the initiation and progression of DKD. For instance, epigenetic modifications such as DNA methylation, histone modifications, and non-coding RNAs can influence the expression of DKD-related genes, thereby regulating the development and progression of DKD. On the other hand, metabolic memory is an important concept in DKD research. Metabolic memory refers to the phenomenon where cells maintain a certain metabolic state even after the disappearance of metabolic stress factors. This state can influence cell function and fate. In DKD, metabolic stress factors such as hyperglycemia can lead to metabolic memory in renal cells, affecting their function and fate, ultimately leading to the development and progression of DKD. Therefore, to further explore the pathogenesis of DKD, research on epigenetics should be strengthened, aiming to provide new ideas and methods for the prevention and treatment of DKD.

Keywords: diabetic kidney disease; epigenetic modifications; Metabolic memory; DNA methylation; non-coding RNAs
**Introduction**

Diabetic kidney disease (DKD) is a clinical syndrome characterized by progressive proteinuria and a gradual decline in renal function [1]. It is reported that DKD accounts for 27% to 53% of diabetes cases and is one of the most common causes of end-stage renal disease (ESRD). DKD is the leading cause of ESRD in developed countries, and its incidence is increasing annually in low- and middle-income countries [2]. Currently, DKD is primarily treated symptomatically, lacking effective therapeutic approaches, making its pathogenesis a continually researched topic. The pathogenesis of DKD is complex and multifaceted, with most studies suggesting its association with genetics, the deposition of advanced glycation end products, activation of the polyol pathway and protein C, abnormalities in lipid metabolism, microcirculatory disorders, oxidative stress, inflammatory factors, and the kallikrein-kinin system. Additionally, research has found that abnormalities in epigenetic modifications and defects in podocyte autophagy [3, 4] play significant roles in the onset and progression of DKD. Approximately one-third of diabetic patients develop DKD, emphasizing the importance of early diagnosis and intervention. Numerous studies suggest that 'metabolic memory' results from epigenetic modifications [5], which refer to changes in gene expression that do not involve traditional alterations in DNA structure. These modifications are often stable and can be inherited, potentially serving as the molecular basis for the "metabolic memory" phenomenon. Consequently, the discovery of 'metabolic memory' [6] has drawn attention to epigenetic inheritance as a prolonged effect mechanism in DKD pathogenesis research. In this article, we aim to delve into the "metabolic memory" phenomenon governed by epigenetic mechanisms in DKD and explore changes in DNA methylation and histone modifications involved in DKD. We hope to provide new directions and perspectives for the prevention and treatment of DKD (Figure 1).

**Metabolic Memory and Diabetic Nephropathy (DKD)**

As early as 2003, strong evidence for "metabolic memory" was provided by the Diabetes Control and Complications Trial (DCCT) and the Epidemiology of Diabetes Interventions and Complications (EDIC) study group [7]. The DCCT discovered that early, intensive blood glucose control could decrease the occurrence and severity of diabetic kidney complications. In the subsequent EDIC observational study, by keeping the intensive treatment unchanged for patients in the intensive treatment group of DCCT and switching patients in the original conventional blood glucose control group to the intensive treatment regimen, and continuing to follow up for 10 years, the results showed that even though the two groups gradually converged to similar levels of glycated hemoglobin after continuing to receive the same intensive treatment, the incidence of kidney complications in the original conventional blood glucose control group was still higher than that in the intensive treatment group. This suggests that if diabetic patients do not correct their hyperglycemic state early on, even if blood glucose control is achieved later, it still cannot prevent a series of complications, including diabetic nephropathy, which is the 'metabolic memory' effect. Subsequently, in 2008, the United Kingdom Prospective Diabetes Study (UKPDS) [8] on T2DM patients also obtained similar results in a multicenter clinical trial, further confirming the existence of the "metabolic memory" effect. In addition, the "metabolic memory" phenomenon has also been demonstrated in various experimental models. EL-OSTA et al. used a short period of high-glucose medium to cultivate endothelial cells and found that there was persistent activation of oxidative stress and high expression of inflammatory factors even after switching to normal glucose concentration [9]. INTINE et al. successfully constructed a zebrafish model of diabetic metabolic memory using a 0.3% streptozotocin (STZ) solution [10]. The proposal of the "metabolic memory" phenomenon has led to a breakthrough in our understanding of the pathogenesis of diabetic complications, including DKD. The pathogenesis of DKD involves multiple factors, such as high blood glucose levels, advanced glycation end products (AGEs), transforming growth factor-β1 (TGF-β1), angiotensin II (AngII), platelet-derived growth factor (PDGF), and inflammatory cytokines. In addition, genetic and epigenetic factors are also important contributors to DKD [11-13]. Numerous laboratory and clinical studies have shown that the generation of the "metabolic memory" phenomenon may be related to cascade effects induced by AGEs and oxidative stress, the accumulation of peroxynitrite, epigenetic modifications, chronic inflammation, and apoptosis [14, 15]. Among them, epigenetics [16] refers to heritable changes in gene expression and function that occur without altering the DNA sequence, mainly including chemical modifications of DNA and RNA molecules, as well as histone modifications. Due to the persistent impact of transient environmental exposure on cellular function, epigenetics [17] has emerged into researchers’ view as a potential molecular means of metabolic memory. Subsequent animal model experiments have demonstrated the critical role of epigenetics in the pathophysiology of diabetic nephropathy.

**Epigenetics and DKD**

**Overview of Epigenetics**

Recently, epigenetic mechanisms have been proposed to explain metabolic memory. Epigenetics is a field that describes functional changes in the genome without altering the DNA nucleotide sequence [18]. There are many epigenetic phenomena, including known ones such as DNA methylation, histone post-translational modifications (HPTM), non-coding RNAs (ncRNAs), maternal effects, gene silencing, nucleolar dominance, dormant transposon activation, and others. Hyperglycemia can induce various epigenetic changes that can persist for several days even after blood glucose levels return to normal [19, 20]. Among several epigenetic modifications, the most commonly studied mechanisms are DNA methylation, histone post-translational modifications, and ncRNAs [21].

![Figure 1 The role of epigenetics in metabolic memory in DKD](/image)

Submit a manuscript: https://www.tmjournals.com/lr
DNA methylation is a critical epigenetic modification that regulates gene expression through chemical alterations. In this process, DNA methyltransferases facilitate the formation of a covalent bond between a methyl group and the fifth carbon atom of cytosine within CpG dinucleotides across the genome. This modification influences gene expression without altering the underlying DNA sequence. It primarily controls gene expression by adding methyl groups to cytosine residues in DNA, typically leading to the suppression of gene activity and impacting cellular differentiation, development, and disease progression [22].

Histone modifications involve a range of covalent alterations, such as methylation, phosphorylation, acetylation, and SUMOylation, occurring at the amino-terminal end of histones [23]. These modifications serve as vital markers of the "histone code", which is recognized by specific proteins and translated into distinct chromatin states, thereby regulating DNA expression [24]. Methylation and acetylation of histones play essential roles in the development of diabetic "metabolic memory" and diabetic kidney disease (DKD). These post-translational histone modifications can adjust the affinity of histones for double-stranded DNA, thus affecting the compaction of chromatin and modulating gene expression [25].

ncRNAs, or non-coding RNAs, refer to RNAs that do not encode proteins. These include various functionally known RNAs such as ribosomal RNA (rRNA), transfer RNA (tRNA), and microRNA. A common feature of these RNAs is that they can be transcribed from the genome but are not translated into proteins, and they can perform their respective biological functions at the RNA level. MicroRNA and long non-coding RNA (lncRNA) play crucial roles in regulating the occurrence and development of kidney diseases.

Following hyperglycemic stimulation, dysregulation of inflammatory mediators, excessive accumulation of reactive oxygen species, increased protein nitration, increased production of advanced glycosylation end-products (AGEs), and sustained high expression of apoptosis-related genes occur in target cells. Numerous studies have shown that these dysregulations are closely related and complementary to epigenetic modifications [26]. The stability and transgenerational inheritance of epigenetic marks have led researchers to believe that epigenetic changes may provide the molecular mechanisms that explain the "metabolic memory" of hyperglycemia. Thus, epigenetic modifications are a frontier in DKD research, including DNA methylation, histone post-translational modifications, microRNA, and chromatin remodeling [27]. These modifications not only play a key role in the pathophysiology of DKD, but also provide new perspectives and potential therapeutic targets for understanding and treating DKD.

**DNA Methylation and DKD**

DNA methylation is the primary modification method in epigenetic modifications [28]. It refers to the process of converting cytosine to 5-methylcytosine through the catalysis of DNA methyltransferase (DNMT) using S-adenosyl-methionine (SAM) as a methyl donor. In mammals, DNA methylation mainly occurs on cytosine in the cytosine-guanine (CpG) dinucleotide sequence. DNA methylation can cause changes in DNA conformation, chromosome structure, and translation processes, ultimately regulating gene expression without altering the base sequence of the genome [21]. In recent years, an increasing number of scholars have begun to focus on the role of DNA methylation in the pathogenesis of various kidney diseases. Both clinical and experimental studies have shown [29] that DNA methylation is associated with the occurrence and development of DKD. Some studies have utilized kidney tissue, peripheral blood leukocytes, and saliva cell DNA from DKD patients to detect methylation changes through methylation chip analysis. These studies have found that DNA methylation changes induced by a hyperglycemic microenvironment play a crucial role in the development of DKD. The expression of CTGF in the glomeruli of patients with DKD is significantly elevated, which is related to the reduction of CpG methylation levels in the promoter region of CTCF-related genes caused by persistent hyperglycemia [30].

Maghbooli et al. used reverse-phase high-performance liquid chromatography (RP-HPLC) to study the overall methylation level of the genome in patients with diabetic nephropathy compared to patients with diabetes alone and found a significant increase in the former group [31]. Wing et al. investigated the genome-wide DNA methylation patterns associated with renal function decline in patients with chronic renal insufficiency using whole blood samples [32]. They found that patients with stable renal function and those with rapid progression had similar ratios of hyper/hypo-methylated CpGs. However, 80% of the CpG sites in patients with stable renal function had higher methylation levels than those with rapid progression, including hypermethylation of CpG islands in genes such as NPHS4, IQSEC, and TCF3, which are involved in pathways promoting epithelial-mesenchymal transition and are related to renal fibrosis. In contrast, only 16% of CpG sites had higher methylation levels in patients with rapid progression. Additionally, genes involved in oxidative stress and inflammation, such as NOS3, NFKBIL2, CLI4, NFKBIB, TGF-B1, and TGF-B2, also showed differential methylation. The results of this study suggest that the rapid loss of renal function in patients with chronic renal insufficiency may be associated with the impact of methylation status on the expression activity of these genes.

**Post-translational Modifications of Histones and DKD**

Post-translational modifications (PTMs) of histones, as a significant epigenetic regulatory mechanism, have potential connections with DKD. These modifications include acetylation, phosphorylation, methylation, ubiquitination, and ADP-ribosylation [33]. Among them, histone methylation and acetylation are particularly important in the "metabolic memory" of diabetes and the progression of DKD. PTMs of histones can alter their affinity for double-stranded DNA, thus changing the loose or dense state of chromatin and regulating gene expression [25]. Current research suggests that the occurrence of DKD is associated with acetylation of histone H3K9 and H3K27 dimethylation of H3K4, and phosphorylation of serine at position 10 of H3 in the kidney. These modifications can cause chromatin to unwind, promoting gene expression [34]. Enzymes such as DNA methyltransferases (DNMTs), histone methyltransferases (HMTs), histone demethylases (HDMs), histone acetyltransferases (HATs), and histone deacetylases (HDACs) are crucial targets for drug development. Studies have found that HDACs can deacetylate non-histone proteins, and HDAC4 levels are elevated in both human and rodent models of DKD. Knocking out the HDAC4 isoform can attenuate podocyte injury, proteinuria, and extracellular matrix deposition in diabetic rats. Further research has shown that HDAC knockout can restore autophagy in podocytes impaired by high glucose [35]. The postulated mechanism is that deacetylation of the transcription factor STAT1 inhibits autophagy in podocytes. Gao et al. discovered that histone H2A ubiquitination increases while H2B ubiquitination decreases in glomerular mesangial cells treated with high glucose [36]. Changes in histone ubiquitination levels can activate the TGF-β signaling pathway, leading to kidney damage. The ubiquitin-proteasome inhibitor MG132 can suppress the activation of the TGF-β pathway by inhibiting abnormal histone ubiquitination.

**Non-coding RNAs and DKD**

MicroRNAs (miRNAs) also play a crucial role in DKD fibrosis. The first miRNA associated with DKD was miR-192, which participates in the expression of TGF-induced collagen 1α2 (Col1α2) in DKD by downregulating the E-box repressor zinc finger-homeobox transcription factor (deltaEIf1) [37]. In mesangial cells of DKD mice, TGF-β reduces the expression of SIP1 (Smad-interacting protein 1) by increasing the level of miR-192 and synergizes with deltaEIf1 short hairpin RNAs to increase the activity of Coll α2's E-BOXHuc [38]. Conversely, LNA-antimiR-192 increases the levels of E-box repressors Zeb1/2 by significantly reducing miR-192 levels, leading to decreased expression of collagen, TGF-β, fibronectin, and urinary protein levels [39].

Studies have found that inhibiting the expression of miR-29c can target the growth factor homolog sprouty homolog 1 (Spry1),...
Reducing proteinuria and matrix deposition in the mesangium of DKD mice. Spy1 is an important regulator of the anti-angiogenic effects of mitogen-activated protein kinases, suggesting a potential link between miR-29c and angiogenesis, affecting microvascular formation in diabetic kidneys [40].

Compared to the former, miR-93 is more directly involved in the development and progression of DKD. Overexpression of miR-93 in podocytes of diabetic mice can target mitogen and stress-activated kinase 2 (Ms2k), affecting the phosphorylation of its substrate histone H3 Ser10 (H3S10), and thereby regulating chromatin remodeling. This significantly improves diabetic nephropathy. Additionally, high glucose inhibits the expression of miR-93 in the kidney, enhancing downstream vascular endothelial growth factor signaling, leading to increased synthesis of collagen and fibronectin, accelerating the onset and progression of diabetic nephropathy [41].

There are differential changes in circulating miRNAs in the serum of DKD patients, along with significant alterations in miRNA complexes and extracellular vesicle (EV) miRNAs. In the serum of patients with diabetic nephropathy, levels of the miR660-Ago-2 (protein Argonaute-2) complex and EV miR-21 and miR-126 are elevated, while levels of the miR-132-HDL (high-density lipoprotein) complex are reduced [42]. The discovery of these non-free differentially expressed miRNAs enhances the sensitivity of miRNAs as clinical markers for diabetic nephropathy. This evidence suggests that miRNAs play a crucial role in the onset and progression of DKD pathology, although the underlying mechanisms remain to be further investigated.

Discussion

Epigenetic modifications, such as DNA methylation and histone post-translational modifications, serve as pre-transcriptional modifications that can regulate gene expression. In the past two decades, epigenetic research has been greatly developed. A large number of studies have shown that epigenetic modifications are indeed involved in the DKD disease process and play an important regulatory role in the development of DKD; at the same time, the reversibility and ease of regulation of epigenetic modifications provide an opportunity to correct their abnormal modifications. These modifications can exacerbate the damage to glomerular podocytes and tubular epithelial cells through various signaling pathways, promote pathological physiological changes like oxidative stress and inflammatory responses in kidney tissue, and mediate the progression of DKD. Research on the epigenetic mechanisms associated with memory phenomena in diabetes has proposed alternative approaches distinct from traditional targeted therapies. Interventions targeting the epigenetic regulatory level may effectively delay the progression of kidney disease and improve diabetic complications and prognosis in patients. However, these benefits may have tissue-specific limitations.

For instance, studies have found that upregulated SIRT expression can improve endothelial cell function in a high-glucose environment. However, other reports have demonstrated that high SIRT1 expression is a significant factor contributing to post-infarction cardiac remodeling, including cardiomyocyte hypertrophy and interstitial fibrosis, in GK diabetic rats. Despite potential tissue specificity, research on epigenetic regulation still provides potential new targets for disease treatment. Therefore, focusing directly on the interactions between different epigenetic modifications, rather than on changes in the epigenetic modifications themselves, may lead to greater discoveries of greater clinical value.

To achieve better therapeutic outcomes and ensure the safety and stability of treatment, there is an urgent need to further elucidate the molecular mechanisms underlying the onset and progression of DKD and continue searching for effective molecular intervention strategies. A more profound understanding of the role of epigenetic regulatory mechanisms in DKD progression may lead to the discovery of novel interventional approaches in the future.

References


17. El-Osta A, Brasacchio D, Yao D, et al. Transient high glucose causes persistent epigenetic changes and altered gene


