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Epigenetic Risk Profile of Diabetic Kidney Disease in High Risk Populations

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Abstract

Purpose of Review—Epigenetic variations have been shown to reveal vulnerability to diabetes and its complications. Although it has become clear that metabolic derangements, especially hyperglycemia, can impose a long-term metabolic memory that predisposes to diabetic complications, the underlying mechanisms remain to be understood. It has been suggested that epigenetics (e.g. histone modification, DNA methylation, and non-coding RNAs) help link metabolic disruption to aberrancies related to diabetic kidney disease (DKD). In this review we discuss the key findings and advances made in the epigenetic risk profile of DKD and provide perspectives on the emerging topics that implicate epigenetics in DKD.

Recent Findings—Epigenetic profiles can be profoundly altered in patients with diabetes, in circulating blood cells as well as in renal tissues. These changes provide useful insight into the mechanisms of diabetic kidney injury and progressive kidney dysfunction.

Summary—Increasing evidence supports the role of epigenetic regulation in DKD. More studies are needed to elucidate the mechanism and importance of epigenetic changes in the initiation and progression of DKD and to further explore their diagnostic and therapeutic potential in the clinical management of patients with diabetes who have a high risk for DKD.

Keywords

Diabetic nephropathy; diabetic kidney disease; epigenetic regulation

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Conflict of Interest

Lixia Xu, Rama Natarajan, and Zhen Chen declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

Diabetic kidney disease (DKD), also known as diabetic nephropathy, is one of the major microvascular complications of diabetes, affecting nearly 50% of all patients with diabetes [1]. With the increase of diabetic population worldwide, DKD has been recognized as the leading cause of end-stage renal disease (ESRD) and is strongly associated with mortality in diabetic patients [2].

DKD is characterized by albuminuria and/or an initially increased glomerular filtration rate (GFR) that decreases in the middle to end stage, and is typically accompanied by hypertension [3-5]. The most common histological changes of DKD include glomerular hypertrophy, glomerular and tubular basement membrane thickening, mesangial matrix expansion, glomerulosclerosis, podocyte effacement, and arteriolar wall thickening [6].

Epigenetic regulation, defined as heritable changes in gene expression and function that occur without an alteration in the DNA sequence [7,8] has been identified as an important mechanism underlying diabetes and its complications, such as DKD [9]. Metabolic derangements, such as hyperglycemia, can cause profound epigenetic alterations (e.g. histone modification and DNA methylation) in various renal cells, which in turn may compromise kidney function. A “metabolic memory” imposed by hyperglycemia has been suggested to contribute to the increased risk of diabetic complications, including DKD [10]. Here we review the recent advances in epigenetic risk profile of DKD and discuss the novel insights into the role of epigenetics in progressive kidney dysfunction and pathologic changes in the kidney of high-risk patients with diabetes.

Metabolic memory and epigenetic changes in diabetes

Longitudinal epidemiologic studies and clinical trials of patients with either type 1 or type 2 diabetes consistently demonstrate improved clinical outcomes resulting from intensive glycemic control *vs.* conventional insulin treatment [11,13,16,17]. After the intervention period, participants in the intensive glycemic control arm continued to have lower risk of vascular complications compared to diabetic patients who received conventional treatment. In particular, the risk of diabetic nephropathy remained significantly higher in the conventional treatment group compared to the intensive control arm [11-17]. Such a phenomenon that early hyperglycemia has persistent and enduring effects in diabetes vascular complications has been described as “metabolic memory” or “legacy effect” [18-22].

In the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC), participants with type 1 diabetes in the intensive glycemic control arm had a 39% reduction in microalbuminuria and 54% reduction in albuminuria compared with with those in the conventional treatment in the initial DCCT phase [11]. Despite conversion to intensive control for almost a decade those originally assigned to conventional therapy experienced a higher prevalence of microalbuminuria and albuminuria compared to those consistently managed with intensive treatment [12,13,18]. Over a median follow-up of 22 years, the risk of impairment in GFR was still significantly

lower in the intensive treatment group than it in the conventional treatment group [14]. The UK Prospective Diabetes Study (UKPDS) and the ADVANCE collaborative group, which have conducted analogous studies in participants with type 2 diabetes, also reported similar phenomena [15-17].

To understand the mechanisms underlying metabolic memory, researchers have begun to investigate epigenetics using samples collected from the DCCT/EDIC trial. Miao et al. profiled the histone modifications in the blood monocytes and lymphocytes obtained from two groups of participants with type 1 diabetes at year 16-17 of EDIC: participants randomized to the DCCT conventional treatment group who had progression of retinopathy or nephropathy in EDIC (case subjects), and participants randomized to the DCCT intensive treatment group who had no progression of retinopathy or nephropathy (controls subjects). The authors found that case subjects had greater number of promoter regions with enrichment in H3K9Ac (an active histone mark), as compared with control subjects. Importantly, the H3K9Ac levels were positively and significantly associated with glycated hemoglobin (HbA1c) levels in all subjects at all time periods ($P < 2.2\times10^{-16}$). Furthermore, among the top genes differentially acetylated were those related to the nuclear factor-κB (NF-κB) inflammatory pathway well known to be associated with diabetes complications, with over 15 genes in this pathway depicting hyperacetylated promoters [23]. These findings provide the first direct evidence of a relationship between epigenetic modifications and metabolic memory in diabetes.

More recently, Chen et al. have analyzed DNA methylation profiles in similar samples from the DCCT/EDIC studies. In addition to the blood monocytes collected from the case subjects and controls subjects as noted above (i.e. samples collected by EDIC year 16-17), the DNA methylation profile in whole blood DNA collected and archived at the end of DCCT ~1993 (and beginning of EDIC) was also analyzed. While hundreds of differentially methylated loci (DML) were identified in both monocytes and whole blood between the two groups, the 3'UTR region of *thioredoxin-interacting protein (TXNIP)*, a gene known to be associated with hyperglycemia and diabetic complications, was found to be significantly hypomethylated in the case group. More importantly, a set of these DML exhibited similar differences that persist for about 17 years from the DCCT into the EDIC Study, again supporting an epigenetic explanation for the metabolic memory linking hyperglycemia to diabetic complications [24].

Epigenetic modifications

Many types of epigenetic processes have been identified and discussed extensively in several excellent reviews [25-27], and the three major types to be discussed here are DNA methylation, histone modification, and non-coding RNA (ncRNA)-associated gene regulation. We will first provide a brief introduction of these epigenetic regulation and the key enzymes that have been investigated in the context of DKD (Fig. 1), before we cover their profile in DKD.

DNA methylation involves the addition of methyl group to DNA molecules, mostly on cytosines [28]. In mammals, DNA methylation is almost exclusively found in cytosine-

phosphate-guanine (CpG) dinucleotides [29]. Methylation in the promoter regions typically leads to gene silencing whereas methylation in the gene bodies allows for active transcription [30,31]. Transfer of methyl groups to DNA is catalyzed by DNA methyltransferases (DNMTs) [32,33].

Histone modifications mainly occur in the exposed histone amino-terminal tails in histone octamers comprising two copies of histone H2A, H2B, H3, or H4. Among over 60 different types of histone modifications, methylation and acetylation of lysine (K) and arginine (R) are the most extensively studied to date [34]. These histone modifications are tightly regulated by enzymes responsible for adding or removing the epigenetic marks, often referred to as “writers”, (i.e., histone acetylases and methyltransferases) and “erasers”, (i.e., deacetylases and demethylases) [35].

Histone methylation on H3K4, H3K9, and H3K36 typically leads to transcriptional activation. In contrast, methylation on H3K27 by enhancer of zeste homolog 2 (EZH2) often denotes transcriptional suppression. These methylation events can be reversed by by demethylases, such as lysine specific demethylases (LSDs) and Jumonji C-domain containing demethylases [36-39].

Histone acetylation or hyperacetylation (e.g. H3K9ac, H3K14ac, H3K18ac, H3K23ac, H3K27ac) is typically associated with transcriptional activation, as the acetyl group can decrease the negative charge of DNA, making chromatin more accessible to transcription factors (TFs) and their coactivators. In contrast, hypoacetylation usually results in a more compact chromatin conformation, leading to transcriptional repression. Histone acetylation and deacetylation are regulated by histone acetyltransferases (HATs) and deacetyltransferases (HDAC) respectively [42]. Among HATs, P300 and cAMP response element binding protein (CREB)-binding protein (CBP) are the best characterized in DKD [40,41]. HDACs, a large protein family consists of many members have been investigated extensively for their role in a variety of disease conditions, including DKD [43,44].

Non-coding RNAs are encoded by and transcribed from the non-protein-coding regions in the genome. While microRNA (miRs) have been intensively studied in DKD in the past decade (please refer to references 45-48 for review), long noncoding RNAs (lncRNAs) have recently emerged to be key regulators implicated in diabetes complications. LncRNAs, with transcript length >200 nucleotides, can epigenetically regulate gene expression through diverse molecular mechanisms [49,50], as we will discuss in the next section using several examples related to DKD.

Epigenetic profile in DKD patients and potential mechanistic link

In this section, we will review current studies performed with different type of samples (i.e. body fluids vs renal tissues) for epigenetic profiling, which likely reveal systemic vs local epigenetic changes underlying DKD.

Epigenetic profile in body fluids

Useful epigenetic information carried in body fluids (e.g., peripheral blood, urine, saliva) of human subjects can be collected non-invasively. Changes in the urine are the most common manifestation of DKD. However, although DNA can be extracted from human urine and DNA methylation has been detected [51-53], to date there is a lack of evidence that DNA methylation or histone modification can be detected in the urine informative of underlying disease process and risk of adverse outcomes in people with DKD .

Attempt has also been made with DNA extracted from saliva. Sapienza et al. profiled DNA methylation in saliva collected from African American and Hispanic participants with diabetes and ESRD undergoing hemodialysis and that in participants with diabetes but without nephropathy. Among 187 genes that were differentially methylated between the two groups of participants, 39 were involved in kidney development or diabetic nephropathy, or have been associated with dialysis-induced changes in gene expression in peripheral blood cells [54]. With only one report to date, it is yet difficult to evaluate the potential use of saliva DNA methylation in the evaluation of DKD risk.

Compared with epigenetic profiling using urine and saliva, tests in peripheral blood seem to be more informative. In addition to the two elegant studies mentioned earlier [23,24] in profiling DNA methylation and histone modification in blood samples collected from DCCT/EDIC, several other studies have also reported the association between epigenetic profiles measured in peripheral blood samples and DKD. Most of these studies have focused on DNA methylation.

Gautier et al. profiled the genome-wide DNA methylation in leukocytes from non-diabetic offspring of mothers with type 1 diabetes (case group) in comparison with offspring of fathers with type 1 diabetes (control group). Among 87 CpG sites differently methylated, DNMT1 (the key enzyme for the maintenance of DNA methylation) was under-methylated in cases, and the 74 under-methylated sites were correlated with GFR in cases and controls [55]. Wing et al. studied the genome-wide DNA methylation pattern in whole blood samples associated with the decline in kidney function among 40 participants in the Chronic Renal Insufficiency (CRIC) study comparing the highest and lowest rates of decline in eGFR. Their results showed that 80% of CpG sites were hypermethylated in individuals with stable kidney function compared with 16% of CpG sites in rapid progressors. Specifically, methylation in CpG islands of *NPHP4, IQSEC1* and *TCF3*, genes involved in pathways known to promote the epithelial to mesenchymal transition, were associated with rapid loss of kidney function [56]. In a more recent well-powered epigenome-wide association study (EWAS), Chu et al. profiled DNA methylation in peripheral blood leukocyte samples from 2,264 (586 cases with diabetes) African Americans participants in the Atherosclerosis Risk in Communities (ARIC) study and 2595 (394 cases with diabetes) in the Framingham Heart Study (FHS). Of 19 CpG sites significantly associated with eGFR/CKD, five were also associated with renal fibrosis in biopsies from participants with CKD and showed concordant DNA methylation changes in kidney cortex. The study further reported that methylation at *PTPN6/PHB2* cg19942083 in kidney cortex associates with lower renal PTPN6 expression, higher eGFR, and less renal fibrosis; these regions are likely enriched

with TF binding sites [57]. Given that *PTPN6* encodes protein tyrosine phosphatase non-receptor type 6, aka Src homology-2 domain-containing phosphatase-1 (SHP-1) and that increased renal SHP-1 expression has been implicated in kidney disease and vascular complications in the setting of diabetes, the dysregulation of methylation at this site may reveal an epigenetic mechanism underlying DKD.

In addition to these genome-wide profiling of DNA methylation, a number of studies have revealed the association of DNA methylation at select gene loci with DKD risk. One example is the let-7a, which is known to decrease collagen (Col) and fibronectin (FN) expression induced by high glucose along with suppression of expression of the target gene ubituitin-like, containing PHD and RING finger domains 1 (UHRF1) essential for DNMT1 activity activity [59,60]. Peng *et al.* found that the methylation of *let-7a* promoter in the blood of DKD group was significantly higher than that in the control groups (including both healthy control and diabetic patients without DKD), whereas its level was lower in the plasma of people with DKD. The average *let-7a* methylation rate was 96.2% in the DKD group, 76.6% in the diabetes without nephropathy group, and 63.2% in healthy controls [58]. It is possible that the link between *let-7a* methylation and DKD is not only associative but may also be a mechanism in the pathogesis of DKD.

Epigenetic changes in people with DKD have also been associated with the manifestation of clinical phenotypes. Maghbooli et al. found that in comparing subjects with diabetes and with or without albuminuria, global DNA methylation in peripheral blood monocytes was significantly higher in those with albuminuria. This finding implicates DNA hypermethylation as an independent risk factor for albuminuria in patients with diabetes [61].

Other studies have suggested that methylation levels in select genes or select regions can also be associated with different manifestation of DKD. For example, methylation states of the tissure inhibitor of metalloproteinase 2 *(TIMP-2)* and aldo-keto reductase family 1, member B1 *(AKR1B1)* genes in peripheral blood was negatively correlated with albuminuria of people with DKD [62]. In another study, methylation in gene promoter of connective tissue growth factor (CTGF), a strong pro-fibrotic factor was significantly decreased in the peripheral blood of participants with diabetic nephropathy compared with participants with diabetes but without nephropathy and with non-diabetic controls. The degree of methylation in the *CTGF* gene is positively associated with CTGF concentration in serum, which is positively correlated with the urinary albumin-to-creatinine ratio, blood urine nitrogen and serum creatinine, but negatively correlated with eGFR [63]. Swan et al. examined the methylation patterns in genes that affect mitochondrial function and found that that the methylation levels of CpG in the *TAMM41 (encoding TAM41 mitochondrial translocator assembly and maintenance homolog)* and *COX6A1* (gene encoding cytochrome c oxidase subunit 6A1) in peripheral blood were significantly different between healthy controls and persons with DKD [64]. Given the role of these genes in mitochondrial function, it is possible that the altered DNA methylation in part mediates the disruption of mitochondrial metabolism in DKD.

Collectively, these efforts in profiling epigenetic states using circulating blood samples strongly suggest the association between epigenetic changes and DKD risk, both in incidence and disease progression. These reports also implicate genes with significant variations in DNA methylation as promising candidates for future experimental studies to illuminate the underlying regulatory mechanisms contributing to the incidence and progression of DKD.

Researchers have also begun to test the changes in lncRNAs using blood samples. Several lncRNAs have been detected in the blood from patients with DKD. For example, the level of lncRNA NR_033515 was significantly higher in the serum of patients with DKD than normal controls, and it was correlated with the severity of DKD and positively associated with diagnostic markers of DKD such as KIM-1 [65]. Plasmacytoma variant translocation (PVT1), a lncRNA significantly upregulated in human mesangial cells by high glucose, has been identified to be a candidate gene for ESRD in type 2 diabetes using a pooling-based genome-wide single nucleotide polymorphism analysis in DNA samples from the participants of Gila River Indian Commnity [66]. Using the Genetics of Kidneys Diabetes (GoKinD) trial database, variants in the *PVT1* gene have been associated with ESRD in type 1 diabetes [67].

Epigenetic profile in renal tissue

Compared to epigenetic profiling using body fluids, it is more difficult to probe epigenetic changes in renal tissues, which are not easily accessible. Nevertheless, investigators have focused on this organ as the relevant site of the disease process. Wang et al. has recently analyzed the gene expression omnibus (GEO) public database archiving data from kidney and reported 121 genes with hypermethylated sites and 579 genes with hypomethylated sites in the kidney tissue. Among these genes with differential methylation states, two hypomethylated genes [Peroxisome proliferator-activated receptor alpha (*PPARA)* and Glutaminase (*GLS)]* were specific for tubular cells and one hypermethylated gene (*PIK3C2B*) were specific for glomeruli [68]. Because tubular cells have abundant mitochondria and are important for acid-base regulation through ammoniagenesis, these findings may suggest epigenetic modulation of mitochondrial biogenesis (PGC-1α) and acid-base regulation (glutaminase) that may contribute to the disease process of DKD.

Hayashi et al. found that KLF4 expression is decreased in proteinuric states in human renal tissues. Restoration of KLF4 expression in diseased glomeruli *in vivo* resulted in restoration of nephrin expression and the attenuation of proteinuria. To explain the mechanism, the authors suggested that KLF4 overexpression leads to demethylation in the *nephrin* promoter region and consequently increases *nephrin* promoter activity. In contrast, KLF4 overexpression caused an increase of DNA methylation in the *vimentin* promoter, which in turn suppresses the expression of vimentin [69].

The association of histone methylation and acetylation in DKD progression have also been examined in renal tissue or cells [70-72]. H3K9ac levels were significantly increased in renal biopsies from patients with DKD, especially in the podocytes [70]. The change of histone methylation/acetylation in different renal cells has been suggested to induce the expression

of potent profibrotic factors such as TGF-β1, CTGF, PAI-1 and Col-1A [41,73,74], which have been correlated with increased renal fibrosis and DKD progress to ESRD [75].

HDAC 2/4/5/9 have been shown to be upregulated in kidney biopsy tissue obtained from patients with diabetes. The mRNA levels of HDAC2/4/5 in patients with DKD were negatively correlated with eGFR. Inhibition of HDACs reduced albuminuria and mesangial expansion, ameliorated podocyte injury, attenuated glomerulosclerosis and decreased production of proinflammatory mediators in diabetic animals [76-79]. The mRNA and protein levels of TFEB, an important regulator of the autophagy-lysosome pathway, were reduced in the renal parenchyma of patients with DKD in comparison to individuals without diabetes or kidney disease. TFEB acetylation was increased by HDAC6 inhibitor in tubular cell. Treating right kidney-nephrectomized rats with an HDAC6 inhibitor attenuated proteinuria, tubule epithelial cell death, and tubulointerstitial collagen IV protein accumulation, concomitantly with an increase in renal protein levels of the TFEB target gene beclin 1 [80]. All of the above indicates that HDAC changes influence progression of DKD, suggesting that HDAC inhibition might be a potential treatment for DKD.

Belonging to Class III HDAC, sirtuins, including SIRT1-7, have been implicated in aging, transcription, apoptosis, and inflammation. The levels of Sirt1, Sirt3, Sirt4, and Sirt6 were reduced in kidneys from patients with diabetic nephropathy. Moreover, Sirt1 expression in proximal tubular and glomerular regions were lower in the kidneys of patients with heavy proteinuria compared with those with moderate proteinuria. Diabetic mice with Sirt6 deletion developed marked albuminuria, significant mesangial matrix expansion, glomerular basement membrane thickening, and podocyte foot process broadening and effacement by increased H3K9ac and activating Notch signaling pathways [70]. Pharmacological targeting of Sirt6-mediated Notch signaling pathways at multiple levels may provide a novel approach for the treatment of proteinuric kidney disease.

The studies about lncRNAs in DKD are limited, but there has been rising interest in their role in the pathogenesis of DKD. In one of the earliest studies, a key lncRNA, lnc-megacluster (*Inc-MGC*) was shown to promote mesangial cell extracellular matrix accumulation and hypertrophy related to early DKD. Lnc-MGC is a host non-coding transcript to a megacluster of nearly 40 miRNAs (miR-379 cluster), which are coordinately increased by HG and TGFβ1 in cultured mouse and human mesangial cells, and in glomeruli of mouse models of type1 and type 2 diabetes. Notably, knockdown of *Lnc-MGC* in diabetic mice with a modified antisense oligonucleotide (GapmeR) reduced glomerular hypertrophy and other features of early diabetic nephropathy, demonstrating the translational potential of targeting renal lncRNAs [81]. LncRNA taurine upregulated gene 1 (Tug 1) was the first lncRNA identified to regulate podocyte function, and through RNA sequencing its transcript levels were shown to be decreased in DKD compared with controls. Lower expression levels of TUG1 was correlated with reduced levels of eGFR in patients with DKD. Podocyte-specific diabetic Tug1 transgenic (Tug^{PodTg}) mice showed a significant reduction in albuminuria. This was associated with a reduction in mesangial matrix expansion, improvement in effacement of podocyte foot process and glomerular basement membrane thickening. The effect of Tug1 overexpression on DKD was shown to regulate the PGC1α and influence mitochondrial bioenergetics [82]. A recent study has identified another

lncRNA, Erbb4-IR, to be a potential key regulator in DKD. Kidney-specific silencing of Erbb4-IR could protect against the development of type 2 DKD, such as elevated microalbuminuria, serum creatinine, and progressive renal fibrosis in *db/db* mice. Inhibition of Erbb4-IR in mouse tubular epithelial cells suppressed TGFβ1-induced collagen I and α-SMA expression inhibiting tubular epithelial mesenchymal transition, extracellular matrix accumulation and significantly reducing the severity of tubulointerstitial fibrosis [83,84]. In another study, Linc01619 was found to be downregulated in renal biopsy tissues collected from patients with DKD. It was negatively correlated with serum creatinine and proteinuria, and positively correlated with eGFR [85]. Additional lncRNAs reported to have functions related to DKD in different renal cell types [86-98] are summarized in Table 1.

Conclusion

Although insulin and a variety of newer medications have been used to control hyperglycemia, microvascular complications such as DKD are still inevitable in many patients with diabetes. Furthermore, many patients with DKD develop ESRD, a devastating outcome of CKD. Currently, there is still a lack of effective measures to predict the risk for DKD development and progression. Epigenetic changes including DNA methylation, histone modification, and noncoding RNAs, are increasingly recognized features of DKD and recent evidence suggests that epigenetic mechanisms may influence the pathogenesis and progression of DKD. Given the rapid progress in epigenetics and epigenomics due to increasingly affordable high-throughput technology and advances in bioinformatics software, more data addressing the epigenetics in DKD can be obtained in a much more cost-effective manner. These advances may reveal additional epigenetic signatures or enhance profiling in the DKD population.

Further investigation is necessary to delineate any causal relationship of epigenetic modification with DKD pathogenesis in light of recent advances in the understanding of basic molecular and cellular mechanisms. In this regard, integration of these data with emerging genome-wide association results for DKD could help place the activation or repression of specific genes on causal pathways. This could enable the application of current knowledge of epigenetics to the evaluation of DKD risk, and facilitate the discovery of new treatment to manage this debilitating disease. A major challenging to the translation of knowledge to the evaluation of patients with DKD is the limited availability of samples and the heterogeneity of renal tissues with multiple cell types. Moreover, epigenetic data generated from patients with DKD in different studies are often affected by heterogeneity in the selection of study population, the nature of the samples collected, and the profiling methods employed. More consistency in study protocols and experimental procedures is needed to promote the reproducibility and comparability of various studies.

Although microRNAs have been showed to be a useful marker in the diagnosis and severity of albuminuria as well as markers of pathological change in the body fluids of patients with DKD, whether DNA methylation, histone modification, and lncRNA levels in urine or serum can be reliable markers to evaluate risks for DKD remains largely unknown. In particular, the role of lncRNAs in the context of DKD is a relative new area for exploration. More research is warranted to elucidate mechanisms by which these non-coding transcripts alter

molecular and cellular processes related to DKD. Several small molecule inhibitors that target epigenetic regulation have been administered in DKD animal models and show promise in attenuating the pathological manifestations of DKD. However, none of them have been tested in patients with DKD. It would be of great interest to investigate whether these therapeutic strategies designed to influence epigenetic profile could delay or prevent the development of DKD and/or even reverse the progression of this condition.

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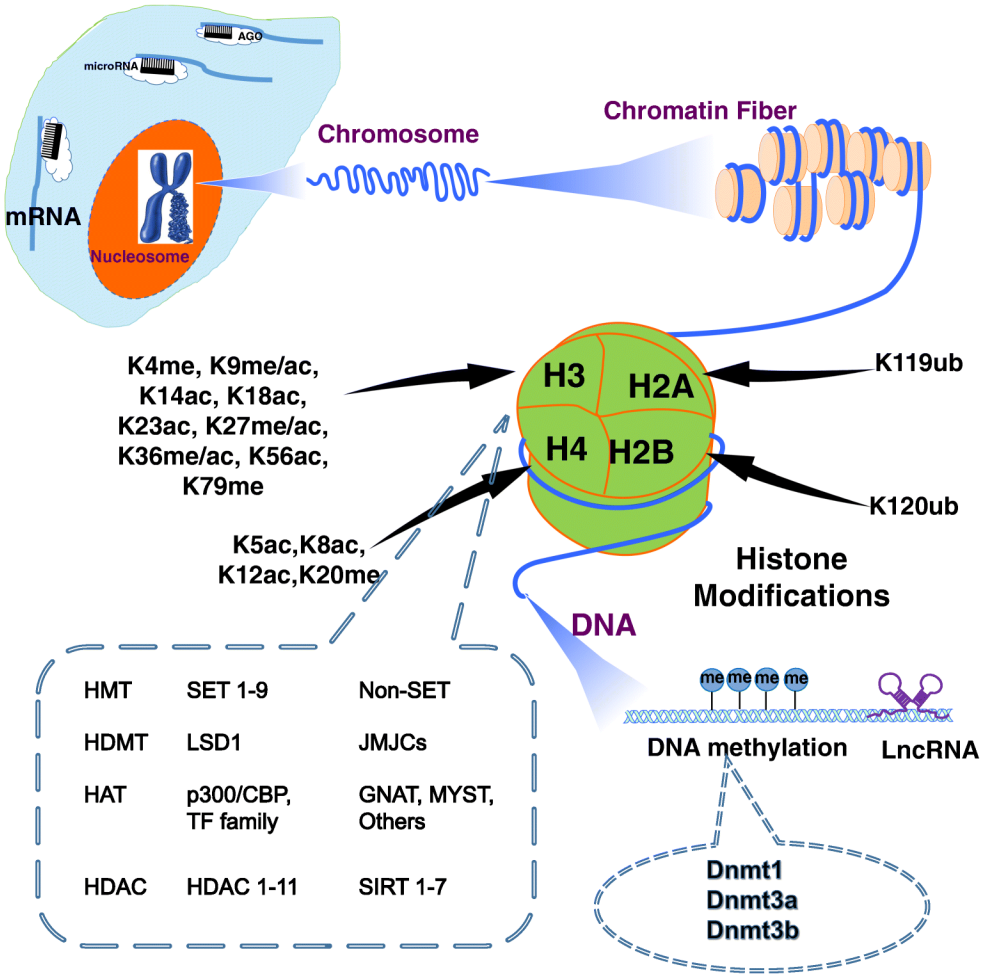

Figure 1. Overview of epigenetic modifications and mediators in DKD.

Chromatins are subjected to epigenetic regulations, including DNA methylation, posttranslational histone modifications, and microRNA (miRNA) and long noncoding RNAs (lncRNAs)-mediated gene regulation. miRNAs typically affect mRNA expression at post-transcriptional level by targeting the 3'-untranslated region of mRNA in the cytoplasm. DNA methylation and histone modification influence gene regulation in the nucleus. These molecular processes are intricately regulated by various epigenetic enzymes as depicted. LncRNAs can regulate gene expression through either nuclear or cytoplasmic mechanism. DNMT, DNA methyltransferases; HMT, histone methyltransferases; HDM, histone demethylase; HAT, histone acetyltransferases; and histone deacetylases (HDACs).

Table 1.

Long non-coding RNAs involved in diabetic nephropathy

Cell type	LncRNA	Cell function	Potential targets	ref
mouse mesangial cell	CYP4B1-PS1-001	overexpression of CYP4B1-PS1-001 inhibited proliferation and fibrosis of mesangial cells	PCNA,cyclin-D1,Col 1,Fibronectin	86
	ENSMUST-00000147869	Reduce cell proliferation, decreased the synthesis of fibronectin and collagen I	PCNA,cyclin-D1,Col 1, Fibronectin	87
	Gm4419	Regulate the pro-inflammatory and fibrosis cytokines and mesangial cell proliferation	NF-kB/NRLP3	88
	lincRNA 1700020I24Rik	affect cell proliferation and expressions of renal fibrosis markers	miR-34a-5p/Sirt1/HIF-1α	89
human mesangial cell (HMC)	Lnc-MGC	inhibit the expression of key cluster miRNAs in the kidney, and regulate TGF-β signaling, cellular hypertrophy, extracellular matrix synthesis and ER stress.	PTEN,CUGBP2,PUM2, TNRC6B,CPEB4, BHC80,EDEM3, ATF3 and others	81
	ASncmtRNA-2	TGF-b and FN expression were up regulated by HG-induced ASncmtRNA-2 in HMC	TGF-b	90
	PVT1	FN1, COL4A,TGFB1,PAI-1 expression were decreased in HMC treated with high glucose	FN1, COL4A,TGFB1,PAI-1	91
podocyte	Lnc TUG1	modulates mitochondrial bioenergetics through PGC-1a in podocyte	peroxisome proliferator–activated receptor g coactivator a (PGC-1 a)	82
	Linc01619	regulates miR-27a/FOXO1 mediated ER stress and podocyte apoptosis	miR 27a/FOXO1	85
	ENSRNOG00000037522	inhibit the high glucose-induced podocyte epithelial mesenchymal transition	PODXL-1,nephrin	92
	MALAT1	MALAT1 regulated by b-catenin, MALAT1 knock-down rectified podocyte damage via down-regulating SRSF1 overexpression	SRSF1	93
	Gm5524 Gm15645	Affect autophagy and apoptosis of podocyte	Atg5,Atg7,Bcl2	98
HK2	MALAT1	Down regulated MALAT1 decreased the expression of the pro-inflammatory cytokines IL-6 and TNF-α	ELAVL1, NLRP3, Caspase-1, TNF-a, IL-1β ,IL-6	94,95
	MIAT	regulated HK2 cell viability via stabilizing Nrf2 expression	Nrf2	96
	Lnc ZEB1-AS1	Inhibition of lnc ZEB1-AS1 may increase HG-induced ECM accumulation by downregulating ZEB1 expression	ZEB1	97
tubular epithelial cell (TEC)/mouse mesangial cell (MMC)	Erbb4-IR	Regulate the collagen expression in TEC and MMC through miR29b	miR29b	83