Epigenetics in Diabetic Kidney Disease

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ABSTRACT
Regulated gene expression by transcription factor networks is critical for normal kidney function. Disruption of these complex networks leads to biochemical aberrations associated with many renal diseases. Epigenetic mechanisms not involving changes in DNA sequence, such as DNA methylation and post-translational modifications of nucleosomal histones, also play a critical role in gene regulation by modulating chromatin access to the cellular machinery for transcription. These epigenetic modifications can be affected by intrinsic and extrinsic environmental factors and play a central role in dictating biologic phenotypes including pathologic disease. Emerging evidence also suggests, apart from traditional genetic predisposition, that epigenetic processes can persist across generations to play a modulating role in the development of renal diseases such as diabetic nephropathy. Recent advances in epigenome research has increased our understanding of epigenetic mechanisms involved in renal dysfunction that in turn may lead to identification of novel new therapeutic targets.

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Epigenetics refers to heritable changes that occur outside the modification of DNA coding sequence, including those conferred mitotically or meiotically. Although the term epigenetics was originally coined to describe programmed changes during embryonic development, more broadly it has been modified to also include the structural adaptation of chromosomal regions to register altered activity states. Epigenetic mechanisms confer transcriptional memory and regulate patterns of cell-specific gene expression during development to maintain cell identity during subsequent cell divisions. Epigenetics also plays key roles in stem-cell plasticity, T cell memory, fetal reprogramming, imprinting, and cellular response to environmental cues. Alterations in epigenetic mechanisms by environmental and other factors can contribute to acute renal injury or lead to chronic diseases such as cancer, diabetes, and cardiovascular diseases. Recent evidence also supports the important notion of transgenerational inheritance of epigenetic changes that influence the well being of future generations.

Epigenetic information is stored in chromatin, a higher order structure of DNA packaged into nucleoprotein complexes consisting of histones and nonhistone proteins. The basic subunit of chromatin is a nucleosome in which DNA is wrapped around an octamer protein complex consisting of dimers of core histone proteins (H2A, H2B, H3, and H4). Chromatin structure plays a critical role in determining the transcriptional status of DNA. Heterochromatin representing transcriptionally silent regions is more compact and thus less accessible to transcriptional machinery, whereas euchromatin representing actively transcribed regions has an open structure that is more permissible. Heterochromatin and euchromatin states, and the dynamic shifts between them, are regulated by epigenetic mechanisms such as DNA methylation (DNAm), histone post-translational modifications (PTMs), small noncoding microRNAs, and long noncoding RNAs (Figure 1). DNA methylation, one of the most stable epigenetic marks, is mediated by DNA methyltransferases (DNMTs) at the 5’-position of cytosine residues in CpG dinucleotides, which tend to be concentrated in regions called CpG islands in genomic DNA. DNMT3A and DNMT3B mediate de novo DNA methylation, whereas DNMT1 is a maintenance methyltransferase that functions to transmit DNA methylation patterns to daughter strands during replication. Methyl-CpG-binding domain proteins bind methylated DNA and recruit transcriptional repressors to mediate gene silencing. DNA methylation patterns are affected by environmental factors, diet, and fetal nutrition and modulate disease susceptibility and embryonic development. In particular, tumor suppressor genes can be silenced by promoter DNA methylation during cancer development, and DNA methylation inhibitors are currently being used to reactivate...
Epigenetic mechanisms can lead to the inhibition of protective genes and activation of pathologic genes associated with renal disease. Chromosomal DNA is tightly packed into higher order nucleoprotein complexes in chromatin consisting of repeating units of nucleosomes made up of DNA wrapped around dimers of core histone proteins. Epigenetic mechanisms including post-transcriptional modifications of nucleosomal histone amino-terminal tails, DNA methylation (DNAme), and noncoding RNAs, collectively referred to as the epigenome, regulate dynamic switching of chromatin between transcriptionally silent compact structure (heterochromatin) and active relaxed structure (euchromatin) to regulate gene expression. Histone lysine acetylation (H3/H4Kac) mediated by histone acetyl transferases such as CBP/P300 and H3 lysine 4 methylation (H3K4me) mediated by histone methyltransferases such as SET7 and MLL can lead to the formation of open chromatin accessible to transcription machinery and active gene expression. In contrast, histone PTMs such as H3K9me3, H3K27me3, and H4K20me3 mediated by HMTs Suv39h1, Ezh2, and Suv4h20, respectively, and DNAme mediated by DNA methyltransferases promote heterochromatin structure associated with transcriptional repression. These modifications are reversible by relevant histone deacetylases, histone demethylases, and DNA demethylases (not shown here). Alterations in the epigenome under disease states such as diabetes or renal injury leads to increased expression of pathologic inflammatory and fibrotic genes and microRNAs (miRNAs) involved in renal diseases or to the inhibition of protective genes. Furthermore, miRNAs can also target epigenetic components to mediate aberrant gene expression. Persistence of epigenetic alterations including decreased H3K9me3 or increased H4K20me3 or H3K4me in diabetes can play key roles in metabolic memory implicated in chronic vascular and renal complications that persist even after glycemic control. HG, high glucose; AGEs, advanced glycation end products; RNA Pol2, RNA polymerase II.

Figure 1. Epigenetic mechanisms can lead to the inhibition of protective genes and activation of pathologic genes associated with renal disease. Chromosomal DNA is tightly packed into higher order nucleoprotein complexes in chromatin consisting of repeating units of nucleosomes made up of DNA wrapped around dimers of core histone proteins. Epigenetic mechanisms including post-transcriptional modifications of nucleosomal histone amino-terminal tails, DNA methylation (DNAme), and noncoding RNAs, collectively referred to as the epigenome, regulate dynamic switching of chromatin between transcriptionally silent compact structure (heterochromatin) and active relaxed structure (euchromatin) to regulate gene expression. Histone lysine acetylation (H3/H4Kac) mediated by histone acetyl transferases such as CBP/P300 and H3 lysine 4 methylation (H3K4me) mediated by histone methyltransferases such as SET7 and MLL can lead to the formation of open chromatin accessible to transcription machinery and active gene expression. In contrast, histone PTMs such as H3K9me3, H3K27me3, and H4K20me3 mediated by HMTs Suv39h1, Ezh2, and Suv4h20, respectively, and DNAme mediated by DNA methyltransferases promote heterochromatin structure associated with transcriptional repression. These modifications are reversible by relevant histone deacetylases, histone demethylases, and DNA demethylases (not shown here). Alterations in the epigenome under disease states such as diabetes or renal injury leads to increased expression of pathologic inflammatory and fibrotic genes and microRNAs (miRNAs) involved in renal diseases or to the inhibition of protective genes. Furthermore, miRNAs can also target epigenetic components to mediate aberrant gene expression. Persistence of epigenetic alterations including decreased H3K9me3 or increased H4K20me3 or H3K4me in diabetes can play key roles in metabolic memory implicated in chronic vascular and renal complications that persist even after glycemic control. HG, high glucose; AGEs, advanced glycation end products; RNA Pol2, RNA polymerase II.

these genes as a therapeutic approach to cancer treatment.6

Histone PTMs are also implicated in both normal cellular function and disease. The exposed amino-terminal tails of nucleosomal histones are subject to several PTMs, including acetylation, methylation, phosphorylation, sumoylation, or ubiquitination.12 Histone lysine acetylation (HKac) marks, such as H3K9ac, H3K14ac, and H4Kac, are generally associated with active promoters. Histone lysine methylation (HKme), on the other hand, associates with either active or inactive promoters depending on the methylated lysine. In general, trimethylation at H3K9, H3K27, and H4K20 associates with inactive genes and trimethylation at H3K4me and H3K36 with promoters and gene bodies of actively transcribed genes, respectively. Pairs of enzymes similar to kinases and phosphatases regulating phosphorylation status dynamically modulate histone modifications. HKac is mediated by histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs). Histone methylation is carried out by histone methyltransferases (HMTs) and erased by histone lysine demethylases. In general HATs tend to be transcription coactivators, whereas HDACs act as repressors. HMTs and histone lysine demethylases can be either positive or negative regulators of transcription depending on the amino acid, position, or extent of methylation (mono, di, or tri). In general, changes in HKac are quite dynamic, whereas HKme are relatively more stable and play a role in long-term cellular memory.14

Epigenetic information carried by histone PTMs can be inherited, but the mechanisms are unclear.2 The epigenetic landscape of the genome including DNAme, histone PTMs, and noncoding RNAs is referred to as the epigenome (Figure 1).13 Recent advances in genomics and sequencing technology reveal diverse features of the epigenome in human stem cells, normal development, and disease.12,13,15

Diabetic Renal Complications and Epigenetic Mechanisms

Diabetes and other risk factors, such as hypertension and hyperlipidemia, can lead to chronic kidney disease (CKD) and ultimately renal failure. Complex interactions between renal endothelial cells, mesangial cells, podocytes, and tubular epithelial cells, as well as infiltrating macrophages, play pivotal roles in a variety of renal diseases. Hyperglycemia, a major risk factor for diabetic nephropathy and its downstream effectors, such as advanced glycation end products, proinflammatory cytokines, and growth factors, promote fibrosis and renal injury through various biochemical mechanisms.16 Although several drugs are currently available for the treatment of diabetic nephropathy,17–22 diabetic patients still reach ESRD at alarming levels. Furthermore, clinical and experimental studies demonstrate the occurrence of a metabolic memory of prior exposure to hyperglycemia, resulting in persistently increased risk for diabetic complications, including nephropathy, long after glucose normalization.23,24 This finding suggests a potential role for epigenetic mechanisms apart from genetic predis-
position in the etiology of diabetes and its complications, as indicated by recent studies. DNA methylation (DNAm) is implicated in the development of diabetes in animal models such as Agouti mice and intrauterine growth retardation. It is also reported in the reduced expression of PGC-1α, leading to reduced insulin expression in islets of diabetes animal models. Recent studies also implicate DNAm in kidney diseases. One study identified a key role for DNAm in fibroblast proliferation and fibrosis in injured kidneys. Differences in DNAm have been observed in patients with CKD, as well as at key genes in diabetic patients with nephropathy.

Epigenetic histone PTMs are also implicated in the regulation of islet-specific gene expression of insulin mediated by the Pdx1 transcription factor in response to changing glucose levels, as well as in adipocyte differentiation. Genetic knockout of the H3K9me2 demethylase, Jhdm2a, leads to obesity and hyperlipidemia. Inflammatory gene expression mediated by NF-κB plays an important role in renal diseases. Evidence shows that epigenetic histone PTMs, including HKac and HKme, and relevant modifying enzymes including HATs such as CBP/P300, key HDACs, and HMTs such as SET7/9 (H3K4me transferase) modulate NF-κB-mediated inflammatory gene expression under normal and diabetic conditions in vascular cells and monocytes. Furthermore, chromatin immunoprecipitation followed by microarrays (ChIP-on-chip) identifies genomewide changes in H3Kme in human monocytes under diabetic conditions, supporting the role of epigenetic modifications in diabetes and its inflammatory complications. However, only limited information is available on the direct role of histone PTMs in renal cells under diabetic conditions.

Recently, TGFβ and high glucose-induced fibrotic gene expression in rat renal mesangial cells were shown to increase H3K4me1–3 (activation marks) and reduce H3K9me2/3 (repressive marks) at these gene promoters. TGFβ also upregulates the H3K4 methyltransferase, SET7/9, in mesangial cells, and SET7/9 gene silencing inhibits TGFβ-induced fibrotic gene expression. Interestingly, a TGFβ antibody blocks high glucose-induced fibrotic gene expression and reverses high glucose-induced histone modifications at their promoters in mesangial cells. These results raise the prospect of evaluating therapeutic modalities targeting TGFβ actions to reverse epigenetic changes associated with diabetic renal complications (Figure 1).

In other studies, H3K4me3 and the chromatin remodeling enzyme Brg1 have been implicated in inflammatory gene expression during renal ischemia reperfusion injury, and increased H3K27me3 is noted at collagen III (Col3a1) promoter in rat models of aging nephropathy. Changes in global histone PTMs in kidneys from diabetic mice were also observed. Together, these studies emphasize the need to fully understand the consequences of variations in DNAm and histone PTMs to identify novel biomarkers and therapeutic targets for renal diseases.

**Metabolic Memory and Epigenetic Mechanism**

There has been considerable interest in identifying the role of epigenetic mechanisms in metabolic memory. Persistently increased expression of p65 (NF-κB active subunit) associates with increased promoter H3K4me1 and SET7/9 occupancy in endothelial cells exposed to short term high glucose treatment even several days after return to normal glucose. A sustained proinflammatory phenotype in vascular smooth muscle cells cultured from type 2 diabetic db/db mice also associates with reduced levels of the repressive mark, H3K9me3, at these gene promoter sites and reduced protein levels of the H3K9me3 methyltransferase Suv39h1, at least in part through upregulation of miR-125b. This latter finding illustrates a novel interaction between two epigenetic components to augment inflammation under pathologic conditions. Promoter levels of the repressive H4K20me3 mark and the corresponding methyltransferase Suv4h20 associate with reduced expression of the manganese-superoxide dismutase (sod2) gene in retinas of diabetic rats exhibiting metabolic memory (Figure 1). Further studies are needed to determine whether diabetes-induced changes in histone PTMs are cell-specific and similarly affect all target renal cells including mesangial cells, podocytes, and epithelial cells. In addition, evaluation of specific mouse models and clinical cohorts in the future will help determine the functional role of epigenetic marks in diabetic nephropathy as well as metabolic memory.

**Summary**

Epigenetic mechanisms that alter chromatin structure play important roles in fine tuning of gene expression mediated by transcription factors. Recent reports of epigenetic mechanisms in renal injury, fibrosis, inflammation, and metabolic memory have set the stage for future research in this area. Epigenomic research has been greatly aided by recent developments in genome technologies including microarrays and next generation sequencing. Major efforts including the Human Epigenome Project and the epigenomics initiative of the National Institutes of Health (http://commonfund.nih.gov/epigenomics/epigeneticmechanisms.aspx) will accelerate our understanding of epigenome alterations relevant to renal and other human diseases. Given the rapid advances in affordable high-throughput methods to quantify genome-wide DNA methylation and histone PTMs, it is anticipated that key chromatin marks among various clinical cohorts will soon be assessed for their role in epigenetic modulation of a wide range of renal diseases. The hope is that these efforts will also lead to much needed new therapies for CKD. Several small molecule inhibitors are already in use as epigenetic therapies for various cancers. Similar strategies may be developed to reverse epigenetic changes associated with CKD and metabolic memory, a major challenge in the prevention of chronic diabetic complications.

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DISCLOSURES
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