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Epigenetics: Deciphering its role in Diabetes and its Chronic Complications

Louisa M. Villeneuve, Marpadga A Reddy, and Rama Natarajan*

Department of Diabetes, Beckman Research Institute of City of Hope, Duarte, CA 91010, USA

SUMMARY

1. Increasing evidence suggests that epigenetic factors may regulate the complex interplay between genes and the environment and affect human diseases such as diabetes and its complications.
2. Clinical trials have underscored the long lasting beneficial effects of strict glycemic control for reducing the progression of diabetic complications. They have also demonstrated that diabetic complications such as diabetic nephropathy, a chronic kidney disorder, can continue even after blood glucose normalization, suggesting a metabolic memory of the prior glycemic state.
3. Dysregulation of epigenetic post-transcriptional modifications of histones in chromatin, including histone lysine methylation, has been implicated in aberrant gene regulation associated with the pathology of diabetes and its complications. Genome-wide studies have demonstrated cell-type specific changes in histone methylation patterns under diabetic conditions. In addition, studies in vascular cells have shown long lasting changes in epigenetic modifications at key inflammatory gene promoters after prior exposure to diabetic conditions suggesting a possible mechanism for metabolic memory.
4. Recent studies have demonstrated roles for histone methylation, DNA methylation, as well as microRNAs in diabetic nephropathy. Whether these epigenetic factors play a role in metabolic memory of diabetic kidney disease is less well understood.
5. The incidence of diabetes is growing rapidly, as also the cost of treating the resulting complications. A better understanding of metabolic memory and the potential involvement of epigenetic mechanisms in this phenomenon could enable the development of new therapeutic targets for the treatment and/or prevention of sustained diabetic complications.

Keywords

Diabetic Kidney Disease; Hyperglycemia; Chromatin; Histone modifications; Metabolic Memory

INTRODUCTION

Various risk factors are associated with chronic kidney disease (CKD) including diabetes, cardiovascular disease (CVD), high blood pressure, glomerular disorders, polycystic kidney disease, as well as other autoimmune diseases. Both diabetic and non-diabetic kidney diseases are quite often associated with proteinuria, hypertension and decreased glomerular

* **Correspondence to:** Professor, Division of Diabetes, Beckman Research Institute of City of Hope, 1500 E. Duarte Road, Duarte, CA-91010. RNatarajan@coh.org Telephone: 626-256-4673 Ext. 62289 Fax : 626-301-8136 .

filtration rates. Diabetes is a leading cause of progressive renal failure and end stage renal disease (ESRD). Even when blood glucose levels are under control, diabetic patients may still develop CKD and renal failure. Current treatment options for diabetic kidney disease include blood glucose and blood pressure control, and treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) to reduce hypertension, proteinuria and progression to renal failure¹. Most of these treatment options only delay progression to ESRD, a debilitating condition requiring dialysis and/or kidney transplantation. As such, it is imperative to evaluate new therapeutic modalities. Recent studies have demonstrated the involvement of epigenetic mechanisms in diabetes and renal complications²⁻³ suggesting that a greater understanding of these phenomena might uncover novel therapeutic targets and strategies.

DIABETIC COMPLICATIONS

Diabetes is a chronic metabolic disease associated with both genetic and environmental factors. The worldwide epidemic of diabetes has greatly increased treatment costs for both the disease and its numerous debilitating complications. Both Type 1 (T1D) and Type 2 diabetes (T2D) are associated with significantly accelerated rates of macrovascular diseases such as atherosclerosis, hypertension and stroke, and microvascular complications such as neuropathy, retinopathy and nephropathy. Furthermore, diabetic nephropathy (DN) is also associated with macrovascular diseases which can ultimately lead to ESRD and CVDs.

Genetic factors and key gene mutations have been implicated in the pathogenesis of diabetes and DN. However, accumulating evidence suggests that complex interactions between genes and the environment may play a major role in many common human diseases such as diabetes and its complications⁴ and this may be due to the involvement of epigenetic factors. Chromatin provides a crucial interface between genetics and environment, and the epigenetic changes in chromatin, such as DNA methylation and post-transcriptional modifications (PTMs) of histone tails, have been linked to gene transcription. Epigenetics refers to the study of heritable patterns of gene expression and subsequent phenotypic changes that occur without alterations in the underlying DNA sequence. Epigenetic factors are now associated with diseases such as cancer and diabetes^{2, 5}. Numerous studies have identified key biochemical pathways triggered by hyperglycemia and diabetes in target cells related to inflammation and diabetic complications⁶⁻⁷. Hyperglycemia and diabetes associated dyslipidemia can alter the expression of inflammatory and other pathologic genes and proteins related to the development of both micro- and macrovascular complications of diabetes. Inflammation also plays a key role in CKD induced by risk factors such as environmental insults, exposure to toxins and infections⁸. However, the exact molecular mechanisms are still not very clear. Increasing evidence suggests that epigenetic mechanisms may be involved in the regulation of inflammatory and extracellular matrix (ECM) genes associated with the pathology of diabetes and its complications such as diabetic nephropathy^{2-3, 9} and this is bound to be a stimulating area of research.

METABOLIC MEMORY: EVIDENCE FROM CLINICAL TRIALS AND EXPERIMENTAL MODELS

Although both T1D and T2D can be controlled through medications, dietary modifications and exercise, subjects with diabetes continue to be afflicted with numerous life threatening complications. This continued development of diabetic complications even after achieving glucose control suggests a 'metabolic memory' of prior glycemic exposure which recent studies have suggested may be attributed to epigenetic changes in target cells. Exploring a role for epigenetics in diabetic complications could allow for new insights clarifying the

interplay between the environment and gene regulation and identify much needed new therapeutic targets.

Several clinical trials have indicated that early intensive glycemic control leads to sustained beneficial effects. The Diabetes Control and Complications Trial (DCCT) demonstrated that T1D patients under intensive insulin therapy had delayed progression of nephropathy, retinopathy, and neuropathy as compared to patients under conventional therapy. At the end of the DCCT, all patients were placed on strict glycemic control and followed up long-term in the Epidemiology of Diabetic Complications and Interventions Trial (EDIC). The EDIC trial demonstrated that patients originally in the intensive treatment group during DCCT and continued on intensive therapy during EDIC continue to have significantly slower progression of key diabetic microvascular complications relative to patients who were in the conventional treatment group during DCCT, even though the latter were placed on intensive therapy and after the differences in glycosylated hemoglobin had normalized several years into the EDIC trial¹⁰⁻¹² (Figure 1). More recently, patients in the continuous intensive treatment group since the beginning of the trials were found to also have better outcomes for macrovascular complications¹³⁻¹⁵. Other trials with T2D patients have also demonstrated that the benefits of intensive glycemic control lasted long after cessation of intervention suggesting a legacy effect. T2D patients with lower fasting blood glucose levels had lesser vascular complications¹⁶⁻¹⁸.

Together, these trials demonstrate the importance of early metabolic control to reduce long term complications and confirm that hyperglycemia can have long lasting detrimental consequences. In addition, postprandial hyperglycemic spikes may also have long term effects¹⁹. The mechanisms responsible for these lasting effects of the prior hyperglycemic state or erratic metabolic control are not well understood and this phenomenon, termed 'metabolic memory' is a major challenge in the treatment of diabetic complications.

Several experimental models have been used to study the molecular mechanisms responsible for metabolic memory. Early studies in dogs found a continuation of retinal complications even after reversing hyperglycemia²⁰. Islet transplantation in rats after twelve weeks of diabetes could not reverse the progression of retinopathy as compared to islet transplantation after six weeks of diabetes.²¹ Studies in streptozotocin (STZ)-induced diabetic rats showed that early glycemic control had protective effects in the retina; however, reinstitution of normal glucose after prolonged diabetes failed to reverse increases in oxidant stress and inflammation which was attributed to metabolic memory²²⁻²³. Similar results were seen in the kidneys of STZ-injected rats²⁴.

High glucose (HG) cultured endothelial cells showed sustained increases in key pro-fibrotic and extracellular matrix proteins even after glucose normalization²⁵. More recently, short-term high glucose culture of endothelial cells displayed sustained activation of inflammatory pathways and oxidant stress that persisted even after glucose normalization²⁶⁻²⁷. Another group demonstrated the persistence of oxidant stress for up to one week after glucose normalization, which could be blocked by antioxidants or NADPH oxidase inhibitors²⁸. Studies in vascular smooth muscle cells (VSMC) derived from T2D obese *db/db* mice demonstrated sustained increases in NF- κ B activation, inflammatory gene expression, migration, and oxidant stress as well as increased monocyte adhesion to relative to control non-diabetic *db/+* VSMC even after in vitro culture for several passages²⁹.

These results suggest a metabolic memory of vascular dysfunction arising from hyperglycemic exposure, and further support the need for strict glycemic control to slow the progression of diabetic vascular complications. They also suggest a role for oxidant stress in perpetuating this metabolic memory by modifying or damaging essential lipids,

proteins, and/or DNA ²⁸. Hyperglycemia and oxidant stress can also increase the accumulation of advanced glycation end products (AGEs) which can further propagate and amplify local inflammation and oxidant stress through irreversible glycation of the various proteins and lipids to promote long term vascular and end-organ damage. Thus AGEs, acting through receptors such as RAGE ³⁰, could also contribute to hyperglycemic memory. Although these studies provide insights into the biochemical aspects of metabolic memory, the nuclear mechanisms responsible for sustained 'memory' over time through multiple cell divisions at the transcriptional and epigenetic level are not fully understood and have elicited much interest in recent years.

EPIGENETIC REGULATION OF GENE TRANSCRIPTION

Chromatin is composed of subunits called nucleosomes containing DNA wrapped around a histone octamer with two copies each of histone H2A, H2B, H3, and H4 ³¹. Transcriptional activation or repression is a dynamic process that depends on the recruitment of protein complexes that alter chromatin structure via enzymatic modifications of histone tails and nucleosome remodeling. These epigenetic histone post-translational modifications (PTMs) include lysine acetylation, methylation, and phosphorylation. Along with other epigenetic factors such as DNA methylation and non-coding RNAs such as microRNAs, these PTMs in chromatin form an added layer of gene regulation without altering the DNA code itself. While microRNAs do play a role in epigenetic regulation as well ³² they are not covered in this review. Numerous combinations of epigenetic modifications allow for chromatin flexibility in response to stimuli and can affect recruitment/ binding of various DNA and histone binding complexes, co-activators and corepressors, thereby dictating chromatin accessibility and transcriptional outcomes ³³.

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are regulators of histone lysine acetylation which usually occurs quite rapidly ³⁴. Histone methylation is generally considered to be more long lasting. Histone lysine methylation is mediated by histone methyltransferases (HMTs) and more complex since lysine residues can be mono-, di-, or tri-methylated. Overall, activation or repression of gene expression depends on the modification, location as well as the residue modified. For example, histone H3 lysine 4 methylation (H3K4me) is generally associated with gene activation; while histone H3 lysine 9 methylation (H3K9me) with gene repression ³⁵ (Fig. 2). Both promoter and coding regions can also be modified, adding another level of complexity to gene transcription outcomes.

The recent discovery of histone lysine demethylases (KDMs) has demonstrated that histone methylation can also be dynamically regulated through the coordinated action of HMTs and KDMs. Numerous KDMs have been identified with varying specificities for different histone lysine residues ³⁶. Characterizing these demethylases and their potential role in disease is currently of great interest ³⁷. In the context of diabetes, a recent report demonstrated that KDM Jhdm2a is associated with obesity affecting genes related to metabolism in rodent models ³⁸.

Histone PTMs are now widely accepted to play a role in epigenetics; however, their specific roles are not fully deciphered. Histone modifications could precede or succeed DNA methylation, and whether they initiate a transcriptional memory or simply maintain it is still debated ³⁹.

DNA methylation, the most well characterized epigenetic mark, is mediated by DNA methyltransferases at the 5' position of cytosine residues in CpG dinucleotides. Hypermethylation of promoter CpG islands generally results in transcriptional repression ⁴⁰. DNA methylation has been widely studied in the context of tumor suppressor genes and cancer ⁵. However, much less is known about DNA methylation in diabetes and its

complications. Cross-talk between various epigenetic mechanisms lends another level of complexity to gene regulation. DNA methylation of CpG islands can promote histone deacetylation and recruitment of the histone methyltransferase SUV39H1 that mediates H3K9me3 to further stabilize the repressive state. Conversely, histone acetylation can prevent DNA methylation by inhibiting the binding of DNA methyltransferases⁴¹. Evidence also exists for the interaction of DNA methylation and other histone modifications⁴². However, the role of these cross-talk mechanisms in diabetes and its complications awaits further investigation.

EPIGENETIC CHANGES ASSOCIATED WITH DIABETIC COMPLICATIONS

Increasing evidence has implicated a role for epigenetic histone PTMs in diabetes and its complications. HATs and HDACs have been implicated in the regulation of several key genes linked to diabetes⁴³. Modulation of NF- κ B transcriptional activity by HATs and HDACs can affect downstream inflammatory gene expression⁴⁴⁻⁴⁵. Interestingly, monocytes cultured in HG showed increased recruitment of HATs leading to histone lysine acetylation at key inflammatory gene promoters with a corresponding increase in gene expression. Similar results were seen in blood monocytes from T1D and T2D patients⁴⁶. Another study demonstrated that oxidized lipids also enhance inflammatory gene promoter histone acetylation associated with increased gene expression⁴⁷. p300 HAT was found to play a role in glucose induced gene expression in endothelial cells⁴⁸. Interestingly, curcumin, a p300 inhibitor, could prevent hyperglycemia induced changes in gene expression levels⁴⁸⁻⁴⁹, but dietary curcumin either before or after the induction of diabetes in mice failed to attenuate albuminuria⁵⁰.

In addition to histone lysine acetylation, active epigenetic changes such as HMT SET7/9 recruitment and H3K4me marks appear to be characteristic of the insulin gene promoter activation only in cells associated with insulin production⁵¹⁻⁵². Studies in diabetic kidneys have shown an important role for HDACs in transforming growth factor-beta1 (TGF- β 1)-mediated ECM production and renal fibrosis⁵³. Overall these studies demonstrate a role for HATs and HDACs in the pathogenesis of CKD and other diabetic complications. Since lysine acetylation is generally a more transient histone modification, it is not clear whether it plays a significant role in hyperglycemic memory.

Since histone lysine methylation is relatively more stable, there has been much interest in exploring its role in diabetic complications and in metabolic memory. Genome-wide location analyses with chromatin immunoprecipitation (ChIP) coupled to DNA microarrays (ChIP-on-chip) demonstrated dynamic changes in both the H3K4me2 and H3K9me2 marks in cultured monocytes treated with HG, with relevant changes also seen in monocytes from diabetic patients⁵⁴. ChIP-on-chip epigenomic studies with white blood cells from T1D patients versus healthy controls revealed a subset of genes in diabetic lymphocytes displaying increased H3K9me2 linked to immune and inflammatory pathways often associated with T1D and its complications⁵⁵. These epigenomic profiling studies suggest that, while a reasonably stable histone methylation pattern is maintained in healthy individuals in a cell-type specific setting, this pattern can be disrupted in disease states.

The mechanisms responsible for aberrant epigenetic histone methylation occurring under diabetic conditions are beginning to be understood. Knockdown of the H3K4 HMT SET7/9 decreased NF- κ B p65 subunit occupancy at monocyte chemoattractant protein-1 (MCP-1) and TNF- α promoters in monocytes with a corresponding decrease in promoter H3K4me and gene expression, suggesting that SET7/9 might co-activate NF- κ B transcriptional activity in response to inflammatory stimuli prevalent in the diabetic milieu⁵⁶. Similarly, a role for SET7/9 in regulating NF- κ B expression in response to HG was also shown in

endothelial cells²⁶⁻²⁷. Recent studies have also demonstrated that TGF- β treatment of renal mesangial cells increased HMT SET7/9 occupancy and the associated active H3K4me marks, but reduced repressive H3K9me marks at the promoters of key fibrotic genes linked to DN. TGF- β antibody treatment reversed both TGF- β and HG induced gene expression and promoter histone methylation changes⁵⁷. These results suggest a role for epigenetic factors in modulating renal gene expression under diabetic conditions (Figure 3).

Changes in epigenetic histone modifications were noted in models of diabetic cardiomyopathy and glomerulosclerosis⁵⁸⁻⁵⁹. Studies in kidney collecting ducts demonstrated dynamic regulation of H3K79 methylation involved in fluid reabsorption essential for blood pressure control and electrolyte homeostasis⁶⁰. Epigenetic changes have also been noted in models of acute kidney injury. Thus, key histone PTMs and related epigenetic events at inflammatory and fibrotic genes were reported in renal ischemia-reperfusion injury and acute renal failure that might confer a memory of sustained inflammation⁶¹⁻⁶².

There is also increasing interest in the role of DNA methylation in kidney diseases. Enhanced blood homocysteine levels were noted in CKD which affects methyl transfer reactions by inhibiting DNA methyltransferases. This would suggest DNA hypomethylation in patients with CKD as noted in some cases. The relationship between homocysteine and DNA methylation in CKD is however more complex⁶³. Another study evaluated peripheral blood leukocytes obtained from three different groups of CKD patients compared to normal controls and demonstrated an association between global DNA methylation, inflammation and increased mortality in CKD⁶⁴.

Some studies have now examined the role of DNA methylation in diabetes and its complications. Epigenetic silencing due to increased promoter DNA methylation has been linked to islet dysfunction and development of diabetes in animal models⁶⁵⁻⁶⁶. Interestingly, renal cells as well as renal tissues from streptozotocin (STZ) induced T1D rats did not show significant differences in DNA methylation at the promoters of HG or diabetes induced genes⁶⁷⁻⁶⁸. However, altered DNA methylation was noted at key gene promoters in T1D patients with DN as compared to T1D without DN including hypermethylation at *UNC13B*, a gene linked with DN in genetic studies⁶⁹. Another recent study used saliva samples from diabetic patients with ESRD versus those without nephropathy and identified DNA methylation differences in several genes including those associated with kidney disease⁷⁰.

Thus epigenetic changes at specific target gene promoters might explain the accelerated development of renal and other complications or occurrence of metabolic memory that seem to persist even after treatment with drugs or glycemic control.

EPIGENETIC MECHANISMS FOR METABOLIC MEMORY

Recent reports suggest that chromatin-based changes in histone methylation patterns may be responsible for metabolic memory. Diabetic *db/db* VSMC cultured *ex vivo* for several passages continued to exhibit increased inflammatory gene expression associated with diabetic complications as compared to non-diabetic *db/+* VSMC^{29, 71}. This corresponded to decreased H3K9me3 repressive marks at the promoters of key inflammatory genes and was associated with decreased protein levels of Suv39h1, a known HMT mediating H3K9me3. Over-expression of Suv39h1 in the diabetic *db/db* VSMC partially reversed the diabetic phenotype. *db/db* VSMC also exhibited increased TNF- α induced inflammatory gene expression with corresponding sustained decreases in promoter H3K9me3 and Suv39h1 occupancy. Normal human VSMC treated with high glucose demonstrated similar changes in chromatin lysine methylation⁷¹.

Interestingly, a recent study reported elevated levels of a microRNA, miR-125b, in diabetic *db/db* VSMC. MiR-125b was shown to target Suv39h1 leading to decreased H3K9me3 and a corresponding increase in inflammatory gene expression as well as enhanced VSMC monocyte binding⁷². These results indicate a sustained loss of chromatin repressive mechanisms in the diabetic state in part through loss of key repressive marks and dysregulation of certain miRs leading to epigenetic regulation of inflammatory genes. This could be due to prior hyperglycemic exposure and thus play a role in metabolic memory (Figures 2 and 3).

Short-term hyperglycemic conditions were also reported to induce long-term changes in chromatin modifications. Endothelial cells exposed to HG for 16 hours and later cultured for several days in normal glucose demonstrated a sustained increase in the expression of NF- κ B p65 subunit which correlated with increased promoter H3K4me1 marks and occupancy of the corresponding HMT SET7²⁶⁻²⁷. These changes could be prevented by blocking components of the mitochondrial electron transport chain²⁷.

It is likely that upcoming studies may uncover similar epigenetic changes in target cells such as podocytes, mesangial and tubular epithelial cells involved in DN or in other diabetic complications. Overall, these emerging studies indicate that prior exposure to hyperglycemia can lead to epigenetic changes in target cells and altered chromatin structure resulting in persisting patterns of gene expression associated with the pathology of diabetic micro- and macro-vascular complications.

CONCLUSIONS

Epigenetic modifications play an important role in altering gene expression patterns associated with various diseases^{4, 9}. Clinical and experimental studies have clearly demonstrated the deleterious effects of hyperglycemia and the importance of maintaining good glucose control to prevent the onset or severity of diabetic complications. Evidence shows that hyperglycemia can induce epigenetic changes to the chromatin structure via activation of signaling pathways that regulate epigenetic factors mediating DNA methylation, histone modifications, chromatin remodeling and microRNAs leading to dysregulated expression of key inflammatory and fibrotic genes in target cells. Persistence of these epigenetic changes may be the mechanism underlying metabolic memory (Figure 3). However, diabetes is a complex disease and other factors such as dyslipidemia, nutrient status and other environmental factors can co-operate with HG to influence the epigenetic state of target cells in damaged tissues. Furthermore, it is still unclear how these epigenetic changes are transmitted through multiple cell cycles. Since epigenetic mechanisms are usually reversible unlike genetic changes, they represent a window of opportunity for therapeutic intervention. Epigenetic drugs such as inhibitors of DNA methylation, HATs and HDACs are already being evaluated for cancer and other diseases^{5, 37, 73}. The Human Epigenome project is expected to greatly enhance our understanding of epigenetic states under normal and disease conditions⁷⁴. Tremendous advances in epigenome profiling aided by high throughput sequencing technologies⁷⁵ will no doubt accelerate the discovery of new therapeutic targets for the treatment of diabetes and associated renal complications.

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Figure 1. Metabolic Memory and Diabetic Complications

In the Diabetes Control and Complications Trial (DCCT) conducted from 1983-1993, Type 1 Diabetic patients were placed either on conventional or intensive insulin treatment for an average of 6.5 years. At the end of the study, patients on intensive control have reduced microvascular complications relative to the conventional group. In the follow up Epidemiology of Diabetes Interventions and Complications (EDIC) trial (1993 to date), both groups of patients were placed under intensive control regimen. EDIC study showed that patients previously in the DCCT conventional treatment group were still at a greater risk for vascular complications compared to those on intensive control in DCCT. These long lasting effects have been suggested to be due to a 'Metabolic Memory' of prior hyperglycemic exposure.

Figure 2. Model of epigenetic regulation of gene expression associated with diabetic complications

Post translational modifications of N-terminal histone tails play essential roles in gene regulation and are regulated by various chromatin modifiers. Tri-methylation of histone H3 lysine 9 and lysine 27 are usually associated with repression, while methylation of histone H3 lysine 4 and acetylation at lysine 9 are both associated with gene activation. In the schematic model shown, diabetic conditions such as hyperglycemia lead to a loss of repressive modifications that normally maintain regulated control of gene expression, while activating histone modifications may be increased, thus leading to relaxation or opening of the chromatin structure around key pathologic genes resulting in increased transcription. Various combinations of histone modifications mediated by several HMTs are likely to be involved. These epigenetic modifications can be maintained through cell division via mechanisms that are not yet clearly understood but may include DNA methylation as well as transmission of histone lysine methylation marks.

Fig. 3. Metabolic Memory and epigenetic mechanisms for diabetic complications

Diabetes, Hyperglycemia and AGEs activate multiple signal transduction pathways including oxidative stress, which regulate of transcription factors (TFs), and epigenetic factors such as Histone methyl transferases (HMTs), Histone acetyl transferases (HATs) and DNA methyl transferases (DNMTs) to alter histone posttranslational modifications (Histone PTMs) and DNA methylation (DNA-me) at promoters of inflammatory and extracellular matrix (ECM) genes (such as MCP-1, IL-6, TGF-beta, PAI-1 and Collagen) leading to changes in their expression. Histone deacetylases (HDACs) and lysine demethylases (KDMs) are also likely to be involved in the regulation of Histone PTMs (not shown). Persistence of these epigenetic marks at target gene regions may be a major mechanism for 'Metabolic Memory' implicated in the continued development of vascular and renal complications despite glucose control.