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Epigenetics of diabetic complications

Louisa M Villeneuve and
Division of Diabetes, Beckman Research Institute of City of Hope, 1500 E. Duarte Road, Duarte, CA-91010, USA

Rama Natarajan[†]
Division of Diabetes, Beckman Research Institute of City of Hope, 1500 E. Duarte Road, Duarte, CA-91010, USA, Tel.: +1 626 256 4673 ext. 62289, Fax: +1 626 301 8136

Rama Natarajan: rnatarajan@coh.org

Abstract

Type 1 and Type 2 diabetes are complex diseases associated with multiple complications, and both genetic and environmental factors have been implicated in these pathologies. While numerous studies have provided a wealth of knowledge regarding the genetics of diabetes, the mechanistic pathways leading to diabetes and its complications remain only partly understood. Studying the role of epigenetics in diabetic complications can provide valuable new insights to clarify the interplay between genes and the environment. DNA methylation and histone modifications in nuclear chromatin can generate epigenetic information as another layer of gene transcriptional regulation sensitive to environmental signals. Recent evidence shows that key biochemical pathways and epigenetic chromatin histone methylation patterns are altered in target cells under diabetic conditions and might also be involved in the metabolic memory phenomenon noted in clinical trials and animal studies. New therapeutic targets and treatment options could be uncovered from an in-depth study of the epigenetic mechanisms that might perpetuate diabetic complications despite glycemic control.

Keywords

diabetes; diabetic complications; epigenetics; hyperglycemia; metabolic memory

Diabetes mellitus is a complex metabolic disease attributed to both genetic and environmental factors. It has become a major healthcare problem as the incidence of diabetes, as well as its debilitating complications, is growing rapidly. While Type 1 diabetes (T1D) is an autoimmune disease characterized by the loss of pancreatic β-cells and insulin production, Type 2 diabetes (T2D) is associated with progressive insulin resistance and β-cell dysfunction. The observed increased risk for the development of T2D with increasing age suggests the accumulation and operation of multiple factors, including the environment. Both T1D and T2D result in dysregulated glucose levels and, while they can be controlled with various medications, changes in dietary habits and increased exercise, subjects with

[†]Author for correspondence: Division of Diabetes, Beckman Research Institute of City of Hope, 1500 East Duarte Road, Duarte, CA-91010, USA, Tel.: +1 626 256 4673 ext. 62289, Fax: +1 626 301 8136, rnatarajan@coh.org.

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diabetes continue to be plagued with numerous complications, some of which are life threatening. This continued development of diabetic complications even after achieving glucose control indicates a missing link in diabetes etiology, which recent studies have suggested may be attributed to a metabolic memory of prior glycemic exposure that is conferred by epigenetic changes in target cells without alterations in gene coding sequences. Although numerous studies have provided a wealth of knowledge regarding the genetics of diabetes, the mechanistic pathways leading to diabetes and its complications remain only partly understood. Studying the role of epigenetics in diabetic complications can provide valuable new insights to clarify the interplay between genes and the environment and uncover new therapeutic targets.

Biochemical & molecular basis for diabetic complications

Hyperglycemia can have overwhelming and long-lasting injurious effects on target cells and organs and thereby contribute to diabetic complications, the risk for which increases with longer disease duration. Vascular disease is one of the most common complications of diabetes. Microvascular complications can result in blindness, renal failure and several neurological disorders, while macrovascular complications include accelerated atherosclerosis and other cardiovascular diseases [1]. Hyperglycemia can lead to the activation of several cellular pathways, including increased oxidant stress [2–4], flux into the polyol and hexosamine pathways [3], activation of protein kinase C [5] and increased formation of advanced glycation end products (AGEs) [6,7]. Studies in endothelial and other cells have linked hyperglycemic activation of these pathways to increased mitochondrial super-oxide anion formation and associated oxidant stress [8]. Thus, while superoxide anions may be transient, they can have lasting effects due to modification or activation of multiple downstream pathways [8]. In addition, hyperglycemia leads to the activation of nuclear factor (NF)-κB proinflammatory transcription factor with resulting increases in inflammatory mediators, in part through oxidant stress, AGEs, protein kinase C and MAPKs [9–11]. Inflammation has been found to contribute to β-cell failure in T1D and insulin resistance in T2D and subsequently lead to accelerated complications. In addition, insulin resistance has been attributed to increased free fatty acids, which, in conjunction with hyperinsulinemia, leads to increased triglycerides and low-density lipoproteins and decreased high-density lipoproteins [12]. The overall consequence of hyperglycemia and dyslipidemia is altered expression patterns of various inflammatory and other pathologic genes and proteins that contribute to the development of diabetic micro- and macro-vascular complications (Figure 1) [13]. However, the molecular mechanisms are still not very clear.

Metabolic memory

Several clinical trials have implicated hyperglycemia in the continued development of diabetic complications and metabolic memory even after glucose levels have been normalized. The landmark Diabetes Control and Complications Trial (DCCT) treated T1D patients with either conventional or intensive insulin therapy and observed that intensive glycemic control could clearly delay the progression of key diabetic complications, including retinopathy, nephropathy and neuropathy [14]. Owing to the relatively young age of the patient population, there was no significant change in macrovascular complications despite a decrease in hypercholesterolemia [14]. Since the benefits of intensive therapy were so pronounced, the study was terminated after 6.5 years, and all patients were placed on intensive therapy for long-term follow-up in the Epidemiology of Diabetic Complications and Interventions Trial (EDIC).

Results from the EDIC trial demonstrated that, relative to patients on prior conventional control, those who were on intensive glycemic control during the DCCT and continued on

intensive control for the EDIC trial had significantly slower rates of progression of microvascular complications, such as retinopathy, nephropathy and neuropathy, even though there was no longer a significant difference in glycosylated hemoglobin levels between the two groups several years into the EDIC trial [15–17]. More recently, they were also found to have better outcomes for macro-vascular complications, such as non-fatal heart attack, stroke or death from cardiovascular disease [18], and decreased progression of intima-media thickness and coronary artery calcification associated with atherosclerosis [19,20].

Similar long-lasting beneficial effects of glycemic control have also been identified in T2D patients. The UK Prospective Diabetes Study (UKPDS) demonstrated that people with lower fasting plasma glucose at the time of diagnosis exhibited decreased cardiovascular risk [21,22]. The Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial showed that intensive glycemic control lead to a decrease in combined macro- and micro-vascular events, primarily due to a reduction in nephropathy [23].

Overall, the findings from these major clinical trials demonstrate the importance of early glycemic control to reduce long-term complications and confirm that hyperglycemia can have long-lasting detrimental consequences. The mechanisms responsible for these enduring effects of the prior hyperglycemic state or erratic metabolic control are as yet unclear, and this phenomenon has been termed ‘metabolic memory’ [18].

Glycated hemoglobin is a biomarker that reflects the mean plasma glucose level over a period of several weeks and is used to monitor glycemic control. However, it may not fully reflect the number or severity of glucose peaks or troughs within that period, including the postprandial hyperglycemic spikes [24]. Recent studies have demonstrated a link between postprandial hyperglycemia and vascular complications [24,25] and suggest that such short glucose excursions, as well as prior hyperglycemic exposure, may in fact confer metabolic memory that might be dependent on epigenetic changes [26–28].

Models of metabolic memory

Along with clinical trials, several experimental studies have shown a similar phenomenon of metabolic memory in animal and cell culture models. These experimental models provide the much needed opportunity to study the molecular mechanisms responsible for ‘metabolic memory’ in order to design better therapeutic treatments for diabetic patients. Early pioneering studies in dogs found that even after reversing hyperglycemia, there was a continuation of retinal complications [29]. Similar results with diabetic rats showed that islet transplantation after 6 weeks of diabetes led to decreased progression of retinopathy compared with rats with longer prior exposure to diabetes [30]. Additional studies in streptozotocin-induced diabetic rats showed that reinstitution of glycemic control after a short period of hyperglycemia had protective effects on nitric oxide levels, lipid peroxides and other parameters in the retina. However, reinstitution of normal glucose after prolonged diabetes and hyperglycemia in the rats failed to reverse increases in peroxynitrites, superoxide anion formation, nitrative and overall oxidative stress and NF-κB activity, as well as inflammation that has been attributed to metabolic memory [31–34]. Similar results were seen in streptozotocin-treated rat kidneys [35].

Early *in vitro* studies with endothelial cells cultured in high glucose showed a continued increase in expression of genes encoding the extracellular matrix proteins fibronectin and collagen, even after normalization of glucose levels [36]. Other recent studies demonstrated the persistence of oxidant stress for up to 1 week after glucose normalization and that antioxidants or NADPH oxidase inhibitors partially blocked the high glucose effects [37,38]. Another recent model of metabolic memory in endothelial cells showed that even short-term

exposure to high glucose resulted in sustained increases in the expression of NF-κB p65 subunit, and inflammatory genes and elevated oxidant stress that persisted despite return to normoglycemia [27,28]. In yet another study, it was found that vascular smooth muscle cells (VSMCs) derived from T2D, insulin resistant, obese *db*/*db* mice exhibited a preactivated phenotype and metabolic memory even after culturing *ex vivo* for a few passages. Relative to cultured VSMCs derived from non-diabetic *db*/+ control littermates, VSMCs from diabetic *db*/*db* mice exhibited increased inflammatory gene expression, migration and oxidant stress, as well as increased activation of NF-κB and CREB transcription factors and key signaling pathways associated with growth and migration [26,39]. Monocyte adhesion to VSMCs from *db*/*db* mice was also enhanced relative to *db*/+cells, probably due to the increase in inflammatory chemokine production [39]. Together, these results suggest a metabolic memory of vascular dysfunction arising from acute hyperglycemic spikes or prior chronic exposure to hyperglycemic conditions.

These findings further confirm that early tight control of glucose levels is essential to slow down the progression of diabetic complications. It also suggests that oxidant stress may play an important role in perpetuating this metabolic memory by modifying or damaging essential lipids, proteins and/or DNA [37,40]. Hyperglycemia and oxidant stress along with increased activity in the polyol pathway can also increase the accumulation of AGEs. AGEs change the structure and function of the vasculature through irreversible glycation of the various proteins and lipids, further perpetuating and amplifying local inflammation and oxidant stress, resulting in long-term vascular damage. Thus, AGEs, acting through receptors, such as the receptor for advanced glycation endproducts (RAGE), could also contribute to hyperglycemic memory [41–43]. Accordingly, while we are beginning to understand some of the biochemical and signaling aspects of metabolic memory and how it may adversely affect target tissues and organs susceptible to diabetic complications, the subtle molecular and nuclear mechanisms responsible for the sustained ‘memory’ over time, through multiple cell divisions at the transcriptional and epigenetic level, need more attention and have emerged as an exciting area of research.

Epigenetic regulation of gene expression

Post-transcriptional modifications of histones in chromatin have been associated with the regulation of gene expression. These epigenetic modifications form an additional layer over the genetic information within DNA and constitute the basis for a ‘histone code’ [44,45]. Chromatin DNA is packaged in a highly organized and compact structure. The DNA is first wrapped around a histone octamer comprised of a histone H3–H4 tetramer and two H2A–H2B dimers followed by a histone H1 linker, which together constitute a nucleosome [46]. Repeating units of nucleosomes make up chromatin. Covalent modifications to histone N-terminal tails alter the chromatin structure, allowing the DNA to become more accessible to transcription factors and RNA polymerase II (euchromatin), or making it inaccessible, compact and silent (heterochromatin). These modifications include acetylation, methylation, phosphorylation, ubiquitination and sumoylation of key histone amino acid residues. Different combinations of histone modifications play important roles in the binding and recruitment of various chromatin-associated proteins and, depending on the local concentration of these factors, may result in chromatin remodeling, leading to either activation or silencing of gene expression [44,45].

Histone modifications, such as phosphorylation, ubiquitination and sumoylation, have been implicated in gene regulation. Phosphorylation is known to facilitate an open chromatin conformation amenable to transcription, as is ubiquitination, while sumoylation is more often associated with transcriptional repression [47]. Phosphorylation plays a wide ranging role in the regulation of numerous signaling pathways. There are reports that ubiquitination

plays a role in insulin signaling [48]. Sumoylation has been found to be involved in regulating insulin receptors, as well as NF-κB signaling, and to play a role in pancreatic β-cell function [49,50]. However, a role for these modifications in epigenetics has not been extensively studied in the context of diabetic complications.

Histone lysine acetylation via histone acetyltransferases (HATs) is a well-known modification associated with gene activation. Most HATs are capable of modifying more than one lysine residue in both histone and nonhistone proteins. HAT-mediated lysine acetylation provides a more open chromatin structure, which enables transcription factor and RNA polymerase II recruitment permissible for transcription. Conversely, histone deacetylases (HDACs) mediate the reverse. For the most part, HDACs do not show specificity for any particular lysine residue and are found to be components of repressor complexes or to be involved in various signaling pathways [51,52]. In general, histone acetylation is quite transient.

On the other hand, histone methylation can be more persistent. It is limited to lysine and arginine residues and has been associated with both gene activation and repression, depending on the residue modified. Protein arginine methyltransferases are responsible for either mono- or di-methylation of arginine residues most often associated with gene activation, while histone lysine methyltransferases (HMTs) mediate lysine methylation [53]. The highly conserved SET domain family (named for a conserved sequence motif found in three *Drosophila* proteins: suppressor of position effect variegation 3–9; enhancer of zeste; and trithorax) of HMTs regulate lysine methylation [54], which is more complex since lysine residues can be mono-, di-, or tri-methylated. HMTs are generally more specific, usually only methylating one particular lysine residue [52,54]. Histone H3 lysine 4 methylation (H3K4me) is typically associated with gene activation and can be mediated by several HMTs, such as SET1, mixed lineage leukemia 1–4, SMYD3 and SET7/9 [55,56]. Histone H3 lysine 9 methylation (H3K9me), on the other hand, is generally associated with gene repression and can be mediated by SUV39H1, G9a and SETDB1/ESET [56]. Modifications at other histone lysine residues have been associated with activating or repressive functions; however, this can depend on the region of the gene being methylated (promoter vs coding region); hence, the outcomes are often difficult to predict. Having multiple HMTs capable of methylating the same residue suggests a degree of fine tuning where other chromatin factors could play additional roles in recruiting specific HMTs, as well as redundancy in the case of loss of a crucial HMT.

While histone lysine methylation was originally thought to be a stable irreversible modification, a significant body of recent work indicates that it can also be dynamic, as demonstrated by the exciting discovery of the first histone demethylase, lysine demethylase 1 (LSD1), which specifically removes H3K4me [57] and, more recently, was found capable of also removing H3K9me [58]. Other lysine demethylases have since been identified with varying specificities for different histone lysine residues [56,59–61]; the nomenclature for which has recently been changed to lysine demethylases (KDMs) [62]. Great interest is now focused on understanding and characterizing these demethylases and the roles they may play in various diseases [61,63]. In this context, the recent report demonstrating that histone demethylase JHDM2A is associated with obesity and affects genes related to metabolism in rodent models [64] is noteworthy. Even though histone lysine methylation is now known to be reversible, it is still one of the most stable epigenetic modifications, with some histone lysine methylation states maintaining very low turnover rates, and hence could be a key factor in metabolic memory.

Epigenetic histone modifications allow for dynamic flexibility of the chromatin by either blocking or recruiting various nonhistone proteins and complexes that recognize specific

combinations of chromatin marks. Histone-binding complexes often contain other chromatin modifying proteins that can further change the chromatin landscape. Crosstalk between modifications has also been suggested where specific histone modifications can facilitate or block additional histone marks. Nucleosome–nucleosome interactions can then be disrupted enabling chromatin to either open and facilitate transcription, or to close and form a compact silent conformation, thereby directing chromatin accessibility and the transcriptional outcome [52].

The original concept of epigenetics involving the inheritance of traits not solely based on DNA sequence has evolved substantially since its inception approximately 50 years ago. DNA methylation has been the best characterized inherited epigenetic modification and it is used as a regulator of gene expression, and some studies have associated changes in DNA methylation with diabetes. The agouti mouse is one example in which DNA methylation and expression of the agouti gene can affect the tendency to develop obesity and diabetes [65]. Another interesting recent study showed that intrauterine growth retardation can lead to T2D due to epigenetic silencing of Pdx1, a key transcription factor that regulates insulin gene expression and β-cell differentiation. Both histone modifications and DNA methylation were implicated [66]. In another study, it was shown that, in diabetic islets, there was increased DNA methylation of the promoter of PPAR-γ coactivator 1α gene (*PPARGC1A*), a factor that plays a key role in regulating mitochondrial genes and in the modulation of diabetes [67]. A recent report showed that treatment of human myotubes with TNF-α or free fatty acids led to DNA hypermethylation of PPARGC1A at non-CpG regions. Similar DNA hypermethylation was also noted in skeletal muscle from T2D subjects relative to normal controls [68].

Lately, the term epigenetics has not focused as much on heredity. The expanded, more broad and unifying definition of epigenetics has been put forward as “the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states” [69]. Thus, histone modifications are widely accepted to play a role in epigenetics; however, there are questions as to what role they specifically play, whether they precede or succeed DNA methylation and whether they initiate the transcriptional memory or simply maintain it [70]. Overall, there has been a substantial increase in recent years in our understanding of the epigenetic mechanisms governing gene-expression patterns without changes in the basic gene coding sequence. However, the relationships to pathological and disease states, such as diabetes and its complications, are currently less clear but of great interest.

Role of epigenetics in diabetes & metabolic memory of diabetic complications

Several exciting studies have now identified a role for epigenetic histone modifications in diabetes. HATs and HDACs have been found to play important roles in the activation and regulation of several key genes linked to diabetes, as reviewed by Gray and De Meyts [71]. There is also increased interest in the sirtuin (SIRT) family of HDACs, particularly SIRT1, which appears to modulate several factors related to diabetes, including metabolism, insulin secretion and adipogenesis [72]. HATs and HDACS are also known to modulate NF-κB transcriptional activity [73,74] and the resulting expression of downstream inflammatory genes [75,76]. Interestingly, high glucose treatment of cultured monocytes increased recruitment of the HATs CPB and p/CAF, leading to increased histone lysine acetylation at the cyclooxygenase (COX)-2 and TNF-α inflammatory gene promoters with a corresponding increase in gene expression [77]. The *in vivo* relevance of these studies was demonstrated through increased histone lysine acetylation at these inflammatory gene promoters in monocytes from both T1D and T2D patients compared with healthy control volunteers [77]. Another recent study demonstrated that oxidized lipids can lead to increased

inflammatory gene promoter histone acetylation in a CREB/p300 (HAT)-dependent manner, with a corresponding increase in gene expression [78]. These studies implicate a role for chromatin histone acetylation in promoting inflammatory gene expression under diabetic conditions. Since acetylation is thought to be one of the more transient and less gene-specific histone modifications, a role for acetylation/deacetylation in hyperglycemic memory remains to be determined.

The role of histone methylation in diabetic complications and metabolic memory has elicited much interest. One study used chromatin immunoprecipitation (ChIP) coupled to DNA microarray (ChIP-on-chip) to analyze alterations to histone methylation patterns in monocytes treated with high glucose. The results revealed dynamic changes in both the H3K4me2 activation mark and H3K9me2 repressive mark at key genes in response to high glucose in the cultured monocytes, with relevant changes observed in monocytes from diabetic patients [79]. ChIP-on-chip results with primary human blood monocytes and lymphocytes demonstrated different cell-type-specific histone methylation patterns between the two cell types that proved to be relatively stable within the cell type regardless of age or gender [80]. This was followed by studies comparing the blood monocyte and lymphocyte H3K9me2 profiles from T1D with healthy controls using both human cDNA and promoter arrays in the ChIP-on-chip studies. A subset of genes in diabetic lymphocytes was found to have increased H3K9me2, analysis of which linked them to immune and inflammatory pathways often associated with the development of T1D and resulting complications [81]. These genome-wide studies demonstrate that, while a reasonably stable histone methylation pattern is maintained in healthy individuals over time in a cell-type-specific setting, this profile can be disrupted in a disease state. Moreover, they also provide a glimpse of the inflammatory cell epigenome under the diabetic state, and suggest that new information regarding diabetes, its complications and metabolic memory can be obtained by such profiling approaches.

Additional *in vitro* experiments have helped to shed light on the mechanisms responsible for the changes in epigenetic marks under diabetic conditions. Studies in monocytes demonstrated that knockdown of the H3K4 HMT SET7/9 attenuated TNF-α induction of key inflammatory genes in an NF-κB dependent manner. Knockdown of SET7/9 also decreased NF-κB p65 subunit and p300 HAT occupancies at monocyte chemoattractant protein (MCP)-1 and TNF-α promoters, along with a corresponding decrease in promoter H3K4me. These results suggest that SET7/9 might coactivate NF-κB transcriptional activity via promoter H3K4me activation in response to inflammatory stimuli prevalent in the diabetic milieu [82]. A role for SET7/9 in regulating NF-κB expression and inflammatory gene regulation in response to high glucose was also shown in endothelial cells [27,28].

Recently, several studies have begun to unravel the potential chromatin-based epigenetic mechanisms responsible for metabolic memory. Relative to VSMCs derived from the aorta of control *db*/+ mice, VSMCs from diabetic *db*/*db* mice cultured *ex vivo* for a few passages continued to exhibit increased inflammatory gene expression associated with the diabetic phenotype and complications [26,39]. In parallel, there was a corresponding decrease in H3K9me3 repressive marks at the IL-6, macrophage colony-stimulating factor and MCP-1 promoters in cells from the diabetic mice relative to control nondiabetic mice. These results indicate a sustained loss of repressive mechanisms in the diabetic state. The loss of repressive mechanisms in *db*/*db* VSMCs was associated with decreased protein levels of SUV39H1, a known HMT mediating H3K9me3. Knockdown of SUV39H1 verified a role for SUV39H1 in regulating inflammatory gene expression by partially reversing the diabetic phenotype. In addition, *db*/*db* VSMCs exhibited increased TNF-α, induced *IL-6* and *MCP-1* expression, and a corresponding sustained decrease in promoter H3K9me3 and decrease in SUV39H1 occupancies at these gene promoters. High glucose treatment of normal human

VSMCs demonstrated similar changes to the chromatin lysine methylation state suggesting the sustained alteration of these epigenetic marks could be due to the prior exposure to a hyperglycemic environment in the diabetic *db*/*db* mice [26].

Furthermore, studies in endothelial cells have demonstrated that even short-term hyperglycemia can induce long-term changes to the chromatin state and suggested that such spikes of hyperglycemia may be a risk for complications independent of glycated hemoglobin. In this model of metabolic memory, endothelial cells treated for 16 h with high glucose followed by a return to normal glucose levels for up to 6 days demonstrated a sustained increase in NF-κB p65 subunit expression and inflammatory genes *MCP-1* and *VCAM-1*. The increase in p65 corresponded to increased H3K4me1 modifications on the proximal promoter of p65 as well as increased Set7 (also known as SET7/9) recruitment [27,28]. Interestingly, further mechanistic studies showed that these epigenetic changes were prevented by reducing mitochondrial superoxide production (by overexpressing uncoupling protein-1 or manganese superoxide dismutase) or by overexpression of glyoxalase 1, an enzyme that degrades α-oxoaldehydes, such as the highly reactive dicarbonyl methylglyoxal known to accumulate in response to high glucose [27]. Supportive animal studies demonstrated that mice exposed for a short term to high glucose via a hyperinsulinemic hyperglycemic clamp followed by a period of glucose normalization exhibited sustained increases in promoter H3K4me1 and p65 expression in aortic endothelial cell isolated from these mice by laser capture microdissection [27]. Similar memory phenomena were also noted in a diabetic $ApoE^{-/-}$ mouse model [28]. Transient high glucose in endothelial cells was also associated with decreased H3K9me2/3 repressive marks along with an increase in LSD1 demethylase recruitment at the p65 promoter [28]. Together, these results support the idea that prior exposure to hyperglycemia and even periods of transient high glucose can result in several epigenetic changes in target cells that alter chromatin structure and thereby have long-lasting effects on the expression of genes associated with the pathology of diabetic vascular complications (Figure 2).

Epigenetic transmission

Clinical studies have shown that diabetic complications can continue in spite of glucose control indicating a metabolic memory of the prior glycemic state. Further investigation into this mysterious phenomenon has provided insight into possible mechanisms. Hyperglycemia has been shown to increase oxidant stress, AGEs, PKC and inflammation. Only recently has there been evidence linking epigenetic chromatin changes to these events. While it is becoming apparent that histone lysine methylation could play a key role in metabolic memory, discovering how these histone modifications are transmitted through multiple cell cycles and modulate diabetic complications is the next looming challenge.

Several studies have demonstrated the transmission of histone lysine methyl marks through DNA replication. Methyl-CpG binding protein can recruit H3K9 HMT SETDB1 to chromatin during replication where it then methylates the newly deposited histones thus coupling the transmission of histone lysine 9 methylation with DNA methylation [83].

The Polycomb complex family of proteins is associated with long-term silencing, components of which have been found to play a role in epigenetic regulation of pancreatic β-cell regeneration associated with diabetes and aging [84,85]. Recent evidence has shown that Polycomb complexes remain bound to chromatin through replication. It is thought that Polycomb complexes bind more than one nucleosome, which would allow them to maintain contact with chromatin as the replication fork passes through; or the Polycomb complex could interact with members of the replication machinery [86]. Another study showed that Polycomb Repressive Complex 2, which contains the H3K27 HMT EZH2, can bind

H3K27me3 modifications at sites of ongoing replication. This could allow transmission or copying of the parental H3K27 methyl marks to the new histones deposited on the daughter strand [87]. However, it is yet to be determined how hyperglycemia-induced changes in epigenetic histone modifications are transmitted through replication.

The discovery of histone variants, especially replication-independent nucleosome assembly pathways, suggests a mechanism for the stable transmission of epigenetic information [88–90]. Histone variants have a different function from canonical histones, ranging from altering the basic properties of the histones to localizing to distinct regions of the genome, and can play a role in either transcriptional activation or repression. There can also be differences in the exposed surface of these histone variants relative to the canonical histones, which can lead to changes in nucleosome–nucleosome and nucleosome–protein interactions, as well as alterations in chromatin folding and consequent changes in the transcriptional state [88,91,92]. Histone variants have been proposed to play a role in establishing and maintaining epigenetic memory [88–90] and are susceptible to post-transcriptional modifications as well, similar to canonical histones. While canonical histones are incorporated into nucleosomes during replication, variant histones demonstrate several mechanisms for nucleosomal incorporation in addition to replication-dependent incorporation, such as transcription-coupled incorporation [89].

In addition, several ATP-dependent chromatin-remodeling complexes have been identified and are known to be comprised of multiple components with a catalytic ATPase subunit. While these complexes do not share the same exact mechanism for remodeling the chromatin, it has been suggested that most remodeling complexes disrupt the DNA–histone interactions by altering, relocating, or replacing the nucleosomes [93,94]. Chromatin-remodeling enzymes have been found in complexes with numerous histone-modifying proteins, including HATs, HDACs, HMTs and DNA methyltransferases [94–97]. This can lead to disruption of the interactions between histones [93]. Chromatin-remodeling complexes can also contain bromodomains known to interact with acetylated histones and suggest a possible targeting mechanism [93,98]. The chromatin-remodeling complex could then disrupt the histone–DNA interactions by loosening the DNA from the histone octamer. In connection with diabetes, the SWI/SNF remodeling enzyme was found to play a role in PPAR-γ induction during adipogenesis [99], while mice with a mutation in Chd2 ATP-dependent chromatin-remodeling enzyme had significantly impaired glomerular function, implicating remodeling enzymes in kidney disease [100]. Overall, these results suggest that further investigation of nucleosome remodeling may increase our understanding of the role of epigenetics and metabolic memory in diabetic complications.

Expert commentary

To date, at the very least, research has shown that epigenetic regulation of chromatin allows for dynamic control of gene expression, turning genes on or off based on cell signaling patterns or environmental stimuli. These epigenetic marks can also result in significant changes to gene-expression patterns often associated with disease states [47]. Clinical and *in vitro* studies have clearly demonstrated the deleterious effects of hyperglycemia and the importance of maintaining good glucose control to prevent the onset or mitigate the severity of diabetic complications. Evidence now indicates that elevated glucose levels can lead to epigenetic changes in chromatin structure through alterations in various factors and pathways. This field of research in the area of diabetes is still in its infancy especially compared with cancer research. So far, studies have mainly focused on epigenetic chromatin changes and histone post-translational modifications affecting the regulation inflammatory genes in vascular and blood cells. Certain key HMTs and KDMs have been implicated, and it is highly likely that in the future other HMTs and KDMs and related epigenetic factors

could also be identified as effectors of hyperglycemia and contributors to the pathogenesis of diabetic micro- and macro-vascular complications (Figure 3). In addition, changes in DNA methylation are also possible. However, diabetes is clearly more complicated than a hyperglycemic state and, particularly, T2D involves a cluster of several risk factors and features including the environment, diet, obesity, insulin resistance and dyslipidemia, along with hyperglycemia. These factors might co-operate with hyperglycemia to lead to epigenetic changes to chromatin in multiple target cells and tissues related to diabetic complications, including blood cells, heart, blood vessels, kidney, retina, nerves and liver. Much progress can be made by using well-defined cell and animal models of diabetic complications with and without treatments with standard diabetes drugs, antioxidants and related interventions.

Five-year view

Recent estimates indicate that the rates of diabetes, metabolic syndrome and associated complications are expected to show significant increases in the coming years. The associated healthcare costs are also expected to show markedly upward trends. As such, new strategies to curb these trends are needed. Research in the area of epigenetics has greatly accelerated in recent years, mainly due to marked advances in basic mechanisms, technologies and bioinformatics. It is well known that the environment, nutrition and lack of exercise play significant roles in the pathology of obesity, diabetes, and its complications; however, there are several key issues that have yet to be addressed. Little is known about how exercise may affect or alter epigenetic marks or whether dietary modifications can reverse these marks. If hyperglycemia-induced oxidant stress promotes epigenetic changes and expression of pathologic genes, it will be essential to determine what kinds of antioxidant therapies would be beneficial. Likewise, the effects of blocking other related factors and pathways, including AGE/RAGE, growth factor signaling pathways [101] and lipids, on epigenetic mechanisms would be worthy of further investigation.

An ambitious plan to characterize the human epigenome is currently in the planning stages [102,103]. This project has been greatly aided by significant advances in technologies and instrumentation for massive parallel next generation sequencing and ChIP-sequencing which was very effectively used to simultaneously map several histone marks in human cells and demonstrate associations with gene-transcription rates [104]. This technology has tremendous power to rapidly sequence vast genomic regions [104]. The human epigenome project will broaden our view of the chromatin landscape by mapping specific histone modifications associated with different cells, tissues and stages of development, with the ultimate goal being to generate a high-resolution reference map to compare to the disease state. It will also include crucial information about the best characterized epigenetic mark, namely DNA methylation. A specific role for DNA methylation in diabetes has remained elusive, although several studies have demonstrated a strong association between aberrant DNA methylation and inflammation, as well as hyperlipidemia [68,105–107]. The question of what type of human samples can be used to analyze DNA or histone methylation poses some difficulties since immortalized cell lines showed different DNA methylation patterns compared with primary cells [108].

Importantly, a more comprehensive understanding of the epigenetic code under normal versus diabetic states will provide better therapeutic opportunities beyond the currently available ones [41]. Epigenetic drugs such as inhibitors of DNA methylation, HDACs and LSD1 are already being evaluated for cancer and other diseases [42,58]. Currently available drugs for diabetic complications could be tested for their ability to alter epigenetic marks. The generally accepted idea is that the histone code is reversible, at least more so than the DNA code. Therefore, a better understanding of the epigenetics of disease could lead to new

therapeutic options for the treatment of numerous ailments, including diabeties and its complications.

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Key issues

- Hyperglycemia is associated with the activation of various pathways, including reactive oxygen species, advanced glycation end products, protein kinase C, and activation of the proinflammatory transcription factor nuclear factor-κB, all of which can lead to altered expression patterns of inflammatory and other pathologic genes contributing to the development of diabetic micro- and macro-vascular complications.
- Several clinical trials have shown that diabetic complications continue even after normalization of blood glucose, indicating a metabolic memory of the prior glycemic state.
- *In vivo* animal studies, as well as *in vitro* studies, have demonstrated long-lasting effects of hyperglycemia related to oxidative stress and inflammation even after a return to normal glucose levels.
- Post-transcriptional modifications of histones are associated with the regulation of gene expression. These epigenetic modifications form an additional layer of regulation over the genetic information within DNA and can be maintained through cell division. Studies have shown aberrant histone-modification patterns associated with numerous diseases, and recent studies have implicated a role for epigenetic histone modifications in diabetic complications and metabolic memory.
- Genome-wide studies have revealed cell-type-specific dynamic changes in histone methylation patterns under diabetic conditions, as well as in diabetic patients.
- Vascular smooth muscle cells derived from diabetic *db*/*db* mice exhibit increased migration, proliferation and inflammatory gene expression relative to control non-diabetic *db*/+ mice even after culture for several weeks outside the animal. This sustained diabetic phenotype has been attributed to a decrease in repressive histone marks.
- Transient high glucose treatment of endothelial cells demonstrated a persistent loss of repressive histone marks and a corresponding increase in activating histone marks leading to a sustained increase in nuclear factor-κB p65 subunit expression, as well as increased inflammatory gene expression.
- Recent results support the idea that prior exposure to hyperglycemia and even periods of transient high glucose can result in epigenetic changes in target cells that alter chromatin structure and thereby have long-lasting effects on the expression of genes associated with the pathology of diabetic vascular complications.
- With the incidence of diabetes, metabolic syndrome and the associated complications growing rapidly, a better understanding of epigenetics and metabolic memory is crucial. Ongoing worldwide efforts to map the human epigenome will prove helpful in these endeavors. A more comprehensive evaluation of the epigenetic code under normal versus diabetic states will provide better therapeutic opportunities for the treatment of numerous diseases, including diabetes and its complications.

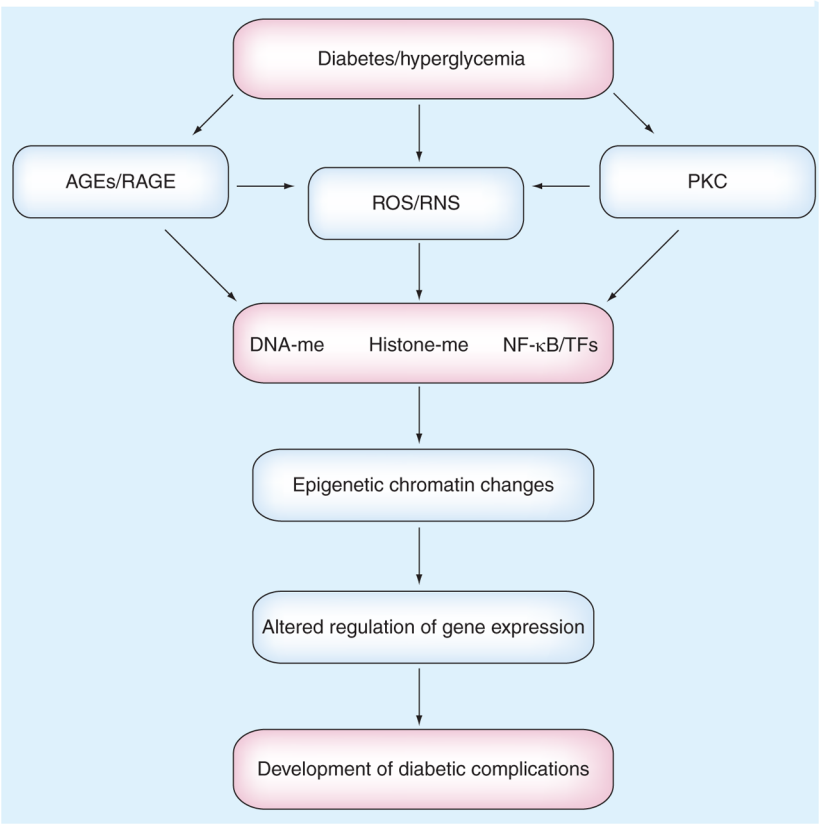

Figure 1. Downstream effects of diabetes and hyperglycemia

Diabetes and hyperglycemia can lead to the activation of multiple pathways, including signaling through AGEs and RAGE, ROS and RNS, as well as PKC. These events could lead to altered DNA-me and histone-me at various genes in target cells in addition to activation of various transcription factors, which over time result in changes to the expression patterns of these genes and ultimately the development of diabetic complications. AGE: Advanced glycation end product; me: Methylation; NF: Nuclear factor; PKC: Protein kinase C; RAGE: Receptor for advanced glycation end product; RNS: Reactive nitrogen species; ROS: Reactive oxygen species; TF: Transcription factor.

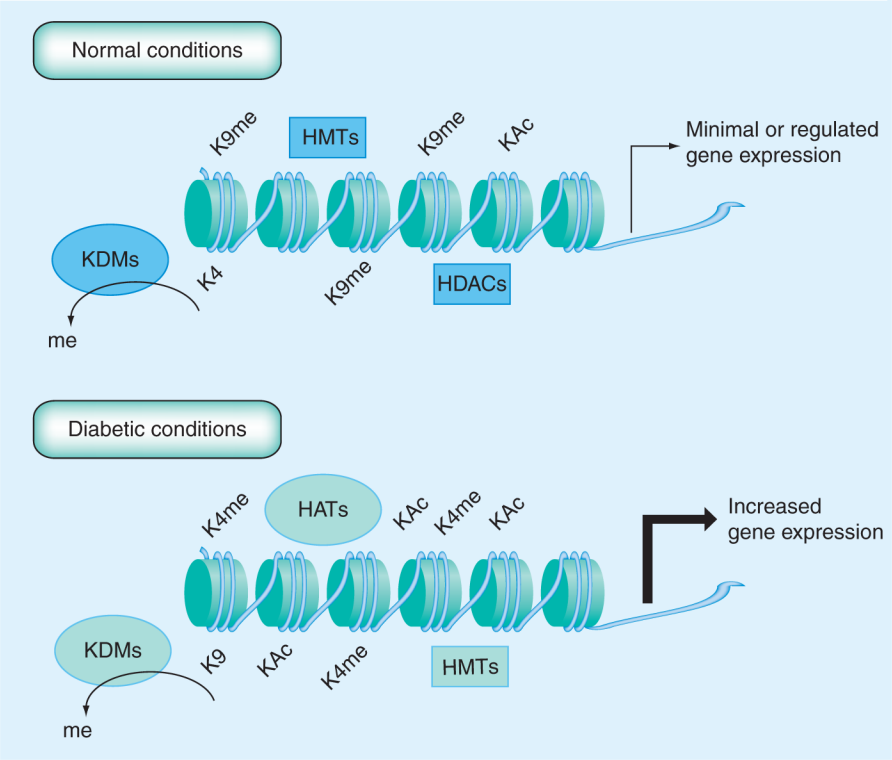

Figure 2. Model for the epigenetic regulation of pathologic gene expression under diabetic conditions

Post-translational modifications, such as lysine methylation regulated by HMTs and KDMs, or histone acetylation regulated by HATs or HDACs on the N-terminal histone tails of chromatin, play important roles in regulating gene expression. In the proposed model, under normal conditions, various chromatin modifiers mediate sufficient levels of repressive histone marks to maintain strict control of pathologic gene expression. However, under diabetic conditions, such as hyperglycemia, the negative regulators may be lost while positive regulators or activating histone marks may be increased, thus leading to relaxing or opening of the chromatin structure around these genes to increase their transcription. Various histone modifications could be involved. Ac: Acetylation; HAT: Histone acetyltransferase; HDAC: Histone deacetylase; HMT: Histone methyltransferase; KDM: Histone demethylase; me: Methylation.

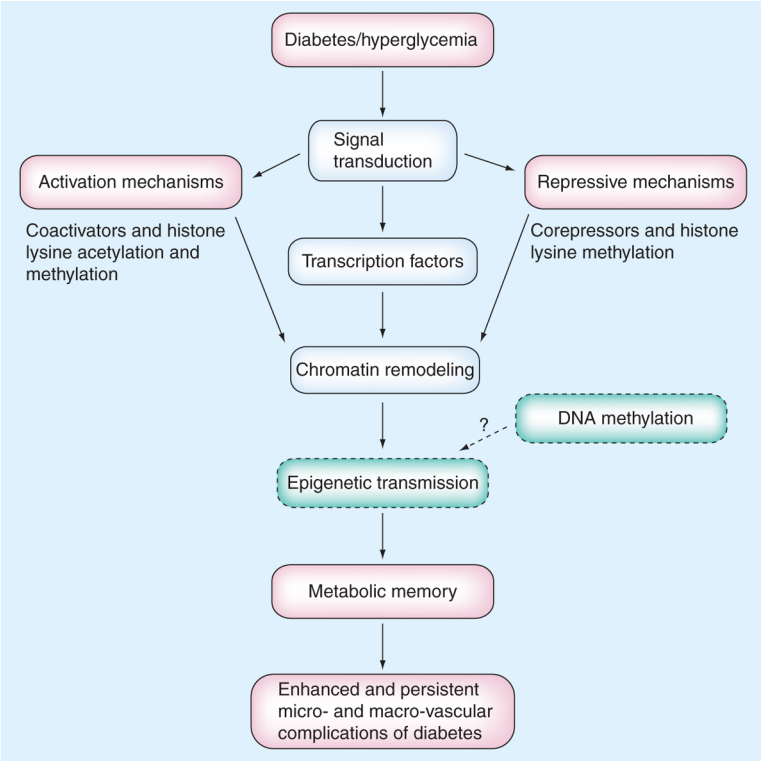

Figure 3. Schematic of epigenetic regulation of diabetic complications

Diabetic conditions or hyperglycemia lead to a loss of repressive mechanisms and a corresponding increase in activation pathways, resulting in long-lasting epigenetic changes through gene promoter histone lysine modifications. Histone lysine methylation can lead to either gene activation (H3K4me) or repression (H3K9me) depending on the specific lysine residue that is methylated, as well as the gene location modified. Other factors, such as the degree of methylation, can also affect whether the methyl mark will result in an open accessible chromatin or a closed condensed chromatin state. These epigenetic modifications are maintained through cell division via mechanisms that are, to date, not clearly understood but may include DNA methylation, as well as transmission of histone lysine methylation marks. These persistent epigenetic changes result in a metabolic memory responsible for the continued development of diabetic complications even after glucose control has been achieved.