Epigenetic Mechanisms in the Pathogenesis of Diabetic Retinopathy

Jun Zeng Baihua Chen

Department of Ophthalmology, Second Xiangya Hospital, Central South University, Changsha City, PR China

Key Words

Diabetes · Retinopathy · Epigenetics

Abstract

Diabetic retinopathy (DR), which arises as a result of an increasing incidence of diabetes mellitus, has gradually become a common disease. Due to its complex pathogenesis, the treatment means of DR are very limited. The findings of several studies have shown that instituting tight glycemic control in diabetic patients does not immediately benefit the progression of retinopathy, and the benefits of good control persist beyond the period of good glycemic control. This has led to the concept of persistent epigenetic changes. Epigenetics has now become an increasingly important area of biomedical research. Recently, important roles of various epigenetic mechanisms have been identified in the pathogenesis of diabetes and its complications. The aim of this review is to provide an overview of the epigenetics and epigenetic mechanisms in diabetes and diabetes complications, and the focus is on the emerging evidence for aberrant epigenetic mechanisms in DR.

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Introduction

Diabetes mellitus, an increasingly common chronic metabolic disorder of insulin production or uptake, is gradually becoming a significant issue worldwide. Diabetic retinopathy (DR), which can lead to blindness if left untreated, is one of the most common microvascular complications of diabetes [1, 2]. The prevalence of DR was 23% in the diabetic group in mainland China [3]. It has been the leading cause of blindness amongst 16- to 64-year-olds [4]. The pathogenetic mechanism responsible for DR is imperfectly understood, but much of the mechanism is apparently reproduced by experimental diabetes in animals and by chronic elevation of blood galactose in nondiabetic animals. It is becoming increasingly clear that epigenetic mechanisms contribute significantly to diabetes and diabetic complications. Although much insight into the epigenetic mechanisms in diabetic complications such as cardiovascular diseases has been gained, there is little understood about the epigenetic mechanisms in the pathogenesis of DR. We may uncover potential therapeutic targets and treatment options to prevent the continued development of DR if we clarify the epigenetic mechanisms in DR. The aim of this review is to discuss the current understanding of how epigenetic mechanisms contribute to the formation of DR.

Pathophysiology of DR

The pathogenesis of the development of DR is extremely complex because of the involvement of multiple interlinked mechanisms leading to cellular damage and adaptive changes in the retina [5]. In the past, retinopathy has been characterized primarily by its microvascular abnormalities, including abnormal hemodynamics, endothelial cell dysfunction, vessel leakage, vascular occlusion and degeneration, and acellular capillary formation [6]. The microvascular dysfunction may result in two main processes: increased capillary permeability and capillary obliteration, which consequently lead to macular edema and retinal neovascularization in DR, respectively. However, recent studies indicate that DR, which can be defined as a form of chronic neurovascular degeneration, is a composite of structural and functional alterations in both microvascular and neuroglial compartments [7, 8]. The explicit mechanisms by which hyperglycemia initiates the neuronal or vascular alterations in retinopathy have not been completely defined [6, 7]. Because of an inverse relationship between extracellular glucose concentrations and glucose transport, cells cannot be directly susceptible to direct hyperglycemic damage. In contrast, vascular endothelial cells, major targets of hyperglycemic damage, show no significant change in glucose transport rate when glucose concentration is elevated, resulting in intracellular hyperglycemia [9]. There are several supposed mechanisms underlying hyperglycemia-induced diabetic vascular damage in the retina. The majority of publications focus on the 5 major mechanisms: polyol pathway flux, increased formation of advanced glycation end products, increased expression of the receptor for advanced glycation end products and its activating ligands, activation of protein kinase C isoforms, and overactivity of the hexosamine pathway, but many of these hypotheses have yet to be validated in human studies or clinical trials [5, 10]. Emerging evidence shows that these metabolic mechanisms are associated with overproduction of reactive oxygen species and depletion of antioxidants in DR [11], and then reactive oxygen species contribute to the DR progression by damaging retinal cells.

Inflammation is another active factor in the pathophysiology of DR. The upregulation of cytokines and other inflammatory mediators, leading to an influx of leukocytes and persistent low-grade inflammation, is supposed to contribute to diabetes-associated damage to the retinal vasculature and retinal neovascularization. Several proinflammatory mediators, such as vascular endothelial

growth factor (VEGF), cytokines, nitric oxide, chemokines, eicosanoid, lipids, angiotensin II and the renin-angiotensin system, have been discovered [12]. Increased expression of vasoactive factors and cytokines probably plays an important role in mediating the structural and functional changes in the retina [13, 14]. Although recent evidence strongly suggests that inflammation is very important in the pathogenesis of early stages of experimental DR [15–17], studies in humans have not found a consistent association between systemic markers of inflammation and retinopathy [16, 18]. Also, it is still uncertain whether inflammation plays a crucial role in the development and progression of DR in humans.

Genetic factors and key gene mutations have been proposed to explain the pathogenesis of DR. However, increasing 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 complications [19–21]. Notably, chromatin is a crucial interface between the effects of genetics and environment, and the epigenetic posttranscriptional modifications of histone tails in chromatin have been linked to gene transcription. While several studies have identified key biochemical pathways triggered by hyperglycemia and diabetes in target cells related to DR, the role of epigenetic mechanisms is only now becoming apparent. Furthermore, the Diabetes Control and Complications Trial and the follow-up Epidemiology of Diabetes Interventions and Complications studies have shown that instituting tight glycemic control in diabetic patients does not immediately benefit the progression of retinopathy, and the benefits of good control persist beyond the period of good glycemic control (GC) [22, 23]. This continued development of DR even after achieving good glucose control suggests a metabolic memory of prior glycemic exposure and indicates epigenetic changes in target cells without alterations in gene coding sequences. Exploring a role of epigenetics in DR could allow for new insights clarifying the interplay between the environment and gene regulation and identify much needed new therapeutic targets.

Epigenetics: An Overview

Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence [24]. Epigenetic mechanisms regulate both long-term (heritable) and short-term (nonheritable) effects and thus have a significant influence on many different disease

processes [25]. Chromatin is composed of subunits called nucleosomes containing DNA wrapped around a histone octamer with 2 copies each of histone H2A, H2B, H3 and H4 [26]. It is a dynamic process of transcriptional activation or repression, depending on the recruitment of protein complexes that alter chromatin structure via enzymatic modifications of histone tails and nucleosome remodeling. An open chromatin conformation results in a greater accessibility of transcription factors to the DNA, allowing for gene transcription, while a closed chromatin structure prevents transcription. These epigenetic histone posttranslational modifications include lysine acetylation, methylation and phosphorylation. Along with other epigenetic factors such as DNA methylation and noncoding RNAs such as microRNAs (miRNAs), these posttranslational modifications in chromatin form an added layer of gene regulation without altering the DNA code itself. Histone modifications, such as methylation, phosphorylation and acetylation, result in conformational changes of the histones. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are regulators of histone lysine acetylation which usually occurs quite rapidly [27]. Histone methylation is generally considered to be more long lasting. Histone lysine methylation is mediated by histone methyltransferases (HMTs) and more complex because lysine residues can be mono-, di- or trimethylated. Generally speaking, 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, but histone H3 lysine 9 methylation (H3K9me) with gene repression [28]. Both promoter and coding regions can be modified, adding another level of complexity to gene transcription outcomes. Although histone modifications occur on all histones, the modifications on histones H3 and H4 are the most widely characterized. Among the different modifications, the most studied is acetylation. Acetylation is maintained by the actions of HATs and HDACs. Hyperacetylation of lysine residues at the ϵ -amino group in the N terminus of histones occurs as a result of the actions of HATs, which normally results in increased gene transcription. Deacetylation by HDACs is normally linked with decreased gene transcription [29]. There are some important positions for histone acetylation, including lysine 9 (K9) and K14 on histone H3, and K5, K8, K12 and K16 on histone H4 [30].

Another mechanism by which epigenetic changes control gene expression is DNA methylation. DNA methylation is regulated by DNA methyltransferases at the 5' po-

sition of cytosine residues in CpG dinucleotides by transferring methyl groups from S-adenosyl methionine. Hypermethylation of promoter CpG islands generally leads to transcription repression [31]. Histone acetylation can prevent DNA methylation by inhibiting the binding of DNA methyltransferases. Conversely, DNA methylation can promote histone deacetylation and recruitment of the histone methyltransferase SUV39H1 that mediates H3K9me3 to further stabilize the repressive status [32]. Evidence also demonstrates the interaction of DNA methylation and other histone modifications [33]. DNA methylation has been extensively studied in the context of tumor suppressor genes and cancer [34]. However, much less is known about DNA methylation in diabetes and its complications. Following cell division, the pattern of DNA methylation in differentiated somatic cells is both stable and heritable. Alterations of DNA methylation can result in changes in the chromatic configuration, making the promoter region either more or less accessible to transcription factors.

Gene and protein expressions are also regulated by miRNA and other small RNAs posttranscriptionally, which have greater temporal flux, making any interpretation variability of these more formidable [35]. miRNAs are short, single-stranded RNA strands which will not translate into protein [36]. Instead, they block gene translation via binding to complementary regions of mRNA. miRNAs are also able to initiate the degradation of mRNA strands to which they are bound [37]. Recent researches suggested a critical role for miRNAs in various diseases. They have been found to play key roles in differentiation, proliferation, development and in cancer, where they can act as tumor suppressors or oncogenes [38–40].

Epigenetic Mechanisms in Diabetes and Diabetic Complications

Recent researches have implicated an important role for epigenetic histone modifications and histone posttranslational modifications in diabetes and its complications. HATs and HDACs, modulating nuclear factor (NF)- κ B transcriptional activity and resulting in changes in downstream inflammatory gene expression levels, have been found in the regulation of several key genes related to diabetes [41–43]. Interestingly, monocytes cultured in high-glucose medium (HG) revealed increased recruitment of HATs resulting in histone lysine acetylation at key inflammatory gene promoters, with a corresponding increase in gene expression, and there were

similar phenomena in blood monocytes from type 1 and type 2 diabetes patients [43]. Another study showed that oxidized lipids can result in increased histone acetylation at the inflammatory gene promoter, with a corresponding increase in gene expression [44]. Further studies suggested that HG increased p300, led to increased histone acetylation at promoters of key extracellular cell matrix genes and vasoactive factors in endothelial cells [45]. However, curcumin, as an inhibitor of p300, could prevent hyperglycemia-induced changes in gene expression levels [45, 46]. Studies in diabetic kidneys demonstrated an important role for HDACs in transforming growth factor- β_1 (TGF- β_1)-mediated extracellular cell matrix production and fibrosis [47]. These results implicate a role for histone acetylation in promoting gene expression correlated with diabetic complications. Except for histone lysine acetylation, H3K4me and recruitment of the HMT SET7/9 also increased in the insulin promoter region, while H3K9me was undetectable [48, 49]. Since histone lysine methylation is relatively more stable, its role in diabetic complications attracts people to explore, and the role of key histone methylation marks in diabetes and its complications needs to be determined. Cultured monocytes treated with HG presented dynamic changes in both the H3K4me2-activating mark and H3K9me2-repressive mark, verifying a role for hyperglycemia in altered histone methylation patterns. The involved changes were also seen in monocytes from diabetic patients [50]. A subset of genes in diabetic lymphocytes displayed increased H3K9me2 linked to immune and inflammatory pathways often associated with type 1 diabetes and its complications [51]. These epigenomic profiling studies suggest that, although the histone methylation pattern is sustained reasonably stable in healthy individuals, it can be disrupted in some disease states. The mechanisms which are responsible for aberrant epigenetic histone methylation occurring in diabetic conditions are further elucidated by additional in vitro experiments. In monocytes, the occupancy of the NF- κ B p65 subunit and p300 HAT at monocyte chemoattractant protein-1 and TNF- α promoters decreased by knocking out of the H3K4 HMT SET7/9, with a corresponding decreased H3K4me at promoter and gene expression, suggesting that SET7/9 might coactivate NF- κ B transcriptional activity by promoter H3K4me activation in response to inflammatory stimuli in the diabetic condition [52]. Likewise, a role in regulating NF- κ B expression for SET7/9 in response to HG was also observed in endothelial cells [53, 54]. Recent evidence has demonstrated that renal mesangial cells treated with TGF- β increased HMT SET7/9 occupancy and the associated

active H3K4me marks, but reduced repressive H3K9me marks at promoters of key fibrotic genes linked to diabetic nephropathy. Both TGF- β and HG induced promoter histone methylation changes, and gene expression reversed by treatment of TGF- β antibody [55]. These results suggest a role for histone modification in modulating gene expression under diabetic conditions.

Recently, some studies have begun to explore the role of DNA methylation in diabetes and its complications. In animal models, epigenetic silencing due to increased promoter DNA methylation has been linked to islet dysfunction and development of diabetes [56, 57]. A recent report demonstrated that the insulin promoter DNA was methylated in mouse embryonic stem cells and only became demethylated when the cells differentiated into insulin-expressing cells, and both the mouse and human insulin promoters were specifically demethylated in pancreatic β -cells, suggesting epigenetic regulation of insulin expression [58]. What is more, DNA methylation and expression of the agouti gene can affect the tendency to develop obesity and diabetes in the agouti mouse [59]. A study showed that both histone modifications and DNA methylation were implicated in the process of intrauterine growth retardation leading to type 2 diabetes, which is due to epigenetic silencing of Pdx1, a key transcription factor that regulates insulin gene expression and β -cell differentiation [56]. In another study, it was shown that increased DNA methylation of the promoter of the peroxisome proliferator-activated receptor- γ coactivator 1 α gene, a factor that plays a key role in regulating mitochondrial genes and in the modulation of diabetes in diabetic islets [56].

Several studies have implicated an important role of miRNAs in the pathogenesis of diabetes [60–63]. However, the role of miRNAs in diabetes vascular complications is less studied. miRNAs are linked with the regulation of genes relevant to insulin secretion, fat metabolism, cholesterol biosynthesis and adipogenesis, crucial pathways in the pathogenesis of diabetes [61, 64, 65]. miRNAs also play a role in TGF- β signaling related to the pathogenesis of diabetic nephropathy, with key miRNAs such as miR-192, miR-216a, miR-217 and miR-377 being up-regulated, resulting in increased fibronectin and collagen expression [66–68]. Currently, there is an area of great interest concerning the role of miRNAs and potential relationships to epigenetic mechanisms in diabetic complications. In a word, epigenetic changes at specific target gene promoters might explain the accelerated development of diabetes and diabetic complications.

Epigenetic Mechanisms in DR

Recently, the role of epigenetic mechanism in the pathogenesis of diabetic complications is gaining increasing attention [69], and there is more emerging evidence for aberrant epigenetic mechanisms in DR. Poor glycemic control (PC) has a close relation to DR. The retinas and retinal endothelial cells (RECs), from streptozotocin (STZ)-treated rats, kept in PC show increased expression of HDAC1, HDAC2 and HDAC8, and reduced activity of a histone H3-specific acetyltransferase; these changes were not reversed with the change of PC returned to good control (GC). It suggests that the epigenetic metabolic memory phenomenon may be the major reason for the continuation of DR even when the blood glucose level returns to normal [70].

Administration of antioxidants or overexpression of *sod2* prevents the development of DR in rodents, suggesting a major role in the development of DR. *Sod2*, a gene encoding manganese superoxide dismutase, undergoes epigenetic regulation, and these modifications fail to reverse after termination of hyperglycemic insult in the pathogenesis of DR [71–73]. Recent evidence demonstrated that epigenetic changes of retinal *Sod2* have an important role in the development of DR and in the metabolic memory phenomenon [74, 75]. Hyperglycemia induced increased acetyl H3K9, H4K20me3 and NF- κ B p65 at the promoter and enhancer of retinal *sod2*, gene expression and upregulated protein of SUV420h2, and increased the interactions of acetyl H3K9 and NF- κ B p65 to H4K20me3. These changes were not prevented by reversal of hyperglycemia. SUV420h2, an enzyme important for di- and trimethylation, is increased in the retina and its capillary cells in hyperglycemia. Silencing SUV420h2 prevented H4K20me3 at *sod2*, which strongly suggests that SUV420h2 is important in the metabolic memory phenomenon associated with DR [74]. Hyperglycemia also reduced H3K4me1 and H3K4me2, which are regulated by lysine-specific demethylase-1 (LSD-1), and increased the binding of LSD-1 and Sp1 at *Sod2*. LSD-1 specifically demethylates mono- or dimethylated H3K4 and H3K9, and removal of methyl groups from H3K4 is related to transcriptional repression [76]. Thus, regulation of LSD-1 by small interfering RNA ameliorated the glucose-induced decrease in H3K4me at *Sod2*, and prevented the decrease in *Sod2* gene expression. In rats, return to GC cannot reverse the decrease in H3K4me1 and H3K4me2 at *Sod2*, and LSD-1 remained active with increased binding of LSD-1 and Sp1 at *Sod2* [77]. De-

creased H3K4me2 and increased LSD-1 at *Sod2* also presented in retinas from human donors with DR [76].

Epigenetic modifications contribute to the mitochondrial damage and are postulated in the development of DR, and also to the metabolic memory phenomenon [77]. Retinal matrix metalloproteinase-9 (MMP-9), activated in the diabetic milieu, damages the mitochondria and augments capillary cell apoptosis. Decreased H3K9me2 and increased acetyl H3K9 and p65 at the retinal MMP-9 promoter were found in the diabetic condition. The enzyme activity and transcripts of LSD-1 were elevated. The glucose-induced increase in p65 and decrease in H3K9me2 at the MMP-9 promoter can be ameliorated by LSD-1 small interfering RNA. LSD-1 small interfering RNA also prevented MMP-9 activation, mitochondrial damage and cell apoptosis [78]. Thus, regulation of MMP-9 by epigenetic modifications has an important role in the development of DR.

Thioredoxin-interacting protein (TXNIP) plays a causative role in diabetes and its vascular complications. In RECs, HG treatment or receptor for advanced glycation end products activation by its ligand S100B induces the expression of TXNIP and inflammatory genes such as *Cox2*, *VEGF-A* and *ICAM1*. Nine modifications of histone H3 lysine and the p38 MAPK-NF- κ B signaling pathway are involved in TXNIP-induced inflammation. HG and advanced glycation end product receptor effects are impeded by silencing TXNIP, while stable overexpression of a cDNA for human TXNIP in endothelial cells elevates inflammation. Overexpression of TXNIP in endothelial cells abolishes H3K9 trimethylation, a gene inactivation marker, and increases H3K9 acetylation, a gene induction indicator, at the proximal *Cox2* promoter bearing the NF- κ B-binding site [79].

In addition to histone modifications, DNA methylation is also one of the mechanisms for the epigenetic control of gene expression, and aberrant DNA methylation patterns of CpG islands can influence normal transcriptional regulation [80]. The epigenetic regulation of DNA methylation in DR has seldom been reported. A recent study showed that the damage to the mitochondrial DNA (mtDNA) replication system, caused by the previous 3 months of PC, cannot be reversed by 3 months of GC. The replication enzymes remain downregulated, and the D loop region of the mtDNA continues to be damaged [81]. And the results of this study suggested that the possible reason for this damage could be continued hypermethylation of the CpG sites at the regulatory region of polymerase- γ , affecting its binding to the mtDNA, and compromising the transcriptional activity. These results

strongly imply that the mtDNA replication system is impaired as a result of continued hypermethylation of polymerase- γ , and this continues even after the hyperglycemic insult is discontinued, DNA hypermethylation as one of the possible mechanisms is responsible for the failure to reverse mtDNA damage after reinstitution of GC and suggests an important role of DNA methylation in the metabolic memory phenomenon associated with the continued progression of DR [81].

The expression of miRNA has also changed in DR. miRNA-200b was found upregulated significantly in a type 1 DR model. Oxidation resistance 1, which attenuates oxidative stress markers and nitration of cellular proteins and ameliorates apoptosis induced by an oxidative stressor, 4-hydroxynonenal, is downregulated by transfection of an miR-200b mimic [82]. When rats treated with STZ were compared with untreated rats, changes in the expression of 37 miRNAs were detected in DR. Six of the confirmed altered miRNAs were differentially expressed over the course of STZ-induced diabetes. Levels of miRNA-182, miRNA-96, miRNA-211, miRNA-204, miRNA-183 and miRNA-124 were significantly increased during the progress of DR, whereas miRNA-10b, miRNA-10a, miRNA-219-2-3p, miRNA-144, miRNA-338 and miRNA-199a-3p were significantly decreased [83].

Another study [84] performed miRNA expression profiling in the retina and RECs of STZ-induced type 1 diabetes rats. They found that key NF- κ B-responsive miRNAs (such as miRNA-146, miRNA-155, miRNA-132 and miRNA-21) were upregulated in the diabetic RECs, and also that key VEGF-responsive miRNA-17-5p, miRNA-18a, miRNA-20a, miRNA-21, miRNA-31 and miRNA-133 and the p53-responsive miRNA-34 family were upregulated in both the retinas and RECs of the diabetic rats. NF- κ B is a key regulator of the immune response and plays an important role in the early pathogenesis of DR by triggering a proapoptotic program in retinal pericytes [85]. miRNAs, which are thought to be transcriptionally regulated by NF- κ B (miRNA-146, miRNA-155 and miRNA-21) [86–88], were also demonstrated to be upregulated in the diabetic RECs in rats [84]. This research confirmed that NF- κ B was able to directly activate miRNA-146 expression. Furthermore, overexpression of miRNA-146 inhibited interleukin-1 β -induced NF- κ B activation in RECs, forming a regulatory negative feedback loop to control NF- κ B and miRNA-146 expression. Thus, overexpression of miRNA-146 may be exploited therapeutically by inhibition of NF- κ B activation in DR [84]. VEGF, promoting angiogenesis

and endothelial permeability, has been shown to be increased in early pathogenesis and to play important roles in DR [89, 90]. The miRNA-34 family is a direct transcriptional target of p53 and contributes to p53-mediated cell cycle arrest, apoptosis and senescence [91]. Upregulation of VEGF- and p53-responsive miRNAs implicates miRNAs in mediating the proangiogenic or proapoptotic effects caused by VEGF and p53 in pathological changes of early DR [92]. In another study, reduced miRNA-200b and increased VEGF have been observed in human umbilical vein endothelial cells and bovine RECs treated with HG [92]. They also confirmed VEGF as a direct target, miRNA mimic treatments in vitro in endothelial cells and in vivo (intravitreal injection) could ameliorate increases in VEGF mRNA and protein levels induced by diabetes. Conversely, miRNA-200b antagonists could increase VEGF production, thus providing further understanding of the role of this miRNA in the pathogenesis of DR [92]. Additional experimentation was performed in animal models: miR-200b mimic injection into the vitreous humor of diabetic mouse eyes resulted in locally decreased VEGF-A expression; in addition, knocking down miRNA-200b inhibits the diabetes-induced upregulation of p300 in the retina, implying crosstalk between two epigenetic mechanisms in DR [92]. Silva et al. [93] examined the role of miRNA-29b and its potential target RAX (an activator of the proapoptotic protein kinase R signaling pathway) in the apoptosis of retinal neurons related to the pathogenesis of DR. They observed that RAX and miR-29b were localized in the retinal ganglion cells and the cells of the inner nuclear layer of the retinas from normal and STZ-induced diabetic rats. Their results suggest that upregulation of miRNA-29b at the early stages of DR in this model may have protective effects against apoptosis of the retinal cells by the RNA-dependent protein kinase pathway. Therefore, intravitreal injections of key miRNAs such as miRNA-29b and miRNA-200b may be developed as translational approaches for the treatment of DR. In a more recent study, a decrease in miRNA-146a was observed in HG-treated endothelial cells from large vessels and retinal microvessels and in retinas from type 1 diabetes rats [94]. Moreover, this research demonstrated that the expression of fibronectin (an miRNA-146a target that can contribute to hypertrophy and fibrosis) was increased under HG conditions, due to decreases in miRNA-146a levels, which in turn was mediated by increases in the coactivator p300.

Conclusions

Currently, studies have suggested an important role of epigenetic modifications in the continued progression of DR [74]. However, the understanding of the impact of epigenetic mechanisms in the pathogenesis in DR is very limited. With epigenetic mechanisms being extremely varied, including changes in DNA methylation, histone posttranslational modifications and miRNA expression, there is an extensive scope for further research in DR. Recent evidence shows that hyperglycemia can induce epigenetic changes to the chromatin structure via activation of various factors and signaling pathways. The specific key enzymes related to active and repressed chromatin states are involved in it, and epigenetic regulation of key inflammatory genes in retinas and RECs has been demonstrated. Well-defined cell and animal models treated with and without related interventions will further our understanding of epigenetic regulation and how to prevent DR. The human epigenome project is expected to

greatly enhance our understanding of epigenetic states under normal and disease conditions [95, 96]. The generally accepted idea is that the histone code is reversible; therefore, greater understanding of the epigenetic basis of disease could enable the discovery of new therapeutic targets for the treatment of numerous human diseases, including DR. Epigenetic drugs such as inhibitors of DNA methylation, HATs and HDACs, and some histone demethylases are already being evaluated for cancer and other diseases [34, 97, 98]. We believe that available drugs could be used for their potential ability to alter epigenetic markers and become a therapeutic method for DR in the future.

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References

- 1 Kempen JH, O'Colmain BJ, Leske MC, Haffner SM, Klein R, Moss SE, et al; Eye Diseases Prevalence Research Group: The prevalence of diabetic retinopathy among adults in the United States. *Arch Ophthalmol* 2004;122: 552–563.
- 2 Saaddine JB, Honeycutt AA, Narayan KM, Zhang X, Klein R, Boyle JP: Projection of diabetic retinopathy and other major eye diseases among people with diabetes mellitus: United States, 2005–2050. *Arch Ophthalmol* 2008; 126:1740–1747.
- 3 Liu L, Wu X, Liu L, Geng J, Yuan Z, Shan Z, et al: Prevalence of diabetic retinopathy in mainland China: a meta-analysis. *PLoS One* 2012;7:e45264.
- 4 Bunce C, Wormald R: Leading causes of certification for blindness and partial sight in England and Wales. *BMC Public Health* 2006; 6:58.
- 5 Frank RN: Diabetic retinopathy. *N Engl J Med* 2004;350:48–58.
- 6 Curtis TM, Gardiner TA, Stitt AW: Microvascular lesions of diabetic retinopathy: clues towards understanding pathogenesis? *Eye (Lond)* 2009;23:1496–1508.
- 7 Villarreal M, Ciudin A, Hernández C, Simó R: Neurodegeneration: an early event of diabetic retinopathy. *World J Diabetes* 2010;1:57–64.
- 8 Barber AJ, Gardner TW, Abcouwer SF: The significance of vascular and neural apoptosis to the pathology of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2011;52:1156–1163.
- 9 Kaiser N, Sasson S, Feener EP, Boukobza-Vardi N, Higashi S, Moller DE, et al: Differential regulation of glucose transport and transporters by glucose in vascular endothelial and smooth muscle cells. *Diabetes* 1993;42:80–89.
- 10 Cheung N, Mitchell P, Wong TY: Diabetic retinopathy. *Lancet* 2010;376:124–136.
- 11 Yang Y, Hayden MR, Sowers S, Bagree SV, Sowers JR: Retinal redox stress and remodeling in cardiometabolic syndrome and diabetes. *Oxid Med Cell Longev* 2010;3:392–403.
- 12 Adamis AP, Berman AJ: Immunological mechanisms in the pathogenesis of diabetic retinopathy. *Semin Immunopathol* 2008;30: 65–84.
- 13 Khan ZA, Chakrabarti S: Cellular signaling and potential new treatment targets in diabetic retinopathy. *Exp Diabetes Res* 2007;2007: 31867.
- 14 Wirotko B, Wong TY, Simo R: Vascular endothelial growth factor and diabetic complications. *Prog Retin Eye Res* 2008;27:608–621.
- 15 Kern TS: Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Exp Diabetes Res* 2007;2007:95103.
- 16 Li Q, Verma A, Han PY, Nakagawa T, Johnson RJ, Grant MB, et al: Diabetic eNOS-knockout mice develop accelerated retinopathy. *Invest Ophthalmol Vis Sci* 2010;51:5240–5246.
- 17 Tang J, Kern TS: Inflammation in diabetic retinopathy. *Prog Retin Eye Res* 2011;30:343–358.
- 18 Nguyen TT, Alibrahim E, Islam FM, Klein R, Klein BE, Cotch MF, et al: Inflammatory, hemostatic, and other novel biomarkers for diabetic retinopathy: the multi-ethnic study of atherosclerosis. *Diabetes Care* 2009;32:1704–1709.
- 19 Ling C, Groop L: Epigenetics: a molecular link between environmental factors and type 2 diabetes. *Diabetes* 2009;58:2718–2725.
- 20 Litherland SA: Immunopathogenic interaction of environmental triggers and genetic susceptibility in diabetes: is epigenetics the missing link? *Diabetes* 2008;57:3184–3186.
- 21 Villeneuve LM, Natarajan R: Epigenetics of diabetic complications. *Expert Rev Endocrinol Metab* 2010;5:137–148.
- 22 The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;329:977–986.
- 23 DCCT Research Group and EDIC Research Group: Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA* 2002;287:2563–2569.
- 24 Wolffe AP, Guschin D: Review: chromatin structural features and targets that regulate transcription. *J Struct Biol* 2000;129:102–122.
- 25 Nightingale KP, O'Neill LP, Turner BM: Histone modifications: signalling receptors and potential elements of a heritable epigenetic code. *Curr Opin Genet Dev* 2006;16:125–136.

- 26 Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ: Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 1997;389:251–260.
- 27 Roth SY, Denu JM, Allis CD: Histone acetyltransferases. *Annu Rev Biochem* 2001;70:81–120.
- 28 Martin C, Zhang Y: The diverse functions of histone lysine methylation. *Nat Rev Mol Cell Biol* 2005;6:838–849.
- 29 Wade PA: Transcriptional control at regulatory checkpoints by histone deacetylases: molecular connections between cancer and chromatin. *Hum Mol Genet* 2001;10:693–698.
- 30 Bjerling P, Silverstein RA, Thon G, Caudy A, Grewal S, Ekwall K: Functional divergence between histone deacetylases in fission yeast by distinct cellular localization and in vivo specificity. *Mol Cell Biol* 2002;22:2170–2181, erratum in *Mol Cell Biol* 2002;22:5257–5258.
- 31 Miranda TB, Jones PA: DNA methylation: the nuts and bolts of repression. *J Cell Physiol* 2007;213:384–390.
- 32 Vaissiere T, Sawan C, Herceg Z: Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutat Res* 2008;659:40–48.
- 33 Murr R: Interplay between different epigenetic modifications and mechanisms. *Adv Genet* 2010;70:101–141.
- 34 Sharma S, Kelly TK, Jones PA: Epigenetics in cancer. *Carcinogenesis* 2010;31:27–36.
- 35 Saetrom P, Snove OJ, Rossi JJ: Epigenetics and microRNAs. *Pediatr Res* 2007;61:17R–23R.
- 36 Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;116:281–297.
- 37 Wutz A: RNA-mediated silencing mechanisms in mammalian cells. *Prog Mol Biol Transl Sci* 2011;101:351–376.
- 38 Bartel DP: MicroRNAs: target recognition and regulatory functions. *Cell* 2009;136:215–233.
- 39 Croce CM, Calin GA: miRNAs, cancer, and stem cell division. *Cell* 2005;122:6–7.
- 40 Kim VN, Han J, Siomi MC: Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* 2009;10:126–139.
- 41 Gray SG, De Meyts P: Role of histone and transcription factor acetylation in diabetes pathogenesis. *Diabetes Metab Res Rev* 2005;21:416–433.
- 42 Ito K, Hanazawa T, Tomita K, Barnes PJ, Adcock IM: Oxidative stress reduces histone deacetylase 2 activity and enhances IL-8 gene expression: role of tyrosine nitration. *Biochem Biophys Res Commun* 2004;315:240–245.
- 43 Vanden Berghe W, De Bosscher K, Boone E, Plaisance S, Haegeman G: The nuclear factor-kappaB engages CBP/p300 and histone acetyltransferase activity for transcriptional activation of the interleukin-6 gene promoter. *J Biol Chem* 1999;274:32091–32098.
- 44 Reddy MA, Sahar S, Villeneuve LM, Lanting L, Natarajan R: Role of Src tyrosine kinase in the atherogenic effects of the 12/15-lipoxygenase pathway in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2009;29:387–393.
- 45 Chen S, Feng B, George B, Chakrabarti R, Chen M, Chakrabarti S: Transcriptional co-activator p300 regulates glucose-induced gene expression in endothelial cells. *Am J Physiol Endocrinol Metab* 2010;298:E127–E137.
- 46 Yun JM, Jialal I, Devaraj S: Epigenetic regulation of high glucose-induced proinflammatory cytokine production in monocytes by curcumin. *J Nutr Biochem* 2011;22:450–458.
- 47 Noh H, Oh EY, Seo JY, Yu MR, Kim YO, Ha H, et al: Histone deacetylase-2 is a key regulator of diabetes- and transforming growth factor-beta1-induced renal injury. *Am J Physiol Renal Physiol* 2009;297:F729–F739.
- 48 Chakrabarti SK, Francis J, Ziesmann SM, Garmey JC, Mirmira RG: Covalent histone modifications underlie the developmental regulation of insulin gene transcription in pancreatic beta cells. *J Biol Chem* 2003;278:23617–23623.
- 49 Mutskov V, Raaka BM, Felsenfeld G, Gershengorn MC: The human insulin gene displays transcriptionally active epigenetic marks in islet-derived mesenchymal precursor cells in the absence of insulin expression. *Stem Cells* 2007;25:3223–3233.
- 50 Miao F, Wu X, Zhang L, Yuan YC, Riggs AD, et al: Genome-wide analysis of histone lysine methylation variations caused by diabetic conditions in human monocytes. *J Biol Chem* 2007;282:13854–13863.
- 51 Miao F, Smith DD, Zhang L, Min A, Feng W, Natarajan R: Lymphocytes from patients with type 1 diabetes display a distinct profile of chromatin histone H3 lysine 9 dimethylation: an epigenetic study in diabetes. *Diabetes* 2008;57:3189–3198.
- 52 Li Y, Reddy MA, Miao F, Shanmugam N, Yee JK, Hawkins D, et al: Role of the histone H3 lysine 4 methyltransferase, SET7/9, in the regulation of NF-kappaB-dependent inflammatory genes. Relevance to diabetes and inflammation. *J Biol Chem* 2008;283:26771–26781.
- 53 Brasacchio D, Okabe J, Tikellis C, Balcerzyk A, George P, Baker EK, et al: Hyperglycemia induces a dynamic cooperativity of histone methylase and demethylase enzymes associated with gene-activating epigenetic marks that coexist on the lysine tail. *Diabetes* 2009;58:1229–1236.
- 54 El-Osta A, Brasacchio D, Yao D, Poci A, Jones PL, Roeder RG, et al: Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *J Exp Med* 2008;205:2409–2417.
- 55 Sun G, Reddy MA, Yuan H, Lanting L, Kato M, Natarajan R: Epigenetic histone methylation modulates fibrotic gene expression. *J Am Soc Nephrol* 2010;21:2069–2080.
- 56 Park JH, Stoffers DA, Nicholls RD, Simmons RA: Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. *J Clin Invest* 2008;118:2316–2324.
- 57 Ling C, Del Guerra S, Lupi R, Rönn T, Granhall C, Luthman H, et al: Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. *Diabetologia* 2008;51:615–622.
- 58 Kuroda A, Rauch TA, Todorov I, Ku HT, Al-Abdullah IH, Kandeel F, et al: Insulin gene expression is regulated by DNA methylation. *PLoS One* 2009;4:e6953.
- 59 Morgan HD, Sutherland HG, Martin DI, Whitelaw E: Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* 1999;23:314–318.
- 60 Muhonen P, Holthofer H: Epigenetic and microRNA-mediated regulation in diabetes. *Nephrol Dial Transplant* 2009;24:1088–1096.
- 61 Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, et al: A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* 2004;432:226–230.
- 62 Mi QS, He HZ, Dong Z, Isales C, Zhou L: MicroRNA deficiency in pancreatic islet cells exacerbates streptozotocin-induced murine autoimmune diabetes. *Cell Cycle* 2010;9:3127–3129.
- 63 Gallagher IJ, Scheele C, Keller P, Nielsen AR, Remenyi J, Fischer CP, et al: Integration of microRNA changes in vivo identifies novel molecular features of muscle insulin resistance in type 2 diabetes. *Genome Med* 2010;2:9.
- 64 Heneghan HM, Miller N, Kerin MJ: Role of microRNAs in obesity and the metabolic syndrome. *Obes Rev* 2010;11:354–361.
- 65 Poy MN, Spranger M, Stoffel M: MicroRNAs and the regulation of glucose and lipid metabolism. *Diabetes Obes Metab* 2007;9(suppl 2):67–73.
- 66 Kato M, Arce L, Natarajan R: MicroRNAs and their role in progressive kidney diseases. *Clin J Am Soc Nephrol* 2009;4:1255–1266.
- 67 Kato M, Putta S, Wang M, Yuan H, Lanting L, Nair I, et al: TGF-beta activates Akt kinase through a microRNA-dependent amplifying circuit targeting PTEN. *Nat Cell Biol* 2009;11:881–889.
- 68 Wang Q, Wang Y, Minto AW, Wang J, Shi Q, Li X, et al: MicroRNA-377 is up-regulated and can lead to increased fibronectin production in diabetic nephropathy. *FASEB J* 2008;22:4126–4135.
- 69 Villeneuve LM, Natarajan R: The role of epigenetics in the pathology of diabetic complications. *Am J Physiol Renal Physiol* 2010;299:F14–F25.
- 70 Zhong Q, Kowluru RA: Role of histone acetylation in the development of diabetic retinopathy and the metabolic memory phenomenon. *J Cell Biochem* 2010;110:1306–1313.

- 71 Kowluru RA, Odenbach S: Effect of long-term administration of alpha-lipoic acid on retinal capillary cell death and the development of retinopathy in diabetic rats. *Diabetes* 2004;53:3233–3238.
- 72 Kowluru RA, Atasi L, Ho YS: Role of mitochondrial superoxide dismutase in the development of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2006;47:1594–1599.
- 73 Kowluru RA, Kowluru V, Xiong Y, Ho YS: Overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes-induced oxidative stress. *Free Radic Biol Med* 2006;41:1191–1196.
- 74 Zhong Q, Kowluru RA: Epigenetic changes in mitochondrial superoxide dismutase in the retina and the development of diabetic retinopathy. *Diabetes* 2011;60:1304–1313.
- 75 Zhong Q, Kowluru RA: Epigenetic modification of Sod2 in the development of diabetic retinopathy and in the metabolic memory: role of histone methylation. *Invest Ophthalmol Vis Sci* 2013;54:244–250.
- 76 Forneris F, Binda C, Battaglioli E, Mattevi A: LSD1: oxidative chemistry for multifaceted functions in chromatin regulation. *Trends Biochem Sci* 2008;33:181–189.
- 77 Kowluru RA: Mitochondria damage in the pathogenesis of diabetic retinopathy and in the metabolic memory associated with its continued progression. *Curr Med Chem* 2013;20:3226–3233.
- 78 Zhong Q, Kowluru RA: Regulation of matrix metalloproteinase-9 by epigenetic modifications and the development of diabetic retinopathy. *Diabetes* 2013;62:2559–2568.
- 79 Perrone L, Devi TS, Hosoya K, Terasaki T, Singh LP: Thioredoxin interacting protein (TXNIP) induces inflammation through chromatin modification in retinal capillary endothelial cells under diabetic conditions. *J Cell Physiol* 2009;221:262–272.
- 80 Schinke C, et al: Aberrant DNA methylation in malignant melanoma. *Melanoma Res* 2010;20:253–265.
- 81 Tewari S, Zhong Q, Santos JM, Kowluru RA: Mitochondria DNA replication and DNA methylation in the metabolic memory associated with continued progression of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2012;53:4881–4888.
- 82 Murray AR, Chen Q, Takahashi Y, Zhou KK, Park K, Ma JX: MicroRNA-200b downregulates oxidation resistance 1 (Oxr1) expression in the retina of type 1 diabetes model. *Invest Ophthalmol Vis Sci* 2013;54:1689–1697.
- 83 Wu JH, Gao Y, Ren AJ, Zhao SH, Zhong M, Peng YJ, Shen W, Jing M, Liu L: Altered microRNA expression profiles in retinas with diabetic retinopathy. *Ophthalmic Res* 2012;47:195–201.
- 84 Kovacs B, Lumayag S, Cowan C, Xu S: MicroRNAs in early diabetic retinopathy in streptozotocin-induced diabetic rats. *Invest Ophthalmol Vis Sci* 2011;52:4402–4409.
- 85 Kowluru RA, Koppolu P, Chakrabarti S, Chen S: Diabetes-induced activation of nuclear transcriptional factor in the retina, and its inhibition by antioxidants. *Free Radic Res* 2003;37:1169–1180.
- 86 Taganov KD, Boldin MP, Chang KJ, Baltimore D: NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA* 2006;103:12481–12486.
- 87 Gatto G, Rossi A, Rossi D, Kroening S, Bonatti S, Mallardo M: Epstein-Barr virus latent membrane protein 1 trans-activates miR-155 transcription through the NF-kappaB pathway. *Nucleic Acids Res* 2008;36:6608–6619.
- 88 Sheedy FJ, Palsson-McDermott E, Hennessy EJ, Martin C, O'Leary JJ, Ruan Q, et al: Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. *Nat Immunol* 2010;11:141–147.
- 89 Sone H, Kawakami Y, Okuda Y, Sekine Y, Honmura S, Matsuo K, et al: Ocular vascular endothelial growth factor levels in diabetic rats are elevated before observable retinal proliferative changes. *Diabetologia* 1997;40:726–730.
- 90 Antonetti DA, Barber AJ, Hollinger LA, Wolpert EB, Gardner TW: Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic retinopathy and tumors. *J Biol Chem* 1999;274:23463–23467.
- 91 He L, He X, Lowe SW, Hannon GJ: microRNAs join the p53 network – another piece in the tumour-suppression puzzle. *Nat Rev Cancer* 2007;7:819–822.
- 92 McArthur K, Feng B, Wu Y, Chen S, Chakrabarti S: MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy. *Diabetes* 2011;60:1314–1323.
- 93 Silva VA, Poleskaya A, Sousa TA, Corrêa VM, André ND, Reis RI, et al: Expression and cellular localization of microRNA-29b and RAX, an activator of the RNA-dependent protein kinase (PKR), in the retina of streptozotocin-induced diabetic rats. *Mol Vis* 2011;17:2228–2240.
- 94 Feng B, Chen S, McArthur K, Wu Y, Sen S, Ding Q, Feldman RD, Chakrabarti S: miR-146a-mediated extracellular matrix protein production in chronic diabetes complications. *Diabetes* 2011;60:2975–2984.
- 95 American Association for Cancer Research Human Epigenome Task Force; European Union, Network of Excellence, Scientific Advisory Board: Moving AHEAD with an international human epigenome project. *Nature* 2008;454:711–715.
- 96 Qiu J: Epigenetics: unfinished symphony. *Nature* 2006;441:143–145.
- 97 Aggarwal BB, Harikumar KB: Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *Int J Biochem Cell Biol* 2009;41:40–59.
- 98 Shi Y: Histone lysine demethylases: emerging roles in development, physiology and disease. *Nat Rev Genet* 2007;8:829–833.