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Epigenetic modifications in diabetes

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Abstract

Diabetes is now considered as a 'silent epidemic' that claims over four million lives every year, and the disease knows no socioeconomic boundaries. Despite extensive efforts by the National and International organizations, and cutting-edge research, about 11% world's population is expected to suffer from diabetes (and its complications) by year 2045. This life-long disease damages both the microvasculature and the macrovasculature of the body, and affects many metabolic and molecular pathways, altering the expression of many genes. Recent research has shown that external factors, such as environmental factors, lifestyle and pollutants can also regulate gene expression, and contribute in the disease development and progression. Many epigenetic modifications are implicated in the development of micro- and macro- vascular complications including DNA methylation and histone modifications of several genes implicated in their development. Furthermore, several noncoding RNAs, such as micro RNAs and long noncoding RNAs, are also altered, affecting many biochemical pathways. Epigenetic modifications, however, have the advantage that they could be passed to the next generation, or can be erased. They are now being explored as therapeutical target(s) in the cancer field, which opens up the possibility to use them for treating diabetes and preventing/slowing down its complications.

Keywords

Diabetes; Complications; Epigenetics

1. Introduction

The incidence of diabetes is rising across the globe at an alarming rate, and it has now become an epidemic of the twenty first century; over 460 million people with diabetes in 2019 to 700 million people with diabetes in 2045. It is considered as the 7th leading cause of death in the United States, and accounts for 4.2 million deaths worldwide in 2020. As per International Diabetes Federation, "Diabetes is a serious threat to global health that respects neither socioeconomic status nor national boundaries". It is a chronic disease, and

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sustained high circulating glucose due to body's inability to produce sufficient insulin, or effectively use it, damages both the small (micro) and large (macro) blood vessel. This results in a number of long-term complications; in fact, diabetes epidemic is considered as an 'epidemic of its complications'. Some of the major complications related to the small blood vessels, 'microvascular complications', include retinopathy (eye disease), nephropathy (kidney disease) and neuropathy (neural damage). Over 80% of patients develop retinopathy after 15 years of diabetes; about 50% of diabetic patients develop neuropathy and the risk of amputation, and end-stage kidney disease is several folds greater in diabetic patients than people without diabetes [1]. The major 'macrovascular' complications (damage to the arteries) of diabetes include accelerated cardiovascular and cerebrovascular diseases; diabetic patients have 70% higher risk of developing cardiovascular disease [2], and are 2–6 times more susceptible to a stroke, compared to nondiabetic individuals [3]. Diabetes and its complications encompass many metabolic, structural, functional and molecular changes, and alter expression of several genes associated with these abnormalities [4–10]. Although high circulating glucose is considered as the main instigator of diabetic complications, other systemic factors, such as hyperlipidemia and hypertension, also contribute to their development [11,12]. Despite extensive research in the field, the molecular mechanism(s) of the development of these complications, however, remains unclear.

2. Epigenetic modifications

Genes have an important role in health and diseases, but external factors, such as behavior and environment, also are critical in health and diseases, and DNA sequence is not the only determinant of clinical phenotype [13,14]. While genetic changes alter which protein is going to be made, epigenetic changes can turn genes "on" and "off", and epigenetic modifications, without affecting the primary DNA sequence, are considered as key regulators of gene expression in a disease state. Epigenetics addresses how behavior and environment can cause changes that affect the way the genes begin to work, and these modifications have the advantage that they could be imprinted within the genome to be passed to the next generation, or can also be erased [15]. The major epigenetic changes constitute DNA methylation, histone modifications and noncoding RNAs; while DNA and histone modifications close or open the chromatin structure regulating access of the transcription factors, noncoding RNAs control gene expression at the RNA level [16].

2.1. DNA methylation

Addition of a methyl group to the 5′ position of the cytosine pyrimidine ring by a family of DNA methyl transferases (Dnmts) forms 5-methyl cytosine (5mC) and condenses the chromatin, and among the Dnmts, while Dnmt1 is the maintenance enzyme, Dnmt3a and 3b are de novo enzymes [17]. DNA methylation is a dynamic process and 5mC can be rapidly hydroxymethylated to 5-hydroxymethyl cytosine (5hmC) by a cyclic enzymatic cascade, dioxygenases-ten-eleven translocation (Tets). In general, methylation of CpG silences gene expression, and hydroxymethylation activates them [18–20]. Interpretation of the correct methylation marks is mediated by a family of proteins that bind methylated DNA, the methyl-CpG binding domain proteins (MBDs) [21]. In addition to the methylation of the

genomic DNA, DNA in the mitochondria (mtDNA) also undergoes methylation, and is considered to play important role in disease processes [9,22].

2.2. Histone modifications

Certain amino acids (e.g., lysine and arginine) in a histone protein are modified by the addition of one, two, or three methyl groups with the help of histone methyltransferases (HMTs), and the balance is maintained by histone demethylases that remove the methyl group. Depending on the site of methylation and number of methyl groups, histone methylation can either increase or decrease transcription of the gene, for example, trimethylation of lysine 4 on histone H3 (H3K4me3) is associated with an active gene expression, but dimethylation (H3K4me2) can result in both inactive and active euchromatic genes, and dimethylation at lysine 9 (H3K9me2) generally results in gene silencing [23,24].

Histones can also be acetylated on lysine residues in the N-terminal tail, and acetylation relaxes the chromatin structure, allowing access to transcription factors for gene transcription. Thus, histone acetylation is generally associated with active gene expression. A balance between histone acetyltransferases (HATs) and histone deacetylases (HDACs) helps maintain steady-state histone acetylation, and aberrant histone acetylation/deacetylation are implicated in many pathologic conditions, including inflammatory and degenerative diseases [25–27].

The histone code is read in part by histone post-translational modifications modules and their associated complexes, leading to chromatin-templated processes, and they have specific readout mechanisms for individual marks, influencing the outcome of the histone modification [28]. Bromodomain containing protein family is one of the epigenetic reader proteins that bind to specific acetylated lysine residues on histone to facilitate the assembly of transcription complexes [29].

Thus, epigenetic modifications (DNA methylation and histone modifications) are introduced by specific enzymes, the “writers”, recognized and interpreted by the specialized domain containing proteins, the “readers”, and can be erased by a dedicated group of enzymes that remove these chemical tags, the “erasers”.

2.3. Noncoding RNAs

The noncoding RNAs, RNAs with no open reading frame for translation, are highly abundant and functionally important RNAs. They include short non-coding RNAs with less than 200 nucleotides (such as microRNAs, miRNAs), RNAs with more than 200 nucleotides (long non-coding RNAs, LncRNAs) and circular RNAs. Human genome project has shown that a large portion of the noncoding RNAs including miRNAs and LncRNAs, play a variety of biological roles in a multitude of cellular processes, such as regulation of DNA replication, transcriptional activity, and translation and stability [30–32]. Although noncoding RNAs are not considered as epigenetic components, they are involved in epigenetic modifications. miRNAs, a class of 21–23 nucleotide single-stranded RNA molecules, is one of the more abundant classes of gene regulatory molecules. miRNAs can influence the output of many protein-coding genes by targeting mRNAs for cleavage or translational repression and control diverse biological processes [33,34], and their

dysregulation is associated with several diseases including cancer, diabetes and Alzheimer's disease [30,31,35]. Contrary to miRNAs, LncRNAs have more than 200 nucleotides, but do not have any protein coding potential, and are either located within the intergenic stretches of the genome or overlap (sense or antisense direction) protein coding genes. RNA-seq data analysis has revealed over 270,000 lncRNA transcripts in humans [36], with their tissue specificity over four fold higher compared to miRNAs [37]. LncRNAs are also involved in a wide range of cellular mechanisms, and they can regulate metabolic processes via several mechanisms including gene expression, binding with proteins and miRNA sponging [38]. Recent research has documented that LncRNAs play crucial roles in the regulation of pathophysiological processes in many chronic diseases including cancer and diabetes & its complications [39–43].

These epigenetics modifications can act independently, or one modification can lead to the other (Fig. 1), ultimately altering the gene expression [20,44]. Although the role of epigenetic modifications in the cancer field is being investigated for few decades, the role of epigenetics in diabetes and its complications is an emerging area of research.

3. Epigenetics and diabetic complications

Nature and nurture interact in a complex manner in the development of diabetes, and epigenetic modifications can modulate the interplay between genes and environment, thus making them as one of the mechanisms by which the environment could be interacting with the genome to modify the risk of diabetes [45]. The role of epigenetics in the development of diabetes is reviewed by many leading investigators [46,47], and is not the focus of this review.

Mounting evidence suggests that DNA methylation, post-translational modifications of histones and long non-coding RNAs play an important role in the initiation, maintenance and progression of both macro- and micro-vascular complications of diabetes [48]. A strong association between DNA methylation and metabolic memory is observed in patients enrolled in The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study [49]. This review will focus on epigenetics and diabetic complications, especially a microvascular complication-diabetic retinopathy.

3.1. Diabetic nephropathy

Diabetic nephropathy, a complex multifactorial disease, is one of the leading causes of chronic kidney disease and end-stage renal disease globally. Kidney disease is prevalent in more than half of the patients with type 2 diabetes and 30% with type 1 diabetes, and a large number of these patients progress to end-stage renal disease [50]. A case control study of 123 type 2 diabetic patients (53 patients with albuminuria and 70 without albuminuria) has shown significantly higher global DNA methylation levels in peripheral blood mononuclear cells of patients with albuminuria compared with those in normal range of albuminuria [51]. Whole blood genomic DNA analysis from diabetic patients with nephropathy have identified differential methylation in a number of genes including Protein Unc-13 Homolog B (*UNC13B0, a gene* linked with diabetic nephropathy), and analysis of the gene expression omnibus public database has shown 121 genes with hypermethylated

sites and 579 genes with hypomethylated sites in the kidney including hypomethylation of Peroxisome proliferator-activated receptor alpha and Glutaminase in tubular cells and hypermethylation of phosphatidylinositol-4-Phosphate 3-Kinase Catalytic Subunit Type 2 Beta,a gene associated with cell proliferation, *in* glomeruli [52]. Oxidative stress is considered to play a major role in diabetic nephropathy, and Enhancer of zeste homolog 2 (Ezh2), a histone-lysine *N*-methyltransferase enzyme important in histone methylation-gene suppression, is implicated in the suppression of endogenous antioxidant inhibitor thioredoxin-interacting protein. In podocytes, histone acetyltransferase is shown to regulate a critical sensor of oxidative stress, p66Shc [53]. In addition, many miRNAs and LncRNAs are also implicated in the development of kidney disease; e.g., miR-192 is implicated in podocyte apoptosis, glomerular and tubular hypertrophy and fibrosis and miR-214 and miR-21 in the regulation of inflammatory gene expression and signaling [54–57]. In addition to miRNAs, LncRNA *MALAT1* expression is also upregulated in kidneys from diabetic patients with nephropathy [58], and while LncRNA *MALAT1* is implicated in inflammation, LncRNA *LINC00462* in apoptosis of renal tubular epithelial cells [57]. Thus, epigenetic modifications are intimately associated with the development of diabetic nephropathy via several pathways including inflammation, cell proliferation and apoptosis and autophagy.

3.2. Diabetic neuropathy

Peripheral nerve dysfunction is commonly seen in 20% of patients with type 1 diabetes and 10–15% patients with type 2 diabetes after 20 years of diabetes [59]. As with nephropathy, the pathophysiology of diabetic neuropathy is also not clearly defined. Whole-genome DNA methylation analysis of 186 patients with type 2 diabetes has shown significantly decreased genomic DNA methylation levels in diabetic patients with peripheral neuropathy compared to patients without peripheral neuropathy [60]. A genome-wide study has shown an association between DNA methylation and diabetic peripheral neuropathy in the transcriptional analysis of the mouse models [61]. In diabetic rodents, while miR29c is upregulated in dorsal root ganglions and sciatic nerve, miR146a and 106a are downregulated [62]. Single nucleotide polymorphisms study has shown that while miR-128a variation rs11888095 is significantly correlated to a higher risk of developing diabetic polyneuropathy, miR-146a variation rs2910164 is associated with a lower risk [63]. Furthermore, increased levels of MiR-199a-3p levels are seen in plasma of diabetic patients affected with polyneuropathy [62,64]. Over four hundred differentially expressed LncRNAs, including LncRNA *MALAT1* and LncRNA *NEAT*2, are identified in diabetic patients with peripheral neuropathy [65].

Thus, epigenetic modifications are involved in various aspects of diabetic neuropathy, and targeting them with therapeutic modalities presents a promising opportunity.

3.3. Diabetic retinopathy

Retinopathy is the leading cause of blindness among diabetic patients in 20–74 age group. The retina is damaged by continuously being bathed by high glucose, and the damage is observed in its vasculature and neuronal cells components. Diabetes is a complex disease, and like its other complications, retinopathy is also a multifactorial disease, making it difficult to identify the molecular mechanism of its development. Many metabolic

abnormalities are implicated in its development including increased polyol and hexosamine pathways and formation of advanced glycation end products, activation of protein kinase C and increase in oxidative stress with increased accumulation of reactive oxygen species (ROS) [66,67]. In addition to dysfunctional mitochondria, hyperglycemia-induced activation of Ras-related C3 botulinum toxin substrate 1 (Rac1)- NADPH oxidase 2 (Nox2), and other metabolic abnormalities including activation of polyol pathway contribute to increased production of ROS. Experimental data have shown that the production of cytosolic ROS by Rac1-Nox2 precedes mitochondrial damage, and cytochrome *c* escapes from the leaky mitochondrial membranes into the cytosol, inducing apoptosis, a phenomenon which precedes the development of diabetic retinopathy [68].

Retinal mitochondria are dysfunctional and have partial cristolysis and leaky membranes, and mtDNA is damaged in diabetes. Activated cytosolic Nox2 continues to produce ROS, damaging the mitochondrial membranes and fueling into the vicious cycle of free radicals [9,66,69]. Furthermore, increased oxidative stress also activates a protease, matrix metalloproteinase-9 (MMP-9), and heat shock protein-mediated translocation of MMP-9 to the mitochondria breaks down mitochondrial integrity [70]. The situation is further worsened by impairments in the protective machinery including mitochondrial ROS quenching enzyme manganese superoxide dismutase (encoded by *Sod2*), mtDNA repair enzyme MutLH (Mlh1), fusion protein mitofusin 2 (Mnf2), and a compromised cytosolic antioxidant system including decrease in intracellular antioxidant glutathione (GSH), reduced activity of the nuclear factor erythroid 2-related factor 2 (Nrf2) and inhibition of catalase and copper-zinc superoxide dismutase [9,66,69].

Diabetes also alters the expression of many genes important in maintaining metabolic, functional and structural integrity of the retina. Diabetic patients with similar risk factors and glycemia can still present variable severity of retinopathy, or even no retinopathy, making genetic variant a good marker. However, despite extensive efforts by some of the leading scientists, good genetic associations in the development of diabetic retinopathy is not yet clearly established. Meta-analyses studies have identified variation in the *AKR1B1* gene, the gene encoding for the rate limiting enzyme of the polyol pathway [4,71–73], and also Ala allele of the *Pro12Ala* polymorphism in the peroxisome proliferator-activated receptor-γ2 gene in type 2 diabetic patients [74]. ‘Diabetic Retinopathy Genetics’ study has shown a novel set of genetic variants involved in the angiogenesis and inflammatory pathways contributing to the progression of diabetic retinopathy, and further investigation of variants is in progress [75]. A recent genome-wide association study of diabetic retinopathy with over 5000 participants, however, has shown no genome-wide significant findings, instead the analysis of protein-protein interaction pathways has suggested possible candidate pathways associated with inflammation for proliferative diabetic retinopathy in African Americans and identified genes implicated in inflammation candidate pathways for proliferative diabetic retinopathy [76]. Thus, any association between genetic factors and diabetic retinopathy remains elusive.

As mentioned above, DNA methylation status is maintained by Dnmts-Tets, and diabetes activates both of these enzymes in the retina and its vasculature [77]. In diabetes, the binding of Dnmt1 is increased at the retinal mtDNA, and this results in its hypermethylation.

Hypermethylation of mtDNA leads to attenuated transcription of mtDNA-encoded genes that are critical for the functioning of the electron transport chain, and the electron transport chain continues to be compromised [9,66,69,78]. Cytosine and 5mC are, however, not very stable; while cytosine can be deaminated to form uracil, 5mC can be spontaneously converted to thymine, and the mutation rate of 5mC is much higher than that of cytosine [79]. Regulation of DNA methylation is shown to attenuate diabetes-induced increase in base-pair mismatches in the retinal mtDNA, implying the role of DNA methylation in mtDNA damage [78]. Diabetes also hypermethylates promoter of the mismatch repair enzyme *Mlh1* and that of DNA polymerase gamma (*POLG*), the only polymerase found in mitochondria, further contributing to a compromised mtDNA repair system and suboptimal mtDNA copy numbers [80,81]. DNA methylation status of the promoters of mitochondrial fusion and fission proteins *Mfn2*, and dynamin-related protein 1 (*Drp1*) are also altered, resulting in transcriptional suppression of *Mfn2* and activation of *Drp1* [81], and unpublished results). Thus, epigenetics has a major role in disturbing retinal mitochondrial homeostasis in diabetes, and the damaged mitochondria continues to self-propagate the vicious cycle of free radicals.

As stated above, in the pathogenesis of diabetic retinopathy, cytosolic ROS production by Nox2 and activation of MMP-9 precede mitochondrial damage [70]. In diabetes, DNA at the promoter of *Rac1* undergoes active methylation-hydroxymethylation, and 5mC formed by Dnmt is rapidly hydroxymethylated to 5hmC by active Tets, resulting in *Rac1* transcriptional activation [82,83]. Similarly, dynamic cytosine methylation- hydroxymethylation of DNA at *MMP-9* promoter plays a major role in its transcriptional activation in diabetes [77].

Peripheral blood from patients with proliferative diabetic retinopathy have higher methylation of mtDNA compared to diabetic patients without any signs of retinopathy. Furthermore, these patients also have significantly higher methylated DNA at the promoters of *MLH1* and *Sod2* [84]. *Global DNA methylation* in blood is also shown to be a predictive biomarker of proliferative diabetic retinopathy including genes associated with inflammation and ischemia [85]. Thus, the role of DNA methylation in the development of diabetic retinopathy is becoming more convincing.

In addition to DNA methylation, many histone modifications have also been implicated in the development of diabetic retinopathy. A Finnish Study has found an association between the polymorphism in the gene encoding histone methyltransferase, suppressor of variegation 39 homolog 2 (*SUV39H2*), and diabetic microvascular complications, including retinopathy, suggesting the role of histone modifications in the development of diabetic retinopathy [86]. Increased levels of trimethylated H4K20 and histone methyl transferase SUV420H2 binding at the promoter and the enhancer of retinal *Sod2* in diabetes is implicated in its gene repression [87], and increased recruitment of lysine demethylase, LSD1, by demethylating H3K4me at *Sod2* promoter plays an important factor in the downregulation of *Sod2*. Decrease in H3K4me3 and H3K4me1 at the promoter of glutamate cysteine ligase-antioxidant response element region 4 is considered to play an important role in regulating the oxidative stress via regulating the production of intracellular antioxidant, glutathione. Increase in H3K4me1 at *Keap1*, an intracellular inhibitor of Nrf2, by SET domain-containing 7 histonely-sine methyltransferase (SETD7) is implicated in its

overexpression in diabetes [88,89], further compromising the antioxidant defense system. In addition, diabetes decreases H3K9me2 at the promoter of *MMP-9*, and increases the recruitment of histone-lysine *N*-methyltransferase enzyme, Ezh2 and elevating H3K27me3 levels [90]. The promoter of *Rac*1 is shown to have increased H3K9me3 and Suv39H1 binding, but decreased levels of H3K9me2 in diabetes [83].

Histone acetylation of many genes is also affected in diabetes; activities of retinal histone acetylases and deacetylases are altered, and global acetylation of histone H3 is reduced [91,92]. Acetyl H3K9 levels are increased at the promoters of retinal *Sod2*, *MMP-9* and *p66Shc* [93]. Epigenetic modifications of thioredoxin interacting protein, an endogenous inhibitor of antioxidant thioredoxin, are implicated in sustained *Cox2* expression seen in the retina in diabetes [94].

Different epigenetic modifications can be interrelated [20], and the same gene can be regulated by DNA and histone methylation and acetylation. In diabetic retinopathy, hypomethylation of H3K9 at *MMP-9* by LSD1 frees up the lysine 9 for acetylation, and acetylated H3K9 allows the recruitment of the transcription factor resulting in *MMP-9* transcriptional activation, suggesting a crosstalk between histone methylation and histone acetylation [95]. H3K9 methylation can facilitate Dnmt1 recruitment at the promoter CpG sites [96], and in diabetes, activation of Ezh2 trimethylates H3K27 at retinal *MMP-9* promoter, which allows the binding of DNA methylation/hydroxymethylation enzymes, resulting in its transcriptional activation [90]. Furthermore, increased *Suv39H1* binding at retinal *Rac1* promoter is shown to assists in the recruitment of Dnmt1, resulting in an active DNA methylation-hydroxymethylation and transcriptional activation [82]. As it is clear from the above discussion, the role of epigenetic tools, the ‘writers’ and the ‘erasers’, in the pathogenesis of diabetic complications is gaining a great deal of attention, the role of the ‘readers’ remain an understudied area of research, and needs further attention.

Expression of many miRNAs are altered in the retina/vitreous/serum in diabetes e.g., miRs 20a, 20b, miR-206 and miR-381–3 are dysregulated in the retina and serum of diabetic mice, affecting the expressions of many important factors associated with diabetic retinopathy including vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor and cAMP response element-binding protein 1 [97]. Upregulation of miR-21 is associated with downregulation of *PPARα*, a ligand-activated nuclear receptor important in regulating the expression of many genes associated with lipid metabolism and insulin signaling [98]. Furthermore, in diabetic patients, expression of miR-216a, miR-34c, miR-410 and miR-203a are significantly upregulated and that of miR-212 is downregulated [99]. Serum from patients with proliferative diabetic retinopathy have altered levels of miR-21, miR-181c, and miR-1179 [100]. Aberrant expressions of several miRNAs are also associated with the metabolic abnormalities considered important in the development of diabetic retinopathy including miR-152, miR-423, miR-146, miR 200b, miR 133b and miR365 [101].

Recent research is also implicating many LncRNAs in the pathogenesis of diabetic retinopathy, e.g., LncRNA *MALAT1* is significantly upregulated in the retina in diabetes, and in addition to contributing to increase in the inflammatory mediators, it is also implicated in the regulation of cellular antioxidant defense system; it facilitates the binding

of the transcription factor at Keap1 promoter, resulting in activation of *Keap1* transcription [41,102]. Upregulation of LncRNA *ANRIL* and LncRNA *NEAT1* is implicated in the nuclear factor NF-*k*B activation and regulation of VEGF and transforming growth factor-β1 [40,103,104]. LncRNA *HOTAIR* is suggested to play a role in angiogenesis, oxidative damage and mitochondrial aberrations in experimental models of diabetic retinopathy, and regulation of LncRNA *HOTTIP* in the retina is shown to regulate the inflammatory mediators [105,106]. Furthermore, knockdown of LncRNA *MIAT* reduces vascular leakage and inflammation by inhibiting tumor necroptosis factor α and intercellular adhesion molecule [32].

Thus, better understanding of the complex epigenetic mechanisms in the pathogenesis of diabetic retinopathy, and better designed studies utilizing prospective samples would open up the field to transition into clinical use.

3.4. Macrovascular complications

Long-term hyperglycemia-induced epigenetic changes are shown to accelerate the development of atherosclerosis by interfering with the physiological activities of macrophages, endothelial cells and smooth muscle cells [107]. NF-*k*B is the key pro-inflammatory transcription factor integral in regulating genes associated with vascular inflammation and atherosclerosis, and hyperglycemia is shown to induce various histone lysine modifications at the promoter of the *RELA* gene (encoding the NF*k*B-p65 subunit); increased H3K4me1 at *RELA* genes is implicated in the upregulation of the inflammatory genes in peripheral blood mononuclear cells of patients with type 2 diabetes [108]. Epigenetic modifications are also considered to play a major role in poor wound healing in diabetics; in addition to the regulation of macrophage plasticity and keratinocyte and fibroblast function during wound repair, epigenetic modifications also affect both immune and structural cells in wounds, influencing cell phenotypes and the healing process [108]. In addition, many LncRNAs are aberrantly regulated in diabetic cardiac disease including LncRNA *MIAT* and LncRNA *MALAT1* [109], and miR-129 and miR-335, via MMP-9, regulate diabetic wound healing [110].

Thus, it is clear that epigenetic modifications occupy an important place in the development of diabetic microvascular and macrovascular complications (Fig. 2).

However, epigenetic changes detected in biological fluids could play an essential role in the diagnosis of diabetic complications, and targeting them may prevent/slow down further progression of these debilitating complications that a diabetic patients is constantly fearful of facing.

4. Epigenetic drugs and diabetic complications

Epigenetics has a major role in diabetic complications; the dynamic nature of these epigenetic modifications and the ability of the epigenome to be reprogrammed, makes them as attractive targets for therapeutic interventions. Many small molecule compounds that can alter DNA and chromatin structure by modulating the activities of enzymes responsible for maintaining methylation status of DNA and histone modifying enzymes are now being

tested in experimental models, and some of them are in ongoing clinical trials. Azacytidine and decitabine, the cytidine analogues that integrate into DNA instead of cytosine forming a covalent bond with Dnmts, are approved by the Food and Drug Administration, and also by the European Medicines Agency for the treatment of acute myeloid leukemia and chronic myelomonocytic leukemia [111–113]. In addition, non-nucleoside analogues, independent of DNA incorporation, including oligonucleotides, natural compounds, S-adenosyl methionine (SAM) competitors, and repurposed drugs, i.e., are also shown to have therapeutic effects incorporation [114]. Curcumin, an active constituent of *Curcuma longa* is shown to demethylate *Nrf2* promoter, increasing its expression [115]. Another phytochemical Sulforaphane, by inhibiting Dnmts, increases the expression of *Nrf2* [116]. Flavonoids from tea, soft fruits and soya are potent inhibitors of Dnmts in vitro, and Folates, a group of water-soluble B vitamins found in high concentration in green leafy vegetables, regulate DNA methylation through their ability to generate SAM; people who regularly consume low levels of folate have a significantly increased risk of developing several cancers and cardiovascular disease [117]. This raises a possibility of potential use of Dnmt inhibitors in ameliorating diabetic complications. In fact, experimental models have shown that inhibition of Dnmts in diabetic rodents ameliorate retinal metabolic and functional abnormalities and prevents the development of histopathology characteristic of diabetic retinopathy [83], and the future use of these compounds looks promising.

HDAC inhibitors are now in use clinically for a wide variety of disorders ranging from hematopoietic malignancies to psychiatric disorders, and some are in clinical trials for other diseases. Therapies for non-oncology indications including HIV infection, muscular dystrophies, inflammatory diseases as well as neurodegenerative diseases such as Alzheimer's disease, frontotemporal dementia and Friedreich's ataxia are achieving promising clinical progress [118]. Vorinostat, an inhibitor of all zinc-dependent HDACs (except HDAC IIa) was approved by FDA in 2006 for refractory cutaneous, Belinostat, a novel and potent class I and II Hb-HDACI, is in a phase II trial, for women with ovarian cancer [119].

As detailed above, noncoding RNAs play crucial roles in gene expression, and their aberrant expression can lead to disease development. This has resulted in major efforts to therapeutically target these noncoding RNAs, and antisense oligonucleotides are considered as the most direct way to target them in a selective manner. In fact, many antisense oligonucleotide–based therapies have been tested in phase I clinical trials, and some have reached to phase II/III. For example, fomivirsen and mipomersen have received FDA approval to treat cytomegalovirus retinitis and high blood cholesterol, respectively [35]. CRISPR-Cas technology, with a crucial single guide RNA, and potential to edit DNA directly to generate therapeutic effects has gained a great deal of interest, and is in clinical trials for cancer, blood disorders and Leber congenital amaurosis [120].

However, there are many challenges in the development of drugs targeting epigenetic modifications including dynamic nature of these modifications, tissue and cell specificity and the possibility of different degrees of toxicity due to wide distribution of epigenetic enzymes in different tissues. It is critical to first identify the epigenetic marker to be targeted in a disease and use its class or isoform-specific enzyme inhibitors, and carefully

determine the optimal dose and identify how the drug should be transferred prior to their clinical applications in bone tissue engineering. Also, targeting noncoding RNAs with oligonucleotides to the correct tissues of interest remains a challenge.

5. Conclusions

It is clear that the human epigenome contributes to diabetic complications and also interacts with, and responds to, various physiological conditions. The dynamic nature of these modifications, and the potential of specific epigenetic alterations to be modified with therapeutics, have paved the way for the emergence of molecular-based epigenetic therapy (Fig. 3). While glycemic control, which may be difficult for some patients to achieve and maintain life-long, undoubtedly remains the best option to avoid or slow down diabetic complications, better understanding and exploitation of the fine-tuning of epigenetic mechanisms operating in diabetic complications are promising in driving forward an unprecedented advance in precision medicine.

5.1. Expert opinion

The discussion provided in this article clearly suggests that multiple metabolic abnormalities, initiated in a hyperglycemic milieu, are affected by epigenetic modifications, and these modifications play a significant role in modifying the course of the development of diabetic complications. Thus, targeting epigenetic modifications appears a viable option to slow down the development or progression of diabetic complications. Several epigenetic drugs have been approved by US Food and Drug Administration for other diseases, or are in clinical trials (Tables 1 and 2). In addition, novel epigenetic drugs with longer halflife and better safety profile and bioavailability and co-administration of two different epigenetic drugs is now gaining attention. Successful results from other diseases should open up the use of such strategies for the treatment of diabetes and its complications. We recognize that the delivery of the drug to the correct site, especially to the back of the eye (retina) also poses a challenge, however, novel tools in collaboration with biomedical engineering and nanoparticle developmental efforts have shown encouraging outcomes for better tissue targeting. The development of innovative modifications and delivery systems will help in future epigenetic-based therapeutics. Thus, the future of drugs targeting epigenetic modifications, although challenging, poses a great promise for treating diabetes and its complications, and provides hope to a diabetic patient to lead a life with less anxiety of losing vision or kidneys, sensory nerve or heart function.

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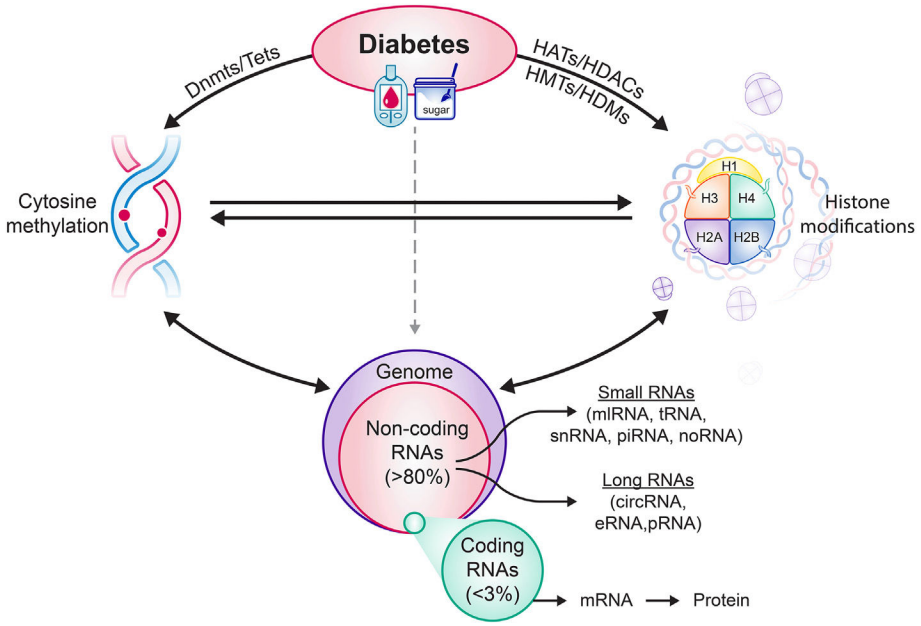

Fig. 1.

Diabetes alters the activities of the enzymes responsible for maintaing DNA methylation status and histone modifications, and this changes DNA methylation and histone acetylation/methylation status of many genes. Levels of many noncoding RNAs are also altered (up- or down regulated), contributing to suppression or overexpression of many genes. Alterations in DNA methylation affect histone modifcations and noncoding RNAs, and vice-versa. Dnmts = DNA methyl transferases, Tets = dioxygenases-ten-eleven translocases, HATs = Histone acetyltransferases, HDACs = Histone deacetylases, HMTs = histone methyl transferases and HDMs = histone demethylases.

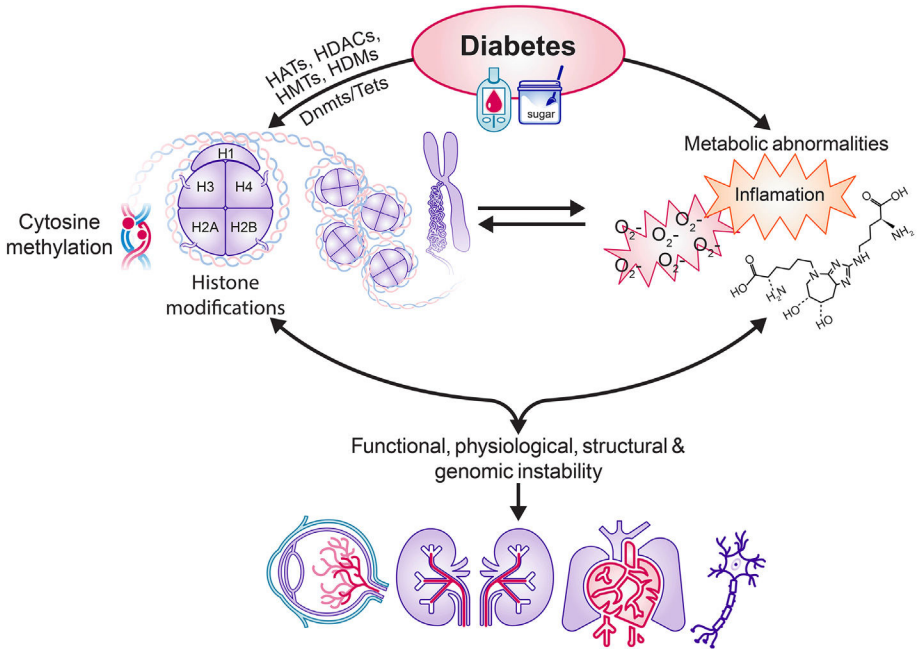

Fig. 2.

Diabetes facilitates many epigenetic modifications and also results in several metabolic abnormalities including increased oxidative stress, inflammation and formation of advanced glycation end products. Epigentic modifications can result in metabolic abnormalities and vice versa. Ultimately, epigenetic modifications/metabolic abnormalities lead to functional, structural, physiological and genomic instability, resulting in diabetic complications.

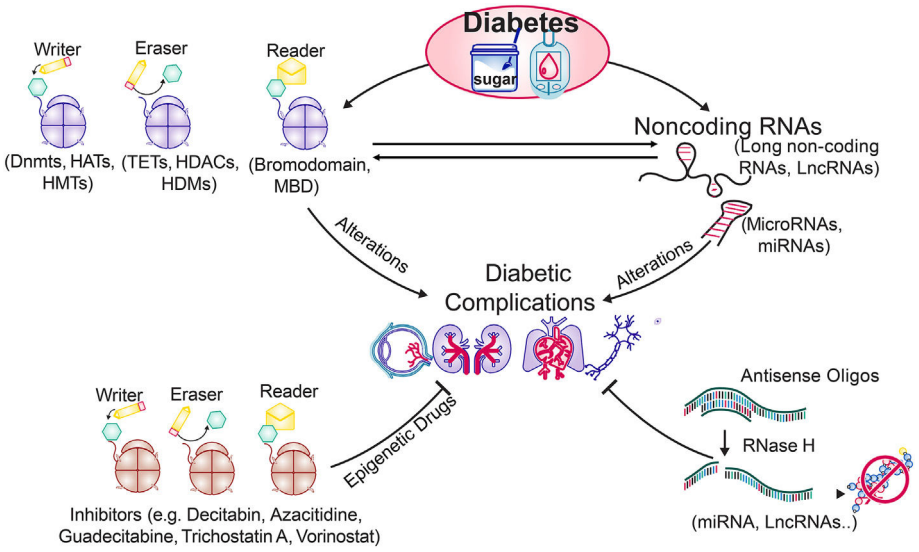

Fig. 3.

The epigenetic tools, writers/readers/erasers, and noncoding RNAs (miRNA, LncRNA etc.) are altered in diabetes, contributing in the development of diabetic complications. Recent technical advances have identified many small molecule componds that can interfere in the activation/inhibition of these epigenetic tools and target noncoding RNAs. Some of them are in pre-clinical or clinical trials for other chronic diseases (e.g., cancer), and the future for such epigenetic drugs for treating diabetic complications looks promising. (MBD = Methyl-CpG binding domain).

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Table 1

Epigenetic drugs approved by US Food and Drug Administration.

Commercial name	Target	Disease
Vorinostat (Zolinza)	Histone acetvlation	Lymphoma
5 Azacitidine (Vidaza, Decitabine)	DNA methvlation	Mvelodvsplastic svndrome
Romidepsin (Ixodax)	Histone acetvlation	Lymphoma
Valproic acid	Histone acetvlation	Anti-depressive Neurologic disorders
Belinostat (Belodaq)	Histone acetvlation	Peripheral T Cells Lymphoma
Panobinostat	Histone acetvlation	Multiple myeloma
Tazemetostat	Histone methvlation	Epithelioid sarcomas
Panobinostat + Bortezomib + Dexamethasone (Farvdak)	Histone acetvlation	Multiple myeloma
Azacitidine+ decitabine or low-dose cvtarabine (Venclexta)	DNA methvlation	Acute myeloid leukemia

Table 2

Epigenetic drugs in on-going clinical trials.

Commercial name	Target	Additional drug	Disease	Trial identifier	Trial phase
Azacitidine	DNA methylation	Lenalidomide	Acute Myeloid Leukemia in Remission	NCT04490707	III
Decitabine	DNA methylation	Carboplatin+ Paclitaxel	Ovarian cancer	NCT02159820	II & III
Decitabine	DNA methylation	Camrelizumab	Hodgkin lymphoma	NCT04510610	II & III
Decitabine	DNA methylation	Carboplatin- Paclitaxel	Malignant neoplasm of ovary	NCT02159820	II & III
Decitabine	DNA methylation	Oxaliplatin	Metastatic renal cell carcinoma	NCT04049344	II
SB939	Histone acetylation		Castration resistant prostate cancer	NCT01075308	II
Panobinostat	Histone acetylation	Bicalutamide	Prostate cancer	NCT00878436	II
Chidamide	Histone acetylation	Tislelizumab	Bladder Cancer	NCT04562311	II
Azacitidine	DNA methylation	Pembrolizumab	Pancreas Cancer	NCT03264404	II
Sodium phenylbutyrate	Histone acetylation		Huntington's disease	NCT00212316	II
Decitabine	DNA methylation	TQB2450 injection+Anlotinib	Digestive system tumors	NCT04611711	II
Sintilimab/ Chidamide	Histone acetylation		Angioimmunoblastic T-cell lymphoma	NCT04831710	II
Valproic Acid	Histone acetylation	Levocarnitine	Spinal muscular atrophy	NCT00227266	II
Decitabine	DNA methylation	Ara-C	Myeloid carcinoma	NCT03417427	II
Entinostat	Histone acetylation	Pembrolizumab	Melanoma	NCT03765229	II
Tazemetostat	Histone methylation		B-cell lymphomas and follicular lymphoma	NCT01897571	I & II
Panobinostat	Histone acetylation		HIV infection	NCT01680094	I & II
Abexinostat	Histone acetylation	Ibrutinib	B-cell and Mantle cell lymphoma	NCT03939182	I & II
Entinostat	Histone acetylation	Tezolizumab + Bevacizumab	Metastatic cancer	NCT03024437	I & II
Azacitidine	DNA methylation	Docetaxel/prednison	Metastatic castration-resistant prostate cancer	NCT00503984	I & II
Decitabine	DNA methylation	Genistein	Leukemias and solid tumors	NCT02499861	I & II
Vorinostat	Histone acetylation	Olaparib	Metastatic breast cancer	NCT03742245	I
Romidepsin	Histone acetylation	Brentuximab vedotin	Cutaneous lymphoma	NCT02616965	I
Tinostamustine	Histone acetylation	Nivolumab	Malignant melanoma	NCT03903458	I
GSK2816126	HMT-Ezh2		B cell lymphoma	NCT02082977	I
Tranylcypromine	Histone demethylation	Tretinoin	Acute myeloid leukemia	NCT02273102	I
Entinostat	Histone acetylation	Enzalutamide	Prostate adenocarcinoma	NCT03829930	I
Valproic acid	Histone acetylation	Bevacizumab	Advanced cancer	NCT00530907	I

Commercial name	Target	Additional drug	Disease	Trial identifier	Trial phase
MS-275	Histone acetylation	Enzalutamide	Castration-resistant prostate cancer	NCT03829930	I
Decitabine	DNA methylation	TQB2450 injection	Digestive system tumors	NCT04611711	I