REVIEW

Epigenetic regulation in diabetic vascular complications

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Abstract

Cardiovascular disease (CVD), the main complication of diabetes mellitus (DM), accounts for a high percentage of mortality in diabetic patients. Endothelial dysfunction is a major causative event in the pathogenesis of diabetes-related vascular disease and the earliest symptom of vascular injury. Epigenetic modification plays a key role in the initiation, maintenance, and progression of both endothelial dysfunction and diabetes. Epigenetic alterations respond to the environment and mediate the 'legacy effect' of uncontrolled hyperglycaemia early in the disease despite thorough glycaemic control in a phenomenon called metabolic memory. Therefore, an understanding of the integrated system of different epigenetic mechanisms in DM and its vascular complications is urgently needed. This review summarizes aberrant epigenetic regulation under diabetic conditions, including histone modifications, DNA methylation, and non-coding RNAs (ncRNAs). Understanding the connections between these processes and DM may reveal a novel potential therapeutic target for diabetic vascular complications.

Key Words

- ▶ epigenetics
- ▶ diabetes mellitus
- ▶ vascular complications
- ▶ endothelial dysfunction
- ▶ metabolic memory

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Introduction

Diabetes mellitus (DM) is a metabolic disorder with significant morbidity that currently affects approximately 150 million people, and the World Health Organization (WHO) predicts that this number could double by 2050 (Lumsden *et al.* 2013). Diabetes is an independent risk factor for cardiovascular diseases (CVD), and the prevalence of CVD in patients with type 2 diabetes mellitus (T2DM) is 2–4 times higher than that in non-diabetic patients (Contreras *et al.* 2000). What's more, the diabetic vascular complications such as heart failure, peripheral arteriopathy, myocardial infarction, angina pectoris, venous and arterial thrombosis have been determined clinically in the T2DM patients. In fact, DM-derived injuries on micro- and macro-vessels are considered the main causes of increased morbidity and mortality in this disease (Wold *et al.* 2005, Jia *et al.* 2018).

Vascular endothelium is an essential metabolic organ that regulates the structure and tone of the vasculature by secreting several vasodilators (e.g., prostacyclin (PGI₂), nitric oxide (NO) and endothelial-derived hyperpolarizing factors (EDHF)) and vasoconstrictors (e.g., endothelin-1 (ET-1), thromboxane 2, angiotensin II and reactive oxidative species (ROS)) (Avogaro *et al.* 2011). Endothelial cells (ECs) remain in a quiescent state under normal conditions; however, the function of ECs is inhibited upon exposure to various harmful stimuli such as high glucose levels, agonists, and shear stress, which leads to the onset of endothelial dysfunction (Ghosh *et al.* 2017). In general, endothelial dysfunction is the underlying mechanism of diabetic vascular disease and the earliest response to vascular injury (Vita & Keaney 2002), which is usually characterized by increased

vasoconstriction factor secretion, decreased cell viability, elevated endothelial permeability, reduced nitric oxide production, and disordered thrombosis (Tabit *et al.* 2010). The high-glucose environment in diabetic patients can undermine endothelial function by diminishing high-density lipoprotein (HDL) uptake, decreasing endothelial nitric oxide (eNOS) level, reducing nitric oxide availability, compromising VE-cadherin expression and generating mitochondrial reactive oxygen species (ROS) (Meng *et al.* 2012). The vasculature becomes vulnerable and enters a pro-atherogenic and pro-thrombotic state under such disorder (Vita & Keaney 2002). In particular, early exposure to hyperglycaemia can exert a 'legacy effect' on ECs that perpetuates endothelial dysfunction even following the in-time return to improved glycaemic control, which is called 'metabolic memory' (Testa *et al.* 2017).

A variety of genes related to T2DM have been identified; however, the expression of these genes *per se* cannot precisely predict the clinical risk of T2DM, implicating the role of other factors in the pathogenesis of T2DM. In addition, the risk of T2DM increases with age (Villeneuve & Natarajan 2010). Moreover, genetically identical twins have different susceptibilities to diabetes if they grow up in different environments (Lipman & Tiedje 2006, Tan *et al.* 2013). All these findings indicate that genetic predisposition is not the only factor accounting for T2DM (Vassy *et al.* 2012). A growing amount of evidence suggests that epigenetic mechanisms (e.g. histone modifications, DNA methylation, and non-coding (ncRNAs)) form a key interface between environmental factors and genetic predisposition, which responds to the environment and mediates the long-term effects of harmful stimuli, such as dyslipidaemia and hyperglycaemia (Keating & El-Osta 2013). Therefore, epigenetic signals driven by the environment at least partly explain the link between early hazardous effects and the later risk of metabolic memory and sustained endothelial dysfunction.

It has been revealed that epigenetics is involved in nearly all disease progression. A great number of studies have demonstrated a crucial role for epigenetic regulatory mechanisms in cardiac homeostasis. It is evident by overt epigenetic modifications in cardiovascular anomalies including hypertension, coronary heart disease, stroke and peripheral vascular diseases in the presence of major pro-epigenetic cardiovascular risk factors such as hyperglycaemia, aging, high fat intake, and exercise. Understanding specific CVD pathologies associated with T2DM and their possible link to epigenetics will be of far-reaching significance (Zhang & Ren 2016).

This article reviews the molecular mechanisms of endothelial dysfunction and metabolic memory in DM, focusing predominantly on the underlying epigenetic mechanisms whose aberrant regulation under diabetic conditions contributes to the pathogenesis of DM and its vascular complications.

Mechanisms of epigenetics regulation involved in endothelial dysfunction

In recent years, an increasing number of studies have emphasized the link between epigenetic factors and T2DM-related CVD. Epigenetics refers to alterations in gene expression and phenotype without changes in DNA sequence, such as post-translational histone modifications, DNA methylation, and ncRNA-mediated translational control. These alterations mediate the accessibility of genes for transcription factor binding and therefore influence their expression, which is essential to a variety of biological processes (De Rosa *et al.* 2018). Epigenetic modification is a cell-specific process with a high degree of responsiveness to environmental stimuli that accounts for many hidden causes of diseases (De Rosa *et al.* 2018).

Current therapies for T2DM primarily focus on reducing its associated cardiovascular risk factors and optimizing glycaemic control, while diabetic CVD and many other irreversible complications have not been fully concerned (Pasquier *et al.* 2015). Addressing the epigenetic links between endothelial dysfunction and T2DM and elucidating their potential pathophysiological role in diabetic CVD will help to identify possible new targets for diabetic CVD treatment.

Histone modifications

Emerging evidence has shown that histone modifications are involved in a crucial mechanism triggered by hyperglycaemia that leads to endothelial dysfunction in diabetes. Modifications to the N-terminal amino acid residues of histones, including acetylation, phosphorylation, ubiquitination, and methylation, are essential for the presence of chronic diabetic complications (Fig. 1).

A recent study identified the glucose-AMPK-TET2-5hmC axis, which is involved in the epigenetic regulation of diabetes. The phosphorylation of TET2 at serine 99 mediated by AMPK is suppressed under a high-glucose milieu, leading to TET2 destabilization and thereby the dysregulation of 5-hydroxymethylcytosine (5hmC)

Figure 1

Aberrant epigenetic modifications associated with T2DM and its vascular complications. Stimulation under diabetic conditions can trigger aberrant epigenetic modifications by recruiting or dissociating several crucial epigenetic factors, such as Set7, HDAC6, SRC-1, JMJD2C, LSD1, making chromatin 'accessible' or 'inaccessible' for transcription factor binding and therefore influencing gene expression (e.g. *P66*, *MMP-9*, *IL-6*, *Rac1*). This series of events leads to 'metabolic memory', oxidative stress, inflammation, mitochondrial damage, and cell apoptosis, which in turn promotes chronic hyperglycaemia characteristic of T2DM and has a further effect on histone modification and DNA methylation. A full colour version of this figure is available at <https://doi.org/10.1530/JME-19-0170>.

in vitro and *in vivo*, suggesting a correlation between diabetes and cancer. Interestingly, the administration of the anti-diabetic drug metformin could rescue these events, which indicates a novel aspect for future T2DM prevention and treatment (Wu *et al.* 2018). The activated demethylase LSD1 was found to be associated with *MMP-9* activation, mitochondrial damage, and cell apoptosis in diabetes. An LSD1-induced altered H3K9 methylation pattern unwinds chromatin, making *MMP-9* accessible to several transcriptional factors, such as NF- κ B that promote the transcription of *MMP-9* and aggravate mitochondrial damage (Mishra *et al.* 2016).

Other stimuli independent of glucotoxicity have also emerged as key players in the induction of epigenetic alterations (e.g. oxidative stress, AGEs, PKC activation). In particular, oxidative stress plays a causative role in the hyperacetylation of the *p66 Shc* promoter and the concurrent repression of CpG methylation (Titchenell & Antonetti 2013). Moreover, the epigenetic role of the methyltransferase SUV39H1 in oxidative stress has recently been discovered. The compromised expression of SUV39H1 in obesity promoted the recruitment of the

acetyltransferase SRC-1 and the demethylase JMJD2C to the *p66 Shc* promoter, resulting in decreased acetylation and di/trimethylation of H3K9, which culminated in the activation of mitochondrial *p66 Shc* and increased oxidative stress (Costantino *et al.* 2019). GLP-1 has a protective effect against oxidative stress in human umbilical vein endothelial cells (HUVECs) by upregulating HDAC6 in a GLP-1R-ERK1/2-dependent pathway, suggesting the protective role of HDAC6 in endothelial dysfunction induced by oxidative stress (Cai *et al.* 2018). Aberrant histone modifications and increased ROS were shown to be associated in human aortic endothelial cells (HAECs). The exposure of HAECs to hyperglycaemia induced methylation (H3K9me3, H3K9me2, H3K4me1) at the *eNOS* and *NOX4* promoters and activated these two genes, which are major sources of ROS, leading to an increased risk of endothelial dysfunction and diabetic vascular complications (Liao *et al.* 2018).

Sirtuins, which are well-known epigenetic metabolic modulators, are also recognized for playing a specific role in regulating EC functions. SIRT6, which is known for deacetylating H3K9Ac and H3K56Ac, was downregulated

in carotid plaques from asymptomatic diabetic patients and in the pancreas islets and retina (Zorrilla-Zubilete *et al.* 2018) of diabetic mice, resulting in inflammation and oxidative stress. SIRT3 is involved in antioxidant pathways and advanced glycation end product (AGE)-induced EPC dysfunction and it has been demonstrated to decrease cellular ROS levels through deacetylating and activating MnSOD (Chang *et al.* 2017). SIRT1 was found to be suppressed by the accumulation of AGEs in human endothelial Eahy926 cells, leading to the acetylation of p53 and the apoptosis of ECs, both of which were ameliorated or decreased by Sirt1 activator (resveratrol) or inhibitor (sirtinol) (Li *et al.* 2015). Indeed, a great number of potential drugs with anti-diabetic and epigenetic activities named epigenetic drugs or epidrugs have been discovered, including HDAC inhibitors (e.g. phenylbutyrate), HDAC activators (e.g. resveratrol, metformin), and acetyltransferase inhibitors (e.g. curcumin), most of which are being intensely studied and may become novel therapeutic targets in diabetic vascular complications (Sommese *et al.* 2017).

DNA methylation

DNA methylation mainly occurs on the cytosine ring of 'CpG islands' in the 5' regulatory regions of many genes through DNA methyltransferases (DNMTs), which always results in gene silencing (Hu *et al.* 2017). DNA methylation is closely related to the stability of gene expression status and the integrity of the genome by recruiting chromatin remodelling complexes that modify nucleosomes (Fig. 1).

DNA methylation analysis of human pancreatic islets identified 1649 CpG sites in 853 genes that were differentially methylated in T2DM patients compared to controls (Dayeh *et al.* 2013). Consistently, in a study using 84 well-matched monozygotic twin pairs, global DNA methylation was linked to higher risk of insulin resistance (IR) independent of other risk factors (Zhao *et al.* 2012).

Fluctuations in glucose levels are associated with DNA hypomethylation of the *P66 Shc* promoter, which causes a significant increase in *P66* level, culminating in persistent oxidative stress and endothelial dysfunction in T2DM patients (Keating *et al.* 2016). The expression of the *PGC-1 α* gene (*PPARGC1A*) is markedly reduced in T2DM patients along with a two-fold increase in DNA methylation of the CpG regions of the *PGC-1 α* promoter, which causes reduced insulin secretion (Ling *et al.* 2008). *IL-6*, a pro-inflammatory cytokine associated with prediabetes and vascular complications in DM (Lowe *et al.* 2014), was reported to alter the DNA methylation patterns of genes

involved in insulin signalling by regulating the DNMT3B and DNMT1 protein expression (Balakrishnan *et al.* 2018). Altered DNA methylation was also found at the proximal promoter and CpG regions near intron 1 of *Edn1*, a prominent gene encoding the endothelin-1 (ET-1) protein that is responsible for the aggravated pathologic state in several diabetic complications (Biswas *et al.* 2018a). Another study showed that hyperglycaemia in diabetic retinopathy could increase 5hmC levels, activate TET2 and facilitate the recruitment of DNMT1 at the *MMP-9* promoter region, which could further activate *MMP-9* and disturb retinal mitochondrial homeostasis (Kowluru & Shan 2017). The DNA methylation-demethylation status of *Rac1* has recently been associated with the regulation of oxidative stress in diabetic retinopathy. Both groups of opposing enzymes, DNMTs and TETs, are activated in diabetic retina; thus, 5mC levels at the promoter region of *Rac1* increase due to DNMT1 activation, yet a concomitant increase in TET2 promptly hydroxymethylates it to 5hmC, allowing NF- κ B to bind and activate *Rac1*. Activated *Rac1* then promotes ROS production via NOX2, which causes mitochondrial dysfunction and capillary cell apoptosis, ultimately resulting in diabetic retinopathy (Duraismy *et al.* 2018). A recent study demonstrated crosstalk between DNA methylation and histone modification in diabetic ECs. Upregulated *EZH2* alters H3K27 methylation pattern and promotes the recruitment of DNMT1-TET2 to *MMP-9* promoter in human retinal endothelial cells (HRECs) in a high-glucose milieu, resulting in transcriptional activation of *MMP-9*, which is one of the earliest events in the pathogenesis of diabetic retinopathy (Duraismy *et al.* 2017).

Non-coding RNAs

Dysregulation of miRNAs

miRNAs are a class of non-coding, single-stranded RNA molecules, approximately 22 nucleotides in length, which usually function as translational repressors. Many miRNAs have been shown to be indispensable in the pathophysiological processes of diabetic complications, including atherosclerosis, microvascular dysfunction, and retinopathy (Table 1) (McClelland & Kantharidis 2014). MiRNAs are now considered a novel group of potential markers in prediabetes and diabetes that provide new insights into potential therapeutic targets in patients with diabetes.

Of the miRNA profiles of various ECs in hyperglycaemic conditions, ten miRNAs (miR-26b-5p, miR-26a-5p, miR-29c-3p, miR-29b-3p, miR-130b-3p,

Table 1 MicroRNAs associated with vascular dysfunction in diabetic patients and models.

miRs	Up-/down-regulation	Cell type	Target gene	Function regulated	References
miR-26b-5p	Up	HUVEC	<i>EphA2, PTEN, RB1, EZH2, MCL1</i>	Vascular leakage, angiogenesis, tumor growth, apoptosis, senescence	Silambarasan <i>et al.</i> (2016)
miR-26a-5p	Up	HUVEC	<i>HMGA2, PTEN, RB1, EZH2, MCL1</i>	Vascular leakage, angiogenesis, tumor growth, apoptosis, senescence	Silambarasan <i>et al.</i> (2016)
miR-29c-3p	Up	HUVEC	<i>MCL1, BCL2, HDAC4, PTEN</i>	Angiogenesis, apoptosis	Silambarasan <i>et al.</i> (2016)
miR-29b-3p	Up	HUVEC	<i>MCL1, BCL2, VEGFA, PTEN</i>	Angiogenesis, apoptosis	Silambarasan <i>et al.</i> (2016)
miR-130b-3p	Up	HUVEC	<i>PTEN, STAT3, PPARG</i>	Pathological angiogenesis, tumor growth, endothelial activation, atherogenesis	Silambarasan <i>et al.</i> (2016)
miR-125b-1-3p	Up	HUVEC	<i>S1PR1</i>	Endothelial dysfunction	Silambarasan <i>et al.</i> (2016)
miR-192-5p	Up	HUVEC	<i>BCL2, RB1</i>	Apoptosis	Silambarasan <i>et al.</i> (2016)
miR-140-5p	Up	HUVEC	<i>VEGFA, HDAC4</i>	Pathological angiogenesis	Silambarasan <i>et al.</i> (2016)
miR-221-3p	Up	HUVEC	<i>PTEN, RB1, PAK1</i>	Angiogenesis, tumor growth, apoptosis, senescence, endothelial barrier function	Silambarasan <i>et al.</i> (2016)
miR-320a	Up	HUVEC	<i>NRP1, MCL1</i>	Endothelial tip cell function, angiogenesis, tumor progression	Silambarasan <i>et al.</i> (2016)
miR-29	Up	Human dermal microvascular endothelial cells (HMVEC)	<i>LYPLA1</i>	NO production, endothelium-dependent vasodilation	Widlansky <i>et al.</i> (2018)
miR-34a	Up	HUVEC	<i>SIRT1</i>	Oxidative stress	Li <i>et al.</i> (2016)
miR-126	Up	HUVECs	Tissue factor	Anti-thrombotic, anti-inflammatory, endothelial homeostasis, vascular integrity	Zampetaki <i>et al.</i> (2010), Witkowski <i>et al.</i> (2018)
miR-19a	Up	HMECs	<i>VCAM</i>	Anti-thrombotic, anti-inflammatory, Angiogenesis	Witkowski <i>et al.</i> (2018)
miR-342-3p	Down	HUVECs	<i>FGF11</i>	Angiogenesis	Cheng <i>et al.</i> (2018)
miR-483-3p	Up	H9c2	<i>IGF1</i>	Apoptosis	Qiao <i>et al.</i> (2016)
miR-200b	Up	HMECs	<i>Ets-1, VEGF</i>	Angiogenesis.	Chan <i>et al.</i> (2011)
miR-221	Up	HUVECs	c-kit	c-kit expression and migration of HUVECs	Bridgeman <i>et al.</i> (2018)
miR-222	Up	VSMCs	p27Kip1	Intimal thickening	Bridgeman <i>et al.</i> (2018)

miR-125b-1-3p, miR-192-5p, miR-140-5p, miR-221-3p and miR-320a) were steadily upregulated with increasing glucose concentration. Four miRNAs (miR-130b-3p, miR-26a-5p, miR-221-3p, and miR-140-5p) were 'glucose-responsive miRNAs' that were positively related to endogenous glucose levels, and three of these miRNAs

(miR-140-5p, miR-221-3p and miR-130b-3p) could lead to the onset of endothelial dysfunction by targeting several genes associated with apoptosis, inflammation, hyperpermeability, senescence, and pathological angiogenesis (Silambarasan *et al.* 2016). Consistently, miR-29 plays a key role in promoting NO production,

restoring endothelium-dependent vasodilation and maintaining endothelial function in diabetic conditions by the downregulation of lysophospholipase I (LYPLA1), a critical protein that is capable of reducing NO production through depalmitoylating eNOS (Widlansky *et al.* 2018). The expression of endothelial miR-34a can be induced by the cooperation of *p53* and *P66 Shc* in a redox-dependent fashion upon the exposure of miR-34a to hyperlipidaemia and hyperglycaemia in DM, which leads to diabetic endothelial dysfunction through an oxidant-sensitive mechanism that targets SIRT1 (Li *et al.* 2016). MiR-126 was highly expressed under normal conditions but downregulated under high-glucose conditions in endothelial apoptotic bodies, suggesting the close relationship between miR-126 and peripheral artery disease in DM patients (Zampetaki *et al.* 2010). MiR-126 can also cooperate with miR-19a to exert anti-thrombotic and anti-inflammatory effects on ECs by regulating the expression of vascular tissue factor in DM patients (Witkowski *et al.* 2018). The expression of miR-342-3p was compromised in the ECs of T2DM mice, while the augmentation of miR-342-3p levels exerted proangiogenic activity by activating FGF11 signalling at post-transcriptional level (Cheng *et al.* 2018). MiR-483-3p was shown to be involved in many biological processes, and the increased expression of miR-483-3p in the vasculature of diabetic subjects had a causative role in impaired endothelial regeneration. Moreover, miR-483-3p downregulates the *IGF1* gene, thereby triggering the apoptosis of ECs in diabetes (Qiao *et al.* 2016). Hyperglycaemia can aggravate endothelial dysfunction by reducing methylation of the miR-200b promoter, leading to a pathologic increase of miR-200b and thus driving diabetic vasculopathy (Chan *et al.* 2011, Singh *et al.* 2017). An inspiring finding suggested that metformin is capable of rescuing endothelial dysfunction in patients with diabetes through regulating several critical miRNAs, including let-7 family miRNAs, miR-221, miR-222, and miR-34a, which are associated with vascular dysfunction in diabetic patients (Bridgeman *et al.* 2018). Moreover, the metformin-induced activation of DICER, a central enzyme involved in miRNA processing, may also explain why metformin administration can rescue endothelial dysfunction (Noren Hooten *et al.* 2016).

Circular RNAs

Circular RNAs (cirRNAs) are a subclass of closed circular ncRNAs that do not have a 3' polyadenylated tail or 5' cap and form a loop structure with covalent bonds.

cirRNAs mainly comprise primary RNA transcripts from an intron or an exon or the back-splicing of both introns and exons (Tang *et al.* 2018). The biological functions of cirRNAs mainly lie in the six activities: (1) interaction with mRNAs, (2) interaction with lncRNAs, (3) transcriptional regulation, (4) miRNA sponge activity, (5) Ramie/Hemp Bath sponge activity, (6) secretion into exosomes (Zhao *et al.* 2016). cirRNAs have been shown to play an increasingly important role in cell mobility, proliferation, differentiation and apoptosis (Legnini *et al.* 2017). Furthermore, cirRNAs are crucial regulators of the pathogenesis of several metabolic diseases such as DM and are associated with the transcriptional and post-transcriptional regulation of endothelial dysfunction under high-glucose conditions (Stoll *et al.* 2018).

cirRNA microarray profiling in human ECs showed a total of 95 differentially expressed cirRNAs in human ECs under hyperglycaemic conditions, which confirmed the important regulatory role of cirRNAs in diabetes (Shang *et al.* 2018). In particular, a variety of miRNAs that interact with these differentially expressed cirRNAs were identified, including miR-3202, miR-1273g-3p, and miR-657, all of which have been demonstrated vital in regulating biological functions of high glucose-cultured ECs (Huang *et al.* 2017).

The cirRNA-miRNA-mRNA interaction network is involved in the pathophysiology of diabetic endothelial dysfunction (Table 2). The cirRNA cPWWP2A functions as an endogenous miR-579 sponge to sequester miR-579 activity that upregulated SIRT1, occludin, and angiopoietin and exacerbated diabetes-induced retinal endothelial dysfunction (Liu *et al.* 2019). Aberrant cPWWP2A expression can also attenuate pericyte-EC crosstalk, leading to vascular obliteration, leakage, and pathological angiogenesis (Boeckel *et al.* 2015). The upregulated expression of cirRNA-cZNF609, which promotes endothelial migration and tube formation by sponging miR-615-5p and promoting the transcription of the miR-615-5p target gene, myocyte-specific enhancer factor 2A (*MEF2A*), was shown in diabetic retinal endothelial cells (RECs) (Liu *et al.* 2017). cirRNA-0054633 exhibits protective effects against diabetic endothelial dysfunction in HUVECs through a similar mechanism via inactivating miR-218, thereby changing the expression levels of ROBO1 and HO-1, which contributes to EC migration, proliferation and angiogenesis (Pan *et al.* 2018). Changes in circHIPK3 levels have been detected in HAECs and HUVECs. The hyperglycaemia-induced inactivation of circHIPK3 upregulates miR-124, resulting in decreased levels of its potential pro-survival targets (STAT3 and

Table 2 Circular RNAs associated with vascular dysfunction in diabetic patients and models.

circRNAs	Cell type	Up-/down-regulation	Sponged miRNAs	Downstream genes	Function regulated	References
cPWWP2A	RECs	Up	miR-579	angiopoietin 1, occludin, <i>SIRT1</i>	Pericyte-EC crosstalk	Liu <i>et al.</i> (2019)
cZNF609	RECs	Up	miR-615-5p	<i>MEF2A</i>	Endothelial migration and tube formation	Liu <i>et al.</i> (2017)
0054633	HUVECs	Up	miR-218	<i>ROBO1</i> , <i>HO-1</i>	Endothelial proliferation, migration and angiogenesis	Pan <i>et al.</i> (2018)
circHIPK3	RECs	Up	miR-124/miR-30a-3p	<i>STAT3</i> , <i>SphK1</i> , <i>WNT2</i> , <i>FZD4</i> , <i>VEGFC</i>	Apoptosis	Liu <i>et al.</i> (2017), Shan <i>et al.</i> (2017), Cao <i>et al.</i> (2018)
circ_0005015	RECs	Up	miR-519d-3p	<i>STAT3</i> , <i>MMP-2</i> , <i>XIAP</i>	Angiogenesis	Zhang <i>et al.</i> (2017b)

SPHK1), which finally triggers apoptosis in ECs (Cao *et al.* 2018). Another eight miRNAs (miR-654, miR-584, miR-338, miR-29b, miR-152, miR-29a, miR-193a, and miR-379) were found to be potential targets of circHIPK3 (Liu *et al.* 2017). CircHIPK3 was found to sponge and suppress miR-30a-3p, thereby activating WNT2, FZD4, and VEGFC and exerting its protective effect against endothelial dysfunction under high-glucose conditions (Shan *et al.* 2017). Furthermore, circ_0005015 can function as a sink for miR-519d-3p to abrogate the repressive effect of miR-519d-3p on XIAP, STAT3, and MMP-2, indicating its potential protective role in endothelial angiogenic function (Zhang *et al.* 2017b). CirRNAs may also serve as transcriptional regulators that mediate chromatin remodelling by binding to RNA-binding proteins (RBPs) such as EIF4A3, FUS and HUR in promoter regions of the host gene (Zhang *et al.* 2017b).

Long non-coding RNAs

Long non-coding RNAs (lncRNAs), the non-coding transcripts more than 200 nucleotides in length (Sun & Wong 2016), have been identified as key epigenetic regulators that participate in a variety of biological processes by guiding certain histone-modifying complexes, promoting the association between enhancers and promoters through chromosomal looping, acting as molecular sponges, and serving as scaffolds for specific molecules (Biswas *et al.* 2018b).

Emerging evidence has authenticated the contributions of various lncRNAs to the biological functions of ECs (Table 3) (Goyal *et al.* 2018). In a systematic transcriptional study conducted to screen lncRNAs in response to the exposure of HUVECs to high-glucose conditions, 100 of the 30586 lncRNAs were appreciably upregulated, while 186 were significantly downregulated, which provided novel insights into the potential regulatory mechanism of hyperglycaemia-associated endothelial dysfunction (Singh *et al.* 2016).

MALAT1, an upregulated lncRNA in the retinas of diabetic rats, is enriched in ECs and regulates many endothelial functions such as migration, proliferation, and tube formation in a p38 MAPK-dependent manner (Liu *et al.* 2014). MALAT1 has also been shown to protect the endothelium from ox-LDL-induced endothelial dysfunction via downregulating miR-22-3p and upregulating CXCR2 (Tang *et al.* 2015). Moreover, MALAT1 can regulate DNA methylation by interacting with methyl-CpG binding protein 2 (MECP2) and thus maintain the dynamic glucose equilibrium (He *et al.* 2017).

Table 3 lncRNAs associated with vascular dysfunction in diabetic patients and models.

lncRNAs	Cell type	Up-/down-regulation	Targets	Function regulated	References
MALAT1	RECs	Up	miR-22-3p, <i>MeCP2</i>	Migration, proliferation, and tube formation	Paneni <i>et al.</i> (2015)
MEG3	RECs	Down	<i>TGF-β1</i> , <i>VEGF</i>	Migration, viability, proliferation and tube formation	Qiu <i>et al.</i> (2016) , You <i>et al.</i> (2016) , Zhang <i>et al.</i> (2018)
ANRIL	HRECs	Up	<i>p300</i> , <i>EZH2</i>	Angiogenesis	Congrains <i>et al.</i> (2013) , Thomas <i>et al.</i> (2017) , Zhang <i>et al.</i> (2017a)
MIAT	ECs	Up	miR-150-5p	Vascular leakage, inflammation and neo-vascularization	Michalik <i>et al.</i> (2014) , Paneni <i>et al.</i> (2013) , Yan <i>et al.</i> (2015)

In addition, the knockdown of MALAT1 in diabetic mice ameliorated retinal inflammation, and MALAT1 repression in RECs inhibited cell migration, proliferation, and tube formation ([Liu *et al.* 2014](#)). Thus, the inhibition of MALAT1 is a potential target for anti-angiogenic therapy.

The lncRNA MEG3 is also related to the pathogenesis of endothelial dysfunction and microvascular dysfunction. This lncRNA is hypermethylated and downregulated upon the exposure of ECs to high glucose and oxidative stress as well as in the retinas of STZ-induced diabetic mice ([Qiu *et al.* 2016](#)). MEG3 knockdown aggravated vascular leakage, increased inflammatory protein levels and triggered acellular capillary formation *in vivo* and promoted cell migration, viability, proliferation and tube formation *in vitro* via activating the PI3K/AKT signalling pathway ([Qiu *et al.* 2016](#)). In addition, the suppression of MEG3 expression was linked with compromised glucose tolerance and impaired insulin secretion ([You *et al.* 2016](#)). Correspondingly, the overexpression of MEG3 has been demonstrated to ameliorate diabetic retinopathy by inhibiting the expression of TGF- β 1 and VEGF ([Zhang *et al.* 2018](#)), which suggests the upregulation of the lncRNA MEG3 as a promising therapy for endothelial dysfunction and diabetic vascular complications.

Antisense ncRNA in the INK4 locus (ANRIL), a crucial functional lncRNA that participates in multiple human diseases, is also upregulated under hyperglycaemic conditions in diabetic subjects. Emerging evidence has revealed that ANRIL facilitates the expression of vascular endothelial growth factor (VEGF), a crucial inducer of angiogenesis, via interacting directly with P300 and EZH2 of the PRC2 complex in HRECs ([Thomas *et al.* 2017](#)). Consistently, ANRIL knockdown in STZ-induced diabetic mice reversed the hyperglycaemia-induced upregulation of P300 and downregulation of miR-200b and abrogated the increase in VEGF ([Thomas *et al.* 2017](#)). In addition to its participation in REC functions, ANRIL also plays a role in other ECs, suggesting its physiological relevance in various vascular diseases such as hypertension and atherosclerosis

([Congrains *et al.* 2013](#)). ANRIL also upregulated VEGF and contributed to angiogenesis through activating NF- κ B signalling pathway in rats with DM ([Zhang *et al.* 2017a](#)).

The lncRNA myocardial infarction-associated transcript (MIAT), which is enriched in ECs, has also been shown to regulate EC function and pathological angiogenesis ([Michalik *et al.* 2014](#)). This lncRNA was found to be upregulated in response to hyperglycaemia and in the pathological processes of some diseases associated with endothelial dysfunction ([Huo *et al.* 2019](#)). Consistently, siRNA-mediated knockdown of MIAT in STZ-induced diabetic mice ameliorated vascular leakage, inflammation, and neo-vascularization ([Paneni *et al.* 2013](#)). Crosstalk among MIAT, miR-150-5p, and VEGF mRNA has also been proposed. MIAT functions as a competing endogenous RNA (ceRNA) that acts as a molecular sponge for miR-150-5p and abrogates the inhibitory effect of miR-150-5p in RECs during pathological angiogenesis, thereby activating VEGF, the target gene of miR-150-5p ([Yan *et al.* 2015](#)).

Epigenetic mechanisms involved in metabolic memory

The metabolic memory phenomenon in diabetic dogs was originally observed in 1987 by Engerman and Kern to describe the deleterious 'legacy effect' of uncontrolled hyperglycaemia early in diabetes despite thorough glycaemic control ([Misra & Bloomgarden 2018](#)). The metabolic memory phenomenon has been duplicated in both *in vivo* and *in vitro* studies. Cell culture studies have confirmed metabolic memory in RECs with persistently increased fibronectin levels, continuous oxidative stress, accelerated apoptosis and the aberrant expression of inflammatory genes despite the normalization of glucose levels, indicating that self-perpetuating varieties of genes have been induced by hyperglycaemia ([Zhong & Kowluru 2013](#)). In some clinical trials, such as the landmark Diabetes Control and Complications Trial (DCCT) and

Epidemiology of Diabetes Intervention and Complications (EDIC) study, tight glycaemic control for 3–5 years was not able to decrease diabetic macrovascular complications. Moreover, metabolic memory has also been confirmed in animal models of diabetic vascular complications such as diabetic retinopathy and microvascular dysfunction (Kowluru 2017).

Metabolic memory has long remained a main obstruction to the effective treatment of diabetic complications. Recently, the importance of epigenetic mechanisms in the long-term effects of hyperglycaemia on the vasculature has been increasingly appreciated. Persistent epigenetic changes can reflect metabolic history, which has been demonstrated in various experimental models including the retinas of diabetic rats in high-glucose conditions (Tewari *et al.* 2012), zebrafish with hyperglycaemia (Olsen *et al.* 2012), and diabetic patient-derived fibroblasts (Park *et al.* 2014). Changes in miRNA and histone methylation at the post-transcriptional level have also been observed after transient exposure to high-glucose conditions, indicating that gene-environment interactions associated with epigenetic changes are closely related to the long-term deleterious effects of metabolic memory (Reddy *et al.* 2015). Genome-wide changes, such as methylated CpG islands and hyperacetylation signatures, are also mediated in primary human vascular cells by elevated glucose levels that trigger the 'legacy effect' through activating several genes related to the physiopathology of diabetes, including C-C motif chemokine 2 (*CCL2*), interleukin-8 (*IL8*), matrix metalloproteinase-10 (*MMP10*), and cystine/glutamate transporter (*SLC7A11*) (Pirola *et al.* 2011).

Hyperglycaemia-induced increased oxidative stress in the vasculature, which is characterized by a damaged cellular antioxidant defence system characterized by the excessive production of ROS, is a central feature of endothelial dysfunction in diabetes mellitus and its associated complications (Cai & Harrison 2000). An increasing number of studies have demonstrated that excessive ROS-mediated metabolic memory contributes to an inflammatory environment and endothelial dysfunction in the aorta (Paneni *et al.* 2012). Interestingly, abnormal histone modifications at the promoters of several genes associated with ROS production after transient hyperglycaemia were recently revealed. For example, hyperglycaemia reduced H3K4me1 and H3K4me2 at *SOD2*, which encodes MnSOD, by promoting the recruitment of SP1 and LSD1 to the *SOD2* promoter in ECs. However, the methylation status had not improved three months after glucose normalization, which caused

mitochondrial damage and excessive production of ROS, suggesting the critical role of histone methylation in the development of diabetic retinopathy as well as metabolic memory (Zhong & Kowluru 2013). Moreover, SET7 and LSD1 have emerged as key influencing factors in metabolic memory via their regulation of H3K4me1, thus attenuating endothelial dysfunction induced by metabolic memory (Liao *et al.* 2018).

Another factor involved in metabolic memory is the sustained pro-inflammatory phenotype of diabetic ECs. Although the mechanism of this involvement is not completely clear, epigenetic modifications seem to act as a key bond connecting environmental and genetic factors that explain metabolic memory (Rana *et al.* 2012). The histone demethylase LSD1 was increasingly recruited to the promoter of NF- κ B-p65 in response to transient hyperglycaemia in ECs, leading to decreased H3K9 and increased H3K4 methylation, which activated NF- κ B and various NF- κ B-dependent inflammatory genes such as *MCP-1* and *VCAM-1*. Moreover, some other hyperglycaemia-induced epigenetic alterations to the promoter of NF- κ B in ECs have been revealed; these modifications include the histone acetyltransferase (HAT)-mediated hyperacetylation of H3K9 and the histone methyltransferase SET7-mediated monomethylation of H3K4 (Brasacchio *et al.* 2009), which elevate NF- κ B subunit p65 levels. On account of these events, pro-inflammatory pathways were activated, culminating in endothelial dysfunction. Furthermore, the repression of HAT (Cordero-Herrera *et al.* 2017) and Set7 (Paneni *et al.* 2015) was shown to abrogate NF- κ B-dependent inflammatory and oxidant signalling by reverting adverse epigenetic remodelling. Therefore, the increased activity and expression of NF- κ B in metabolic memory is a therapeutic target.

Nonenzymatic protein glycosylation in which the Maillard reaction leads to the formation of advanced glycation end products (AGEs) is another pathogenic event in metabolic memory (Yamagishi *et al.* 2017). Increased levels of AGEs have various detrimental effects on ECs through the binding of AGEs to the AGE receptors (RAGEs) on cell surface, which further enhances oxidative stress, decreases endothelial NO synthase, increases vascular oxidized low-density lipoprotein (LDL) deposition and attenuates inflammatory response, triggering various microvascular and macrovascular complications in DM (Stirban *et al.* 2014). Furthermore, the cytotoxicity of AGEs persists after being transferred to new medium without AGEs, indicating the potential role of epigenetic alterations, at least to some extent,

in AGE-mediated metabolic memory (Ravelojaona *et al.* 2007).

Epigenetic changes have therefore been proposed to be involved in the mechanism of the abovementioned components of metabolic memory (oxidative stress, an inflammatory phenotype, and nonenzymatic protein glycation), which may account for the long-term detrimental effects of metabolic memory (Testa *et al.* 2017).

Prospects

The evidence discussed above suggests that epigenetic factors have a crucial function in the pathophysiological process of diabetes and its vascular complications. However, there is a long way to go for the clinical application of epigenetics in diabetes and its vascular complications, and further cohort studies in diabetic patients with different complications are needed to validate these results. The present diabetic parameters are not sufficient to predict the development of long-term complications of T2DM. However, with the rapid development of bioinformatics analysis platforms and high-throughput sequencing, epigenetic factors are showing more potential acting as new non-invasive biomarkers, which enables the early diagnosis of prediabetes and T2DM. Hence, it is a profound understanding of individual epigenetic landscape, genetic variability, intercellular communication mechanisms, chromatin architecture and concrete mechanisms by which histone modifications, DNA methylation, and ncRNAs interact that will contribute to the identification of diagnostic biomarkers and the design of specific molecules to regulate epigenetic markers and modulate chromatin accessibility. Moreover, future study to determine whether and how high glucose-induced epigenetic changes take part in endothelial dysfunction and their effects on inflammatory responses and oxidative stress is merited. Further study in this field will advance personalized therapeutic interventions and individualized risk assessment in diabetic patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

J J drafted and wrote the manuscript. J J and X W collected the literature. X Z and D M conceived and organized the work, and revised the manuscript.

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