Cardiovascular Diabetology

REVIEW

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MicroRNAs in diabetic macroangiopathy

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Abstract

Diabetic macroangiopathy is a leading cause of diabetes-related mortality worldwide. Both genetic and environmental factors, through a multitude of underlying molecular mechanisms, contribute to the pathogenesis of diabetic macroangiopathy. MicroRNAs (miRNAs), a class of non-coding RNAs known for their functional diversity and expression specificity, are increasingly recognized for their roles in the initiation and progression of diabetes and diabetic macroangiopathy. In this review, we will describe the biogenesis of miRNAs, and summarize their functions in diabetic macroangiopathy, including atherosclerosis, peripheral artery disease, coronary artery disease, and cerebrovascular disease, which are anticipated to provide new insights into future perspectives of miRNAs in basic, translational and clinical research, ultimately advancing the diagnosis, prevention, and treatment of diabetic macroangiopathy.

Keywords miRNAs, Diabetes mellitus, Diabetic macroangiopathy, Macrovascular dysfunction

Introduction

Diabetes mellitus (DM), a disease of the endocrine system characterized by hyperglycemia and insulin resistance, is one of the most common and fastest-growing chronic diseases worldwide, projected to affect 1.3 billion people (approximately 9.8%) by 2050 [1]. As a systemic metabolic disorder, DM affects multiple systems and organs in the body, frequently resulting in numerous complications. Under typical circumstances, diabetic vasculopathy emerges as the earliest and most common complication of DM [2]. In particular, hyperglycemia can directly destroy the vascular microenvironment and injure blood vessels, subsequently triggering organ damage, including damage to heart, brain, kidney and retina. These vascular-related complications are the primary cause of morbidity and mortality associated with DM [3].

DM impairs the vascular system by causing both microvascular lesions and macrovascular lesions, leading to complications such as diabetic kidney disease (DKD), diabetic retinopathy (DR), diabetic foot ulcers, peripheral artery disease (PAD), coronary artery disease (CAD), and cerebrovascular disease (stroke) [4]. Diabetic macrovascular complications, also known as diabetic macroangiopathy, include peripheral artery disease (PAD), coronary artery disease (CAD), and cerebrovascular disease (stroke) [5] (Fig. 1). Clinical studies suggest that PAD and heart failure are the most common initial manifestations of cardiovascular disease in individuals with type 2 diabetes mellitus (T2DM) [6]. These conditions are

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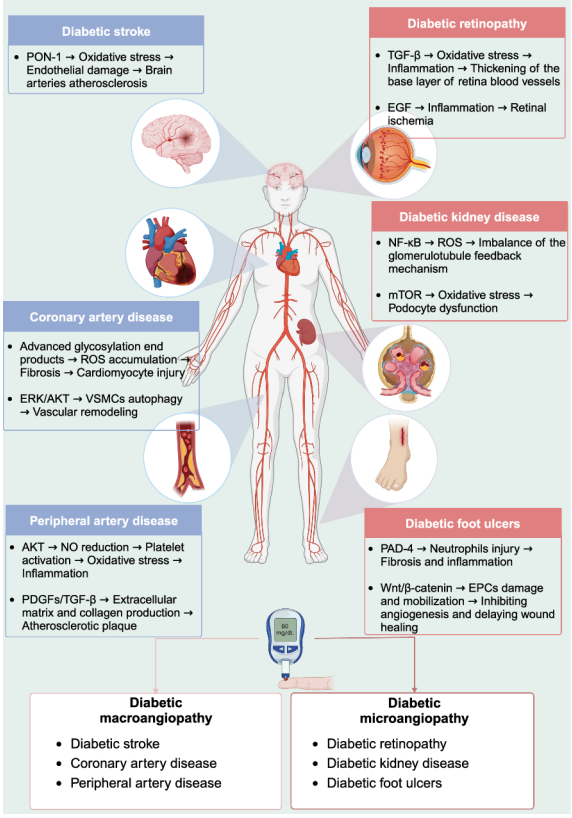

Fig. 1 The pathogenesis of cardiovascular complications in diabetes mellitus. Hyperglycemia induces pathological changes in vital organs through circulatory system, including cerebral, cardiac, renal, ophthalmic, peripheral systems and foot

closely linked with macrovascular dysfunction, including dysfunction of coronary arteries and peripheral large vessels. Due to the characteristics of high mortality risk and early onset, diabetic macroangiopathy is a vital factor affecting the prognosis of DM patients. Frustratingly, simple glycemic control cannot completely prevent or even reverse macrovascular damage. The current mainstay of treatment for diabetic macroangiopathy involves a combination of hypoglycemic agents and cardiovascular protective agents, which is reluctantly acknowledged as an accurate and effective approach. Thus, knowledge about molecular mechanisms underlying diabetic macroangiopathy pathogenesis, especially the identification of new druggable targets, is of significant importance for preventing and treating the diabetic macroangiopathy pandemic. Encouragingly, the identification of microRNAs (miRNAs) provides novel insights into the pathogenesis of DM and its complications, including diabetic vasculopathy [7–10].

MiRNAs constitute a still incompletely explored class of molecules that have been shown to play a fundamental role in DM and its cardiovascular complications. In this review, we will provide a comprehensive overview of the characterization of miRNAs, specifically focusing on their roles and underlying mechanisms in diabetic macroangiopathy. Moreover, we will emphasize their intricate interactions with cardiovascular system cells under diabetic conditions, including endothelial cells, vascular smooth muscle cells and macrophages.

Non-coding RNAs

Approximately 90% of the human genome sequence is transcribed into RNA, yet less than 2% can be translated into functional proteins. The remaining untranslated portion, often regarded as "dark matter", contains a diverse range of functional non-coding RNAs (ncRNAs), as revealed by the ENCODE project [11]. ncRNAs can be classified into several size-based categories. Small ncRNAs (sncRNAs) include miRNAs, piwi-interacting RNAs (piRNAs), and tRNA-derived small RNAs (tsRNAs). Long ncRNAs (lncRNAs), comprising ncRNAs longer than 500 nucleotides, encompass pseudogenes and circular RNAs (circRNAs) [12, 13]. ncRNAs play a crucial role in regulating biological processes. In recent decades, ncRNAs, particularly miRNAs, Y-RNAs and Vault RNAs, have sparked significant research interest in the cardiovascular field [14–17]. Although the potential mechanisms are incompletely known, miRNAs have shown a universal and critical role in cellular processes across various diseases, including metabolic disease, underscoring their importance [18].

miRNAs

The discovery of miRNAs is closely related to a fascinating phenomenon known as RNA interference (RNAi). In 1990, scientists artificially upregulated a pigment synthesis enzyme in order to create dark purple petunias, but instead ended up with predominantly white flowers. Research suggested that both transgenic and endogenous genes were coordinately repressed, but the underlying mechanism remained unclear. Consequently, this intriguing phenomenon was described as "co-suppression" [19]. Since then, RNA silencing has been recognized as an effective approach to regulate cellular activity. Ambros and Ruvkun discovered the first miRNA, lin-4, from *C. elegans* in 1993. They conjectured that lin-4 complementarily paired with the 3' untranslated region (3' UTR) of lin-4 mRNA, thereby regulating the silencing of the target mRNA [20, 21]. Incredibly, this theory, which was validated eight years later, accurately predicted the biological mechanism of miRNAs. The exploration of miRNAs reached its pinnacle in the early twenty-first century. In 2004, Matthew et al. identified the first metabolic-related miRNA, miR-375, which regulates insulin secretion [22]. With the completion of the Human Genome Project and the beginning of the postgenomic era, miRNAs have aroused great interest in various

research domains. As a result, thousands of miRNAs and other ncRNAs have been identified in diverse species and disease models, including cancer and metabolic disease.

Biogenesis and regulation of miRNAs

As a class of small (20–24 nucleotides) non-coding RNAs, miRNAs are formed differently from other conventional RNAs, and can be roughly summarized with several key points: five steps (transcription, cleavage, transport, cleavage, and maturation), two processing sites (nucleus and cytoplasm), two engines (RNA polymerase II/III), and two scissors (Drosha and Dicer), abbreviated as '5–2–2–2' [23] (Fig. 2). Firstly, the target genes, including individual (monocistronic) genes, clustered (polycistronic) genes, or from introns of protein-coding genes, are initially transcribed by RNA polymerase II/III to form hairpin-shaped precursor miRNA molecules, the primary miRNA (pri-miRNA) transcripts [24]. Concurrently, the RNase III endonuclease Drosha combines with essential cofactor DiGeorge syndrome critical region 8 (DGCR8) subunits to form a multimolecular complex.

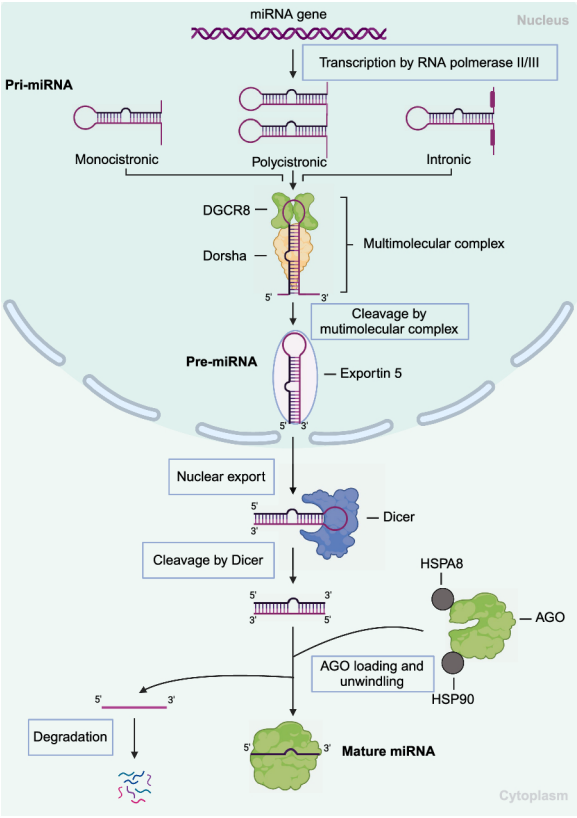

Fig. 2 Canonical miRNA biosynthesis. Initially, primary miRNA transcripts (pri-miRNAs) are transcribed by RNA polymerase II from miRNA gene. Then, pri-miRNAs are processed to generate precursor miRNA (pre-miRNA) by DGCR8 and Dorsha, and exported in to the cytoplasm by Exportin 5. Subsequently, Dicer recognizes and cleaves the pre-miRNA to produce a ~ 20-bp miRNA duplex. Finally, the resulting miRNA duplex binds to the AGO protein and matures into a single stranded miRNA

Secondly, the pri-miRNAs are recognized and processed by the multimolecular complex to generate ~ 70-nucleotides-long precursor miRNAs (pre-miRNAs). Notably, these processes occur within the nucleus. Subsequently, the GTPase RAN-dependent protein Exportin 5 recognizes and transports the pre-miRNAs from the nuclear into the cytoplasm. Right after that, RNase III endonuclease Dicer recognizes the pre-miRNAs, and cleaves them to form a ~ 22-nucleotide miRNA/miRNA duplex. Finally, in the presence of the heat shock protein HSPA8 and HSP90, the resulting miRNA duplex is unwound and bound to Argonaute (AGO) proteins [25]. Upon loading, the AGO proteins stabilize the mature single stranded miRNA to initiate its biological functions.

Evolutionarily conserved miRNAs, due to their host genes proximity to cell-specific enhancers/super-enhancers, typically exhibit cell-, tissue-, or developmental-specific expression and thus contribute to the spatiotemporal transcriptome and proteome. Additionally, the expression of miRNAs fluctuates with human pathophysiological states, suggesting potential functional outcomes [26, 27]. For example, the expression of miR-92a-2-5p is decreased in heart tissues of T2DM rats, but DM-induced myocardial damage could be significantly alleviated by injection of miR-92a-2-5p agomiR in vivo [28]. Certain miRNAs, including miR-21-5p, miR-218-3p, and miR-132-3p, are dysregulated in patients with conditions of combined high glucose and high fat but not solely with high fat or high glucose alone [29, 30]. Significantly, the alteration of miRNA expression may indicate the etiology of disease or complications. For example, endothelial cell dysfunction, a prevalent issue in diabetic patients, constitutes the fundamental pathological alteration in diabetic cardiovascular disease. Among the endothelial cell-enriched miRNAs, miR-126-3p and miR-132 exhibit significant downregulation in the myocardial tissue of diabetic patients with the non-diabetic heart [31]. Numerous studies have demonstrated that exercise ameliorates the onset and progression of diabetic heart disease in mouse models through normalizing the cardiovascular system-enriched miR-126 [32]. In addition, it can be speculated that miRNAs expression may undergo further changes when complications occur in diabetic patients. Plasma miR-126-3p levels differ in patients with DM and CAD to those of healthy controls. The expression of miR-126-3p exhibits increased levels in DM, and the occurrence of CAD on the basis of DM will further increase its expression [33]. Although glucose homeostasis dysfunction spurs miRNAs expression chaos, the underlying mechanisms remain puzzling. Logically, anything that affects the synthetic element adjacently could influence the expression and function of miRNAs. As described above, Dicer is a key enzyme in miRNA biogenesis. Previous studies have suggested that Dicer

mRNA and protein levels are significantly decreased in platelets from diabetic patients and mice but elevated in the hearts of diabetic mice [34, 35]. In addition, variation in the organism, such as exercise, may stimulate the upregulation of Dicer and several miRNAs in adipocytes, which is thought to be an adaptive response to coordinate whole-body metabolic signals [36]. These findings will provide new insights into the regulation of miRNAs biogenesis.

Biological functions of miRNAs

In animals, miRNAs predominantly act as post-transcriptional inhibitors of gene expression. Gene silencing induced by miRNAs depends on the RNA-induced silencing complex (RISC). The macromolecular protein complex, RISC, is composed of AGO proteins loaded with the miRNA guide strand and several effector proteins required for target RNA silencing, which can ultimately mediate translational repression or decay of target transcripts [37]. The specificity of the silencing reaction is primarily determined by the base pairing between the single-stranded miRNA in the RISC and the target mRNA, most commonly within the 3′ UTR of transcripts. The degree of complementarity between the miRNA-RISC complex and the target mRNA is a major determinant distinguishing the mode of gene silencing [38]. Under most circumstances, the complementarity between the miRNA-RISC complex and the target mRNA is partial, often predominantly based on a six to eight base sequence [27]. AGO recruits the adaptor protein TNRC6, interacts with the poly(A)-binding protein (PABPC) associated with the mRNA poly(A) tail and also recruits deadenylase complexes, including PAN2–PAN3 deadenylase complex and CCR4–NOT deadenylase complex. The deadenylases catalyze the shortening of the poly(A) tail, resulting in mRNA destabilization through decapping and 5′-to-3′ exonucleolytic decay in most systems [39, 40]. In addition, the recruitment of DDX4 and 4E-T mediated by the CCR4-NOT complex can nonetheless repress translation initiation in cells through a parallel mechanism [41]. This partial base pairing causes the mRNA to be translated into protein with lower yield. For example, miR-199-5p, an elevated miRNA in T2DM patients and its complications, binds with SIRT1 mRNA through partial base pairing to reduce its translation [42, 43]. In another exceptionally rare condition of near-perfect complementarity to the miRNA, target mRNA can be cleaved and degraded to achieve expression inhibition by AGO [44]. Notably, AGO2 is the most highly expressed in mammals and retains its ancestral ability to catalyze the endonucleolytic cleavage characteristic of RNAi; thus, the miRNA directs slicing of the target transcript [40]. This mode of repression, characterized by slicing, serves as the foundation for siRNA-mediated mRNA-knockdown technologies that have revolutionized biomedical research and hold promise in clinical applications. However, only miRNA-directed slicing involving no more than 30 cellular transcripts has been reported in humans and animals [41]. The interaction between miR-196 and the target gene HOXB8 is a rare example of a high match in animals that results in the cleavage of the target mRNA [45]. Thus, the identity of targets and the magnitude of their restraint ultimately define the function of each miRNA.

Admittedly, the overwhelming majority of research rests on the dogmatism that miRNAs regulate the expression of specific target mRNAs by inhibiting mRNA translation or promoting mRNA decay by RISC. The available evidence indicates that miRNAs also exhibit unconventional regulatory functions and cellular localizations, extending beyond the established canonical paradigm. Nuclear miRNAs employ different functional mechanisms. For instance, nuclear miR-446c-3p activates the transcription of the VEGFA gene by targeting of the promoter-associated transcript on at the VEGFA promoter [46]. Microglia cell nuclear miR-137-3p binds to the transcription factor Pu.1, thereby controlling chromatin accessibility and inhibiting Pu.1-mediated gene expression [47]. Another miRNA, miR-126-5p, which is closely associated with metabolism and the cardiovascular system, exhibits an aptamer -like binding to caspase-3 in the nucleus. This interaction impedes caspase dimerization and suppresses its activity, thereby attenuating endothelial cell apoptosis and ameliorating arterial AS [48]. Furthermore, a subset of nuclear miRNAs, including miR1 and miR-126-5p, exhibit binding affinity for ion channel proteins such as potassium channel Kir2.1 and Kv2.1, thereby elucidating a novel evolutionarily conserved biophysical action of endogenous miRNAs in modulating cellular electrophysiology [49, 50]. The aforementioned studies reveal noncanonical mechanisms underlying miRNAs.

Genetic deletion of miRNAs in organisms and pathological models has shown that few physiological and pathological processes are absolutely dependent on a single miRNA [44, 51]. The human genome is estimated to encode up to thousands of miRNAs. Indeed, the significant redundancy within miRNA families is considered to serve as a buffer against deleterious variations in gene expression programs [52]. On the other side, the initiation of miRNAs function predominantly relies on the six to eight base pairs with mRNA. Given the prevalence of such complementary sequences in mRNA transcripts, each miRNA exhibits the peculiarity of post-transcriptionally regulating multiple targets. MiR-21, a star molecule which has been claimed to be a specific predictive or prognostic biomarker for at least 29 diseases, is involved in numerous pathological processes through regulating

diverse targets in humans [53]. For instance, miR-21-5p in islet β cells MIN6 regulates the expression of the downstream target Pdcd4, thus promoting insulin secretion in T2DM [29]. In addition, many key factors, such as PTEN, Cdc25a, KRIT1, and BDH2, have been proven in vivo to be targets of miR-26-5p in various disease [54–56]. The multiplicity of miRNAs, mRNAs, and protein products contributes to a distinctive regulatory network.

miRNAs in diabetic macroangiopathy

The concordance between increased miRNAs and pathological diversity suggests a possible contribution of miRNAs to the development of disease. Indeed, with the identification of miRNAs in various disease, increasing evidence indicates that evolutionarily conserved miRNAs play an important role in various physiological and pathological processes, including developmental process, virus invasion, immune function, and diseases of various organs/systems [27, 57, 58]. Due to DM doubles the risk of vascular disease, and the species and quantity of miRNAs vary dramatically in diabetic individuals, the initiation and development of diabetic macroangiopathy, such as AS, CAD, PAD and stroke, may also be regulated by miRNAs [50, 59, 60] (Table 1). Moreover, the role of miRNAs in diabetic macroangiopathy has been extensively demonstrated by the striking phenotypes associated with the ablation of critical miRNAs [61–63]. For instance, atherosclerosis (AS) is a common pathology in diabetic macroangiopathy. An appreciable quantity of miRNAs has been reported to be involved in the development of AS, including immune cells activation, endothelial cells dysfunction, and foam cells formation. Although the interpretation of many miRNAs in diabetic macroangiopathy remains incomplete, the identification and summary of novel miRNAs are valuable to further understand the pathogenesis of diabetic macroangiopathy and develop novel targeted treatments.

miRNAs in diabetes induced AS

The pathology of complications in diabetes exhibits a significant degree of vascular-level commonality, with AS representing one of its primary clinical manifestations [64] (Fig. 3). Many dysregulated miRNAs, such as miR-503-5p, let-7d-5p, miR-106b-3p, miR-93-5p, and miR-10a-5p, are involved in the formation of arterial plaque phenotype in DM [56]. Therefore, we will focus on precise vascular cell fate to elucidate the roles of miRNAs in characteristic pathological alterations of diabetic AS (Fig. 4).

Endothelial cells

Endothelial cells, a highly dynamic monolayer of cells that lines the vascular network, play a crucial role in the development and maintenance of the functional circulatory system. This includes the initiation and regulation of angiogenesis, control of vascular tone, and provision of paracrine support [65]. Due to their anatomical location, endothelial cells are continuously exposed to flowing blood containing many pernicious substances, especially in anomaly condition. Hyperglycemia is a fundamental pathological manifestation of DM, which can induce a series of detrimental effects on endothelial cells. Endothelial cell dysfunction is an inchoate pathogenetic step in hyperglycemia-induced macrovascular disease. Under hyperglycemic conditions, numerous studies have revealed the role of miRNAs on endothelial cells, mainly concerning cell function and survival [66, 67].

Various regulators and signaling molecules, such as fibroblast growth factor (FGF), signal transducer and activator of transcription 3 (STAT3), vascular endothelial growth factor (VEGF) and protein kinase B (AKT), are associated with endothelial cells function [68–70]. MiR-210-3p plays a protective role in cardiovascular homeostasis and is decreased in whole blood of T2DM mice and patients. Overexpression of miR-210-3p restores endothelial cells function in hyperglycemia, whereas inhibition of miR-210-3p in the same cells impaired this improved effectiveness. Mechanically, reduced miR-210-3p promotes the expression of PTP1B in T2DM. PTP1B, a target of miR-210-3p, is an enzyme that regulates insulin signaling through the IR/AKT/GSK3β pathway, promoting peripheral insulin resistance and endothelial dysfunction by accumulating oxidant in DM [69, 70]. In mice, miR-210-3p mimics can attenuate endothelial dysfunction through downregulating vascular PTP1B and oxidative stress in vivo [71]. Additionally, decreased miR-210-3p promotes the expression of fibroblast growth factor receptor-like 1 (FGFRL1), a member of the FGF family that exerts a biological function through the FGF signaling pathway. Elevated FGFRL1 significantly attenuates the phosphorylation of AKT, ERK and STAT3 in human vein endothelial cells, which are downstream FGFs, impairing endothelial cells proliferation and migration [68].

'Metabolic memory', the long-lasting damaging effects of persistent hyperglycemia in DM, refers to adverse effects on micro- and macrovascular complications that are not reversed by improved blood glucose control [72]. Indeed, the process of 'metabolic memory' is associated with miRNAs. For example, miR-27a-3p always remains upregulated under both persistent and past hyperglycemia, impairing endothelial cell function [73]. In diabetic mice, miR-27a-3p reduces the expression of nuclear factor erythroid-2 related factor 2 (NRF2), a transcription factor that regulates antioxidant genes and cytoprotective phase II detoxifying enzymes. NRF2 improves cardiovascular pathological remodeling and dysfunction by suppressing oxidative stress genes expression. MiR-27a-3p

Table 1 Relevant examples of miRNAs in diabetic macroangiopathy

Cells	Role in diabetic AS	miRNAs	Functions	Targets	Locations	References (PMID)
ADSCs	Aggravated	miR-144-3p	Reversing the protective effects of circ-Snhg11 on ECs	HIF-1α, NFE2L2	–	36076572 36503078
AT-MSCs	Improved	miR-126	Downregulating SPRED1 and PIK3R2 and increasing secretion of angiogenic cytokines	SPRED, PIK3R2	PAD	35151721
BMDMs	Improved	miR-181a-5p	Inhibiting BMDMs inflammation and attenuating atherosclerosis	STING/NFκB	Aorta	36108984
	Aggravated	miR-486-5p	Inducing cell proliferation and reprogramming energy metabolism	ABCA1	–	34381972
ECs	Aggravated	miR-200 miR-466	Regulating ECs permeability	CLDN5	Aorta	35892090
		miR-206-3p	Inhibiting atheroprotective functions of CXCR4	CXCR4	Aorta	37910599
		miR-351-5p	Regulating apoptosis, lipid accumulation, and oxidative stress	ITGB3	–	36180828
		miR-27a-3p	Regulating cardiac perivascular fibrosis and restoring cardiovascular function	NRF2/ROS/TGF-β/EndMT	CAD	33483741
		miR-29a	Regulating perfusion recovery, skeletal muscle injury, muscle function, and cleaved Tie 2 and AKT phosphorylation	ADAM12	PAD	35008854
		miR-204-5p	Participating in adhesion of monocytes to ECs, and inflammatory molecules secreted in the medium	SIRT1	–	35508613
	Improved	miR-210-3p	Protecting cardiovascular homeostasis	PTP1B	Carotid artery	34753800
		miR-146a-5p	Mitigating ECs senescence and stimulate angiogenesis	Src	–	34675187
		miR-130b-3p	Promoting angiogenesis, migration, and proliferation	INHBA	PAD	37097749
		miR-375-3p	Improving new vessel growth and arteriogenesis in muscle tissues	KLF5	PAD	36074222
		miR-126-3p	Regulating angiogenesis and the homing and retention of EPCs in injured vascular walls	–	Carotid artery	36875160
		miR-425-5p	Enhancing the survival of ECs exposed to ischemia-mimic conditions	DACH1, PTEN, RGS5, VASH1	–	35474734
		miR-24 miR-126	Biomarkers	–	–	33680027
HSCs	Improved	miR-181a-5p miR-181b-5p	Improving revascularization in limbs, attenuating skeletal muscles ischemia	Plac8/CXCR4	PAD	36821386
HUVECs	Aggravated	miR-425-5p	Inducing lactate accumulation and apoptosis in HUVECs	MCT4	–	31711985
		miR-429-3p	Abolishing antioxidative stress and anti-ferroptosis effects of HSYA	SLC7A11	–	37410531
	Improved	miR-29b	Aortic root fibrotic cap thinning	LYPLA1	Aorta	35965413
		miR-29a	Triggering the mitochondrial apoptotic pathway	Bax	–	30520097
		miR-147a	Relieving HG-induced endothelial cell injury	MyD88/TRAF6/NF-κB	–	34369659
		miR-210-3p	Intensifying the tube formation, proliferation, and migration of HUVECs	FGFRL1	–	37062273
		miR-181b	Improving endothelium-dependent vasodilation in aortas but suppressing superoxide overproduction and vascular inflammation markers	–	Aorta, Renal arteries	35740034

Table 1 (continued)

Cells	Role in diabetic AS	miRNAs	Functions	Targets	Locations	References (PMID)
Macrophages	Aggravated	miR-375-3p	Reducing resolving M2 macrophage but increasing pro-inflammatory M1 macrophage markers expression	KLF4	Aorta	33545131
	Improved	miR-33-5p miR-378a-3p miR-146-5p miR-99a-5p miR-21-5p	Reducing hematopoiesis and myelopoiesis, and favorably reprogramming inflammatory signaling and metabolism	PPARγ-GLUT4, UCP1	– – – – –	35292359
		miR-145	Repressing cell proliferation and inducing apoptosis	OPG, KLF5	CAD	30941422
		miR-181a-5p miR-181b-5p	Impairing revascularization in limbs, reducing accumulation in ischemic skeletal muscles	Plac8/CXCR4	PAD	36821386
		miR-425-5p	Stimulating adiponectin expression and resolving inflammation	–	–	38379282
VECs	Aggravated	miR-335-3p miR-495-3p miR-548o-3p	Improving glucose tolerance	IRS1	–	33297755
	Improved	miR-21-3p	Promoting the cleaved form of sRAGE and inhibiting RAGE/NADPH oxidase signaling	ADAM10, RAGE/NADPH	–	32196704
VSMCs	Aggravated	miR-29c	Promoting the phenotype transformation of VSMCs	–	Carotid artery	38374991
		miR-126	Conferring "diabetic" phenotype to non-T2DM VSMCs	MEK/ERK	–	34298200
		miR-217	Inhibiting excessive proliferation and migration of VSMCs	ROCK1	Aorta	33283391
		miR-325-5p	Hindering the cholesterol efflux and enhancing HA-VSMC viability	SREBF1, KDM1A, PPARγ-LXR-ABCA1	–	33811891
		miR-504	Promoting VSMCs dysfunction	Grb10, Egr2	Aorta	26941017
		miR-221-3p miR-222-3p	Participating in the monocyte adhesion on endothelial cells, M1 polarization of macrophages, and vascular inflammation and atherosclerotic plaque development	ICAM-1, $p27^{Kip1}$/ RhoA	Aorta Aorta	37179303
	Improved	miR-223-3p	Regulating VSMCs phenotypic switching	PDGFRβ	–	30645204
		let-7c/g-5p	Inhibiting the proliferation and migration of VSMCs	Lin28a	LEAD	37433785
		miR-143-3p	Reducing the migration and proliferation of VSMCs	Ras	Aorta	35696095
		miR-125a-5p	Suppressing HG-induced excessive activation of the mevalonate signaling pathway in VSMCs	HMGCR	Aorta	32319638

induced NRF2 reduction attenuates the suppression of oxidative stress genes and induces reactive oxygen species (ROS) accumulation in endothelial cells [73]. Additionally, decreased NRF2 mediates endothelial-to-mesenchymal transition and perivascular fibrosis through the TGF-β signaling pathway during mice hepatic fibrosis in vivo [74]. Notably, hyperglycemic stimulation is memorized by endothelial cells and is not erased when transitioning to a hypoglycemia state, causing sustained cardiovascular system. MiR-29b-3p is downregulated in hyperglycemic patients and is negatively modulated by miR-27-3p, though the mechanism is unclear [73]. Another study suggests that miR-29b-3p inhibits its direct target LYPLA1, which would otherwise decrease nitric oxide (NO) production [75]. In contrast to ROS, NO improves vasodilation and endothelial cells function [76]. Additionally, miR-29b-3p serves as a master inhibitor of at least 16 extracellular matrix gene expression and may attenuate tissue fibrosis [77]. Liang et al. developed chitosan-packaged and chemically modified miR-29b-3p mimics, which could significantly improve the cardiovascular complication in diabetic mice, including AS, hypertension, and cardiac fibrosis [75]. However, Zhang et al. reported that pancreatic β cell-released exosomal

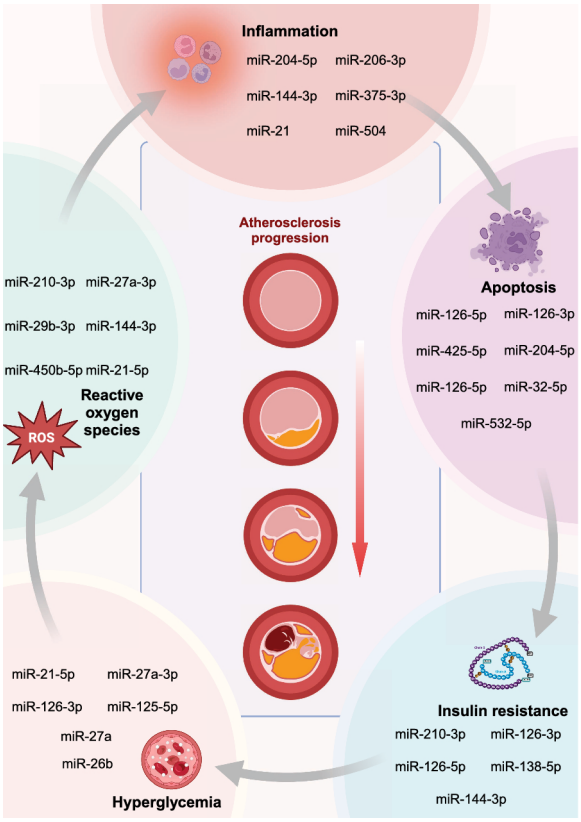

Fig. 3 The interconnections and commonalities among diverse pathological aspects of diabetic AS. Through the mediation of miRNAs, diverse pathological manifestations synergistically contribute to the progression of diabetic AS

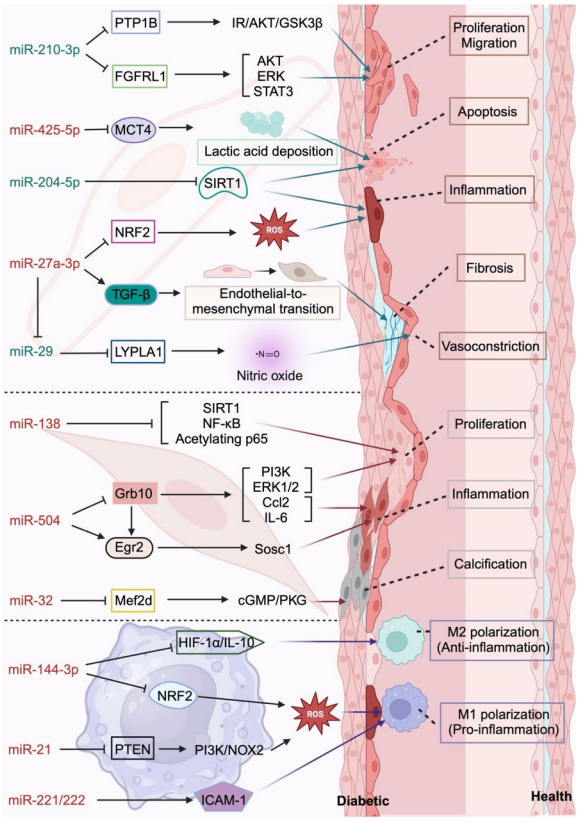

Fig. 4 Pathology and molecular mechanisms of miRNAs in diabetic AS. Diabetes mellitus is a well-established independent risk factor for AS, and involved with the development of AS. In endothelial cells, vascular smooth muscle cells and macrophages, hyperglycemia induced miRNAs chaos are closely related to oxidative stress, inflammation, abnormal metabolism and apoptosis. These aberrant cellular activities facilitate the pathological progress of AS

miR-29 family negatively regulates hepatic insulin sensitivity and glucose homeostasis [78]. The miR-29 family in human includes miR-29a, hsa-miR-29b and miR-29c. In vivo, decreased miR-29 expression in β cells improved HFD-induced insulin resistance. Moreover, isolated miR-29 family enriched exosomes inhibits insulin signal transduction in the liver and increase hepatic glucose production [78]. The dual properties of miR-29 family, which both aggravate metabolism dysfunction and improve diabetic complications, appear somewhat contradictory. The role of miR-29 family remains to be further explored.

Endothelial progenitor cells (EPCs) are precursors to endothelial cells and possess endothelial specification and vascular tropism. The destruction of EPCs leads to endothelial cells dysfunction and subsequent vascular abnormalities, which may be regulated by miRNAs. For example, miR-126-3p modulates the expression of proangiogenic cytokines, such as Spred-1, Syndecan-1, VCAM1 and PIK3R2, thereby regulating angiogenesis and the homing and retention of EPCs in injured vascular walls of T2DM patients [79]. However, EPCs derived from DM patients contain down-regulated miR-126-3p and exhibit impaired function. In vivo, transfection of miR-126-3p increases migration and promotes homing and stemness maintenance in EPCs, thus repairing vascular injury in diabetic mice [80]. CXCR4, a chemokine receptor, is crucial for vascular integrity through regulating vascular cell viability, proliferation, and migration. In atherosclerotic mice, miR-206-3p decreases CXCR4 expression in endothelial cell and VSMCs in blood vessel walls, inducing vascular permeability and monocyte adhesion to endothelium, and promoting the development of diet-induced AS [81]. Indeed, miRNAs are also involved in EPCs function by mediating CXCR4 to regulate AS progression. Recent evidence suggests that both strands of miR-126, including miR-126-3p and miR-126-5p, modulate target mRNA and subsequently regulate atheroprotective function [82]. In EPCs of DM patients, decreased miR-126 inhibits the expression of p-ERK、VEGF、p-AKT and eNOS, though the underlying mechanisms remain unclear. These dysregulated factors downregulate CXCR4, inducing EPCs dysfunction, especially migration impairment. Additionally, KLF-8, a

target of miR-126, negatively regulates EPCs stemness. KLF-8 inhibits stemness gene expression in miR-126-depleted EPCs, promoting intimal hyperplasia of injured arteries [83]. Interestingly, miR-126 is observed in endothelial microparticles released from apoptotic endothelial cells. MiR-126 is transported into recipient human coronary artery endothelial cells by endothelial microparticles, functionally regulating SPRED1, CXCL12 and CXCR4 expression [84, 85]. In response to tissue damage such as AS, the CXC chemokine CXCL12 and its receptor CXCR4 counteract apoptosis, and recruit EPCs and confer plaque stability on atherosclerotic mice.

Moreover, several miRNAs modulate endothelial cells survival in diabetic AS progression through regulating metabolism and autophagy. Unlike other canonical cells, endothelial cells mainly rely on glycolytic energy metabolism [86]. The final metabolite, lactic acid, is transported out of endothelial cells by the translocator MCT4 [87]. MCT4 is specifically targeted by miR-425-5p, which is significantly upregulated in endothelial cells treated with high glucose. Decreased MCT4, induced by increased miR-425-5p in DM patients, leads to lactic acid accumulation [88]. Excess lactic acid promotes apoptosis of endothelial cells and worsens AS. Additionally, other studies suggest that topical administration of miR-425-5p-modulated small extracellular vesicles significantly enhances epidermal wound healing in diabetic mice, which may be explained by tissue-specific character of miRNAs [89, 90]. SIRT1 is an important gene related to autophagy and apoptosis [91]. MiR-204-5p is downregulated by acting as competing endogenous RNA of lncRNA LYPLAL1-DT in T2DM patients. Decreased miR-204-5p promotes the expression of SIRT1, an anti-autophagy factor that can reduce cell death, ameliorating endothelial cells inflammation and effectively improving vascular endothelial injury in DM-associated complications [92]. Simultaneously, autophagy serves as a form of self-degradation that not only functions as a downstream mechanism of miRNA but also governs its functionality [93]. Autophagy regulates miRNA biogenesis by degrading Dicer, thus controlling subsequent downstream events [94]. The atheroprotective factor miR-126-5p is enriched in the nucleus during autophagy, which attenuates endothelial cells apoptosis and then sustains endothelial integrity [48].

Vascular smooth muscle cells

Vascular smooth muscle cells (VSMCs) are integral to all phases of atherosclerotic plaque formation. In accordance with the "damage response" and "vulnerable plaque" hypotheses, adaptable VSMCs undergo phenotypic conversion to achieve an optimal state, such as transitioning into proliferative or inflammatory cells, thereby actively participating in plaque synthesis and stabilization [95]. These transformation processes can be expedited under specific pathological conditions, such as DM [96]. Although the phenotypic plasticity of VSMCs in DM-induced AS is well-established, the precise fate of VSMCs and their phenotypic states in this chronic metabolic disease remain unknown [97]. Numerous dysregulated miRNAs induced by DM regulate the phenotypic conversion of VSMCs and mediate atherosclerotic plaque formation.

The proliferation of VSMCs significantly contributes to intimal thickening, which represents the initial step in atherosclerotic plaque formation and is regulated by key molecules and pathways, including AKT, SIRT1, PI3K and ERK signaling pathway [50, 98, 99]. Vessels affected by T2DM exhibit increased remodeling due to enhanced migration and proliferation of VSMCs [50]. The expression of miR-126 is predominantly observed in endothelial cells and its transfer from endothelial microparticles to VSMCs occurs, particularly in the context of vascular injury [100]. However, overexpression of miR-126-5p increases proliferation and migration rates in non-T2DM VSMCs by inhibiting the expression of two known targets of miR-126-5p: PI3KR2 and AKT. Consequently, miR-126-5p may participate in the 'metabolic memory' of VSMCs through regulation of proliferation-related signaling pathway, potentially imparting the "diabetic" phenotype to non-T2DM VSMCs [50]. Similarly, miR-504 elevated in VSMCs exposed to hyperglycemia [98]. The expression of miR-504 is further augmented in the presence of the coexistence of hyperglycemia and hyperlipidemia. Grb10, a member of the adapter proteins family, is a direct target of miR-504. Increased miR-504 inhibits Grb10 expression and activates PI3K and ERK1/2 pathways, thereby promoting VSMCs proliferation and phenotypic transition. Moreover, Grb10 knockdown leads to increased expression of inflammatory genes Ccl2 and IL-6, resulting in a proatherogenic phenotype of VSMCs. Additionally, decreased Egr2, another target of miR-504, upregulates the anti-inflammatory Socs1 in diabetic mice while inducing a pro-inflammatory VSMC phenotype. Grb10 can also positively regulate Egr2 expression through mechanisms that are not fully understood [98]. Elevated levels of miR-221/222 in VSMCs of diabetic patients exacerbate vascular inflammation and atherosclerotic plaque formation [101]. Besides mitigating endothelial cell inflammation, previous research indicates that SIRT1 can also inhibit calcification in VSMCs of mice and reduce neointima formation following injury by decreasing cell proliferation and migration [102]. Diabetic mice exhibit a thick aortic smooth muscle layer compared to DM wild type mice. Intriguingly, this thickening of aortic smooth muscle layer increases with age, and the expression of SIRT1 in VSMCs is decreased in a time-dependent manner. SIRT1 has been identified as a

target of miR-138 in db/db mice, which promotes VSMCs proliferation and migration through inhibiting SIRT1 expression and acetylating p65 and NF-κB, thereby deteriorating DM-induced AS [99]. Moreover, miR-138-5p, a strand of the miR-138, inhibits the synthesis and secretion of insulin during obesity in mice by upregulating islet-specific genes Ins2, NeuroD1, and Creb1 [103]. These results demonstrate that miR-138 act as a negative role in DM and its complications. In advanced lesions, the proliferative phenotype of VSMCs are generally considered to have atheroprotective plaque–stabilizing properties [104]. For example, platelet-derived miR-223 plays a dual role in vascular injury repair through modulating VSMCs proliferation, initiating an immediate repair process and, concurrently, a delayed process to prevent excessive repair [105]. Beyond proliferation regulation, a reduced number of VSMCs can also impact vascular functions, such as autophagy. The transcription factor Mef2d is a key target gene of miR-32-5p that inhibits VSMCs autophagy [106]. Under conditions of hyperglycemia, macrophages may promote the expression of miR-32-5p in VSMCs via exosome pathway. Increased miR-32-5p inhibits Mef2d expression and reinforces the autophagy inhibition of VSMCs, thereby promoting VSMCs calcification by cGMP/PKG pathway [106].

Macrophages

Atherosclerosis (AS) is characterized by progressive inflammation of the arterial vessel wall, marked by extensive macrophage infiltration, which can be either beneficial or detrimental. As AS progresses, macrophages typically adopt a pro-inflammatory phenotype, influencing plaque formation stability [107]. However, macrophage dysfunction induced by certain pathological conditions accelerates the occurrence of AS and cardiovascular system diseases, such as carbohydrate metabolism disturbance [108]. Increasing evidence suggests that miRNAs may mediate interactions between macrophages and diabetic AS [101].

In the setting of hyperglycemia, miRNAs regulate macrophage polarization and AS progression through several vital molecules, such as PI3K, HIF and ICAM-1 [109, 110]. Hyperglycemia induces miR-21 expression and macrophage M1 polarization, though the relationship between miR-21 and M1 polarization of macrophage remains uncharted [109]. Recent studies have identified PTEN as a direct target of miR-21 in vivo murine skin wound model [111]. MiR-21 downregulates PTEN, leading to activation of the PI3K/NOX2 pathway. NOX2, in turn, promotes inflammation progression by enhancing ROS production. Therefore, miR-21 facilitates M1 polarization of macrophages by the PI3K/NOX2/ROS signaling cascade, contributing to sustained inflammation in vessels and wounds [109]. Zinc-induced glucose dysmetabolism and T2DM may lead to more serious complications. This anomaly-induced T2DM results in an elevation of plasma miR-144-3p level. Prior research has confirmed that zinc-stimulated miR-144-3p suppresses the target gene NRF2, a crucial redox regulator that enhances antioxidant enzymes activity and protects cells from oxidative damage. Therefore, miR-144-3p-mediated NRF2 inhibition exacerbates insulin resistance in T2DM [112]. Additionally, miR-144-3p is involved in diabetic complications. HIF-1α, another target of miR-144-3p, plays a role in macrophage M2 polarization through the HIF-1α/IL-10 pathway [113]. MiR-144-3p can inhibit macrophage M2 polarization, a regenerative or pro-remodeling macrophage phenotype, and promote endothelial cells dysfunction through sponging HIF-1α [114]. Fortunately, vascular damage caused by miR-144-3p can be alleviated by delivery of circ-Snhg11, an inhibitor of miR-144-3p [110]. Several anti-DM medications, including metformin, regulate miRNAs to relieve cardiovascular complications associated with DM. Metformin reduces the expression levels of miR-221 and miR-222, thereby helping to mitigate cardiovascular complications in diabetic patients by delaying intimal thickening [115]. VSMCs-derived exosomal miR-221/222 promotes endothelium activation, induces monocyte adhesion and increases the expression of endothelial cell adhesion molecules, such as ICAM-1, which contrasts with previous reports showing miR-221/222 target ICAM-1 mRNA and reduce its expression [101, 116]. Consequently, miR-221/222 drives the pro-inflammatory polarization of monocytes into M1 macrophages and contributes to atherosclerotic plaque development.

On the other hand, macrophage-derived foam cells play a vital role during the pathogenesis of AS. Macrophages uptake oxidized low-density lipoprotein (ox-LDL) through scavenger receptors to form foam cells, which is widely recognized as a hallmark of atherosclerotic lesions [117]. The phagocytosis of ox-LDL by macrophages is mediated by several vital molecules. For example, intermedin (IMD), a calcitonin peptide, inhibits macrophage phagocytosis by promoting lipoprotein efflux, thereby preventing foam cells formation [118]. Moreover, IMD acts as a vasodilator directly in the cardiovascular system. However, the mechanisms that mediates those favourable roles of IMD remains unclear. Recent research by Hu et al. revealed that IMD inhibits Dnm3os, leading to increased miR-27b-3p levels. This miRNA improves AS by suppressing the expression of scavenger receptors involved in lipid uptake by macrophages, thereby alleviating AS in diabetic patients [119].

miRNAs in AS associated diabetic macroangiopathy

AS is the fundamental mechanism responsible for the initiation and progression of macrovascular complications.

DM accelerates all atherosclerotic lesions by intensifying sundry pathological processes, leading to increased plaque formation. When atheromatous plaque suffocates the major vessels, these pathological changes in diabetic patients might result in CAD, PAD, and stroke.

CAD

Cardiovascular disease, especially CAD, is considered as one of the leading causes of morbidity and mortality in DM patients. Different degree of coronary artery obstruction is the main cause of the imbalance of blood supply and oxygen demand of cardiomyocytes. Therefore, CAD always induced by AS [120]. Hyperglycemia continuously damages the cardiovascular system, leading to diabetic cardiovascular complications, including AS. As described above, the DM-induced dysregulation of miRNAs modulates vascular cells function and AS progression, thereby regulating the occurrence and development of CAD. Indeed, altered expression of miRNAs has been identified in individual with CAD. MiRNAs also appear to serve as biomarkers to predict cardiovascular complications of DM (Fig. 5).

To investigate the expression profile of serum-derived miRNAs, a clinical study collected sixteen serum samples, including eight patients with CAD and hyperglycemia and eight patients with CAD and normoglycemia. The results of miRNA microarray analysis suggest that let-7b-5p is up-regulated in patients with CAD and hyperglycemia, indicating that Let-7b-5p may serve as a noninvasive biomarker to distinguish the severity of coronary stenosis [121]. The NOX family is a vital source of ROS and plays a significant role in modulating redox signaling, particularly in response to hyperglycemia and DM. Ewida et al. suggest that miRNA-342 and NOX-4 are upregulated in T2DM and CAD group, with the highest expression found in those suffering from T2DM with

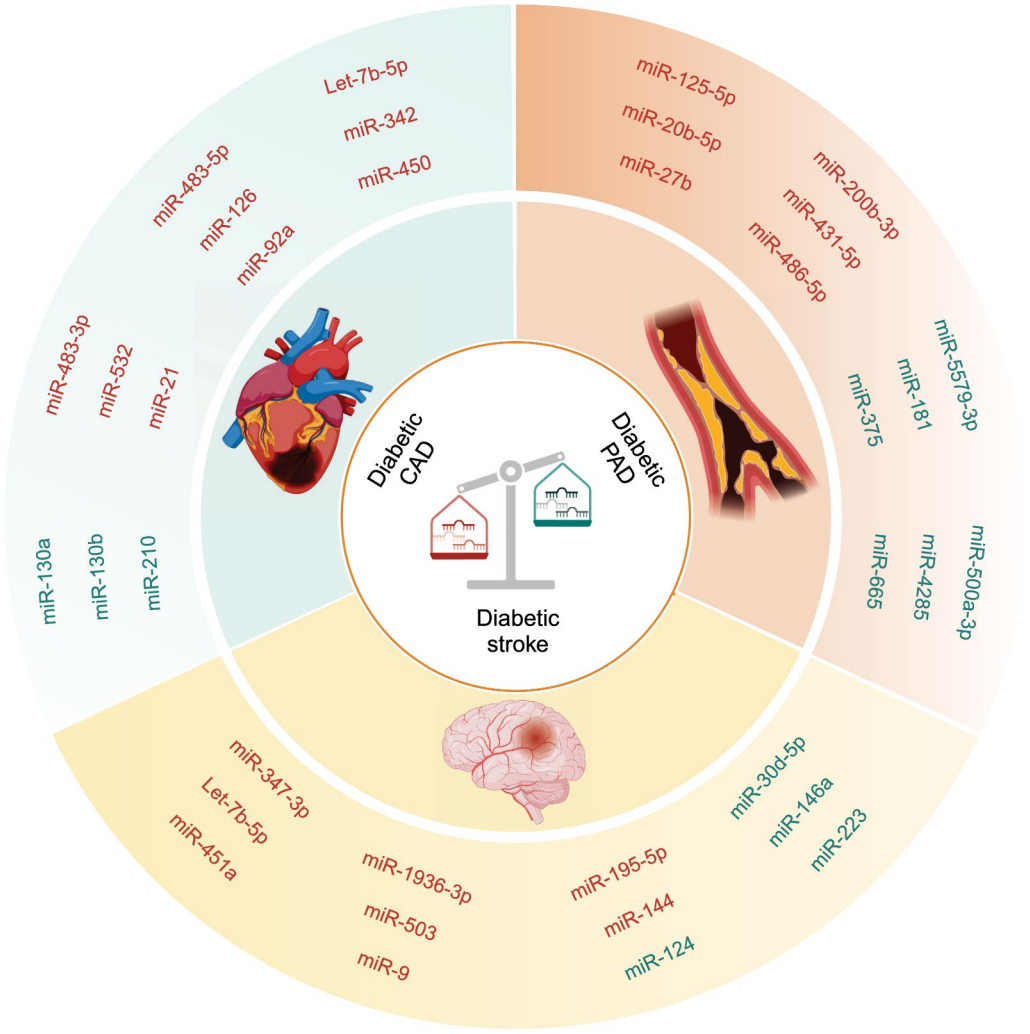

Fig. 5 Dysregulated miRNAs in diabetic macroangiopathy. Many miRNAs disorders in diabetic macroangiopathy which including CAD, PAD and stroke. These miRNAs may serve as potential biomarkers in diabetic macroangiopathy. (The red font indicates up-regulated miRNAs, and the green font indicates down-regulated miRNAs)

CAD group. These results indicate that profiling miRNA-342 and NOX-4 expression may help identify individuals at high risk for developing CAD among those with T2DM [122]. Additionally, numerous experiments have identified several miRNAs related to cardiometabolic risk factor profile, including miR-450-5p, miR-92a and miR-483-5p [123, 124].

In addition to vascular events, miRNAs are also involved in the regulation of cardiac pathological changes in DM. The expression of miR-532-3p is significantly increased in the right atrial appendage of patients with T2DM after coronary artery bypass surgery [125]. Meanwhile, elevated level of miR-532-3p has also been identified in diabetic mice at an advanced stage of the disease. High glucose-induced dysregulated miR-532-3p promotes cardiomyocyte apoptosis, which can be attenuated through miR-532-3p inhibitors in vitro. These results suggest miR-532-3p plays a critical role in accelerating diabetic cardiomyocytes apoptosis and may act as a potential therapeutic target to overcome the DM-induced apoptotic cardiomyocytes death [125]. Diabetic individuals are also at higher risk for CAD and metabolic disorders, such as hyperglycemia and dyslipidemia, which can lead to endothelial dysfunction by reducing NO bioavailability and increasing ROS levels [126]. Interestingly, the flow-mediated vasodilation (FMD) medium in patients with CAD switches from NO-dependent to hydrogen peroxide (H_2O_2)-dependent. MiRNAs could act as regulators of the NO-to-H_2O_2 switch, and subsequently regulate vascular function during hyperglycemia. Yin et al. found that miR-21-5p is abundantly expressed in endothelial cells of diabetic mice, where it governs NO production and ROS-homeostasis by suppressing SOD2 [127]. Above all, these findings indicate that miRNAs modulate CAD progression through regulating the macrovascular cells function in DM.

PAD

PAD is generally considered a group of diseases affecting one or more peripheral arteries, leading to narrowing or blockage of the large arteries due to AS, except for coronary arteries, aortic arch, and brain arteries [128]. DM is an independent risk factor for PAD, and the risk further increases with age [129]. Moreover, patients with DM complicated by PAD display the highest risk of critical limb ischemia (CLI) and amputation, yet the underlying mechanisms remain incompletely understood [130]. Latest studies suggest that miRNAs may be involved in the development of PAD in diabetic patients [130, 131].

The miR-181 family members, mainly miR-181a2b2, are downregulated in experimental PAD in diabetic mice and diabetic patients with a high risk of PAD. Additionally, miR-181 deficient mice suffer from impaired hind limb perfusion in hyperglycemia due to the abrogation of circulating Ly6Chi monocytes, resulting in reduced accumulation in ischemic skeletal muscles [130]. Furthermore, miR-181 deletion promotes macrophages M2 polarization, which fails to produce proangiogenic cytokines under diabetic conditions. Mechanically, overexpression miR-181 prevents its target Plac8 expression, and up-regulates CXCR4, leading to increased downstream myeloid and lymphoid cells. Moreover, CXCR4 sustains macrophage polarization to promote angiogenesis, potentially through the activation of the CXCL12-CCR4 axis that promotes recruitment of myelin-reactive T cells to the bone marrow, thereby maintaining hematopoietic system homeostasis [132, 133]. As with miR-181, miR-375-3p is significantly downregulated in diabetic patients and mice during progression to CLI. Moreover, overexpression of miR-375-3p is pro-angiogenic in endothelial cells through inducing endothelial migration, proliferation, sprouting and vascular network formation. KLF5 is identified as a critical potential target of miR-375-3p and associated with angiogenesis and skeletal muscle repair [131]. Under condition of hyperglycemia, decreased miR-375-3p forfeits the pro-angiogenic and anti-inflammatory function in endothelial cells, which may be mediated by KLF5/phospho-p65/NF-kB pathway [134]. Adipose-derived mesenchymal stem cells (ADSCs) are a promising treatment option for lower limb ischemia that may serve as mediators for delivering miRNAs. Li et al. suggest that exosomes from adipose-derived mesenchymal stem cells (ADSC-Exos) accelerates diabetic hindlimb ischemia repair [131]. MiR-125b-5p is the most abundant miRNA in exosomes from ADSC-Exos and could be delivered into C2C12 cells, a kind of muscle cells. MiR-125-5p promotes proliferation and migration of C2C12 cells through suppressing the expression of its target ACER2 and elevating AMPK expression [135]. Although ACER2 is derived from mice with diabetic hindlimb ischemia through bioinformation analysis, the function of the miR-125-5p/ACER2 axis has not been verified under hyperglycemia. In another study, Feinberg et al. have identified miR-130b in diabetic patients with PAD and diabetic mice with limb ischemia. Local delivery of miR-130b mimics promotes proliferation, migration, and sprouting in endothelial cells in vivo and in vitro. Furthermore, miR-130b facilitates revascularization by enhancing angiogenesis and significantly improving limb necrosis and amputation rate, possibly mediated by the miR-130b/INHBA signaling axis [136]. While the above studies have demonstrated the roles of several miRNAs in diabetic PAD, the further potential function of miRNAs therapy on other aspects of PAD are yet to be explored.

Stroke

The pathogenesis of stroke is complex, involving intricate consequences that ultimately lead to mortality [137]. DM facilitates the development of cerebrovascular disease and stroke, with coronary AS contributing significantly. Obviously, sustained hyperglycemia significantly accelerates the atherogenic and stroke process. It is established that DM is associated with poor prognosis after acute stroke, and the various biological factors that mediate inferior recovery profiles in diabetic patients remain unknown [138]. Although the mechanisms are not fully understood, numerous miRNAs have been identified in DM-induced stroke, and scientists suggests that miRNAs may serve as potential diabetic stroke biomarkers [139].

Compared with stroke patients, DM patients and controls, the expression of miR-124 is significantly decreased in the coexisting group of stroke and DM. Additionally, miR-124 shows a significant negative association with inflammatory markers CRP and TNF-α. Zidan et al. suggest that miR-124 could be used as a diagnostic marker of stroke in diabetic patients [140]. MiR-503 is significantly upregulated in hyperglycemia and ischemia but does not remain at high levels after 3 months. Although higher miRNAs are associated with more disability in the acute phase, they do not affect long-term outcomes in ischemic patients. Therefore, miR-503 may serve as a potential diagnostic marker and novel therapeutic agent for diabetic ischemic stroke [141]. Hyperglycemia after acute stroke induced Fas and let-7b-5p accumulation in plasma exosomes is demonstrated to be a favourable prognostic biomarker for predicting poor neurological outcomes in ischemic stroke patients [142].

In addition, several miRNAs may be involved in the pathogenesis or prognosis of stroke in DM. Chen et al. extracted a kind of exosomes from bone marrow stromal cells of T2DM rats. These exosomes, through decreasing miR-9 expression and increasing its target ABCA1, may improve brain white matter remodeling and inflammatory responses in diabetic stroke [143]. Due to the intricate pathological mechanisms and anatomical structures involved, an unequivocal understanding of stroke in the context of DM remains elusive. Increasing awareness and involvement of neurologists in the management of DM and other related vascular complications is imperative for improving patient outcomes.

miRNAs in therapeutic manipulation of diabetes and angiocardiopathy

The intricate pathogenesis of diabetic macroangiopathy has led to a dearth of fully elucidated specific mechanisms and effective molecular intervention targets and tools. As a result, the current management of diabetic macroangiopathy primarily relies on comprehensive symptomatic therapy. MiRNAs function as potent regulators, exemplified by their ability to orchestrate entire cellular pathways through interactions with a diverse array of target genes. This peculiarity renders miRNAs as highly intriguing therapeutic manipulation to restore cell function that are altered as part of a disease phenotype.

A compound inhibiting miRNA-92a in clinical development aims to decrease the functional levels of miRNA-92a to promote the growth of new blood vessels. The beneficial effects of miR-92a silencing in myocardial damage and for the promotion of angiogenesis have been described above. The safety and tolerability of antimiR-92 (S 95010) administration via intravenous injection to 49 healthy volunteers has been assessed in a phase I trial (NCT03494712) [144]. Additionally, a phase I trial investigating the intradermal injection of miR-92 antimiR for wound healing and incision complications (NCT03603431) is conducted simultaneously. The synthetic oligonucleotide CDR132L, which acts as an inhibitor of miR-132-3p and is recognized as a crucial regulator in cardiac remodeling processes, has recently entered clinical phase II trials (NCT04045405, NCT05350969) to evaluate its safety and efficacy in patients with ischemic heart failure [145].

In addition, tremendous progress has also been made in the field of miRNAs-based drugs for DM. The most intriguing clinical trials in the field of diabetes involve RG-125/AZD4076 (NCT02612662, NCT02826525) conducted by Regulus Therapeutics/Astra Zeneca, utilizing an inhibitor of miR-103/107 conjugate for the treatment of non-alcoholic steatohepatitis in patients with T2DM. The expression of miR-103/107 is upregulated in obese mice. Repression of miR-103/107 results in enhanced glucose homeostasis and insulin sensitivity [146]. However, the experiment indicates that anti-miR-103/107 leads to a reduction in cardiac function, cardiomyocyte size, and mitochondrial oxidative capacity in mice [147]. Further clinical trials are warranted to substantiate the clinical safety of RG-125/AZD4076. Compared with common antidiabetic and prokinetic medications, the synthetic miR-10b-5p mimic have been proven to be more effective in improving glucose homeostasis and gastrointestinal motility [148]. Nexturn Bioscience is currently in the preclinical stage of developing RSVI-301, an agonist targeting miR-10b-5p. If the results of RSVI-301 are positive and meet the requirements, it may proceed to the subsequent stage of clinical trials, wherein its efficacy and safety will be assessed on a larger scale and in real-world settings.

Despite the development of miRNAs therapeutics with significant strides, there remain numerous pressing challenges that necessitate immediate attention. For example, off-target effects result in suppressing genes other than the desired target due to sequence similarities or overdosing along with undesired immune responses.

Additionally, safeguarding the target cells during delivery and ensuring efficient uptake are also fundamental considerations. Nevertheless, irrespective of the circumstances, the promising outcomes attained thus far offer immense potential for establishing miRNAs-based therapeutic approaches in diverse application domains encompassing disease such as diabetic macroangiopathy.

Conclusion and perspectives

MiRNAs play a crucial role in regulating and maintaining metabolic homeostasis and are intricately involved in the development of DM and its cardiovascular complications, including diabetic macroangiopathy. This review delineates the biosynthesis and canonical functions of miRNAs, elucidates their adaptive changes in DM, and exhaustively describes their role in diabetic macroangiopathy, particularly in DM-related CAD, PAD and stroke. Specifically, we exclusively focus on the cytopathological changes induced by miRNAs in diabetic macroangiopathy.

However, understanding the full range of miRNAs roles in diabetic macroangiopathy requires extensive investigation and clarification. Firstly, the molecular functions of miRNAs are not yet fully elucidated. For example, many miRNAs are secreted into the circulation in diabetic patients with macroangiopathy and can be taken up by distant cells [149]. There is evidence suggesting that circulatory miRNAs may act as 'RNA hormones' to regulate metabolism upon their uptake, but their definitive fate in recipient cells is still poorly understood. Secondly, preliminary studies on miRNAs phenotype in diabetic macroangiopathy have largely been conducted in immortalized cell line [150]. However, the clinical relevance of these miRNAs must be corroborated with rigorous studies in animal models, particularly primates, and human samples. Finally, most studies have investigated the mechanisms of miRNAs in diabetic macroangiopathy by primarily referencing their typical function, namely AGO-dependent gene silencing. However, increasing evidence suggests that miRNAs could play other roles, such as nuclear ncRNAs inhibition, protein interaction and translational activation [41, 151–153]. A deeper understanding of these roles may provide new hypotheses for diseases with unknown mechanism.

Nevertheless, it is clear that, despite some limitations, new larger cohort studies and further insights into the mechanisms of action offer a promising perspective for future functional research related to the cardiovascular complications in diabetic patients.

Abbreviations

ADSCs	Adipose-derived stem cells
ADSC-Exos	Exosomes from adipose-derived mesenchymal stem cells
AGO	Argonaute
AT-MSCs	Adipose tissue mesenchymal stem cells
AKT	Protein kinase B
AS	Atherosclerosis
BMDMs	Bone marrow-derived macrophages
CAD	Coronary artery disease
CLI	Critical limb ischemia
DGCR8	DiGeorge syndrome critical region 8
DKD	Diabetic kidney disease
DM	Diabetes mellitus
DR	Diabetic retinopathy
EPCs	Endothelial progenitor cells
FGF	Fibroblast growth factor
FGFRL1	Fibroblast growth factor receptor-like 1
FMD	Flow-mediated vasodilation
H_2O_2	Hydrogen peroxide
IMD	Intermedin
miRNA	MicroRNA
mRNA	Messenger RNA
ncRNA	Non-coding RNA
NO	Nitric oxide
NRF2	Nuclear factor erythroid-2 related factor 2
Ox-LDL	Oxidized low-density lipoprotein
PAD	Peripheral artery disease
pre-miRNA	Precursor miRNA
pri-miRNA	Primary miRNA
RISC	RNA-induced silencing complex
RNAi	RNA interference
ROS	Reactive oxygen species
STAT3	Signal transducer and activator of transcription 3
T2DM	Type 2 diabetes mellitus
VEGF	Vascular endothelial growth factor
VSMCs	Vascular smooth muscle cells
3'UTR	3'Untranslated region

Author contributions

YT, JT and XF conceived the project, designed the structure and made critical revisions of the manuscript. GR wrote the manuscript. BP and GZ prepared the figures and tables. All authors have read and approved the final version of the manuscript for submission.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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