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Novel insights regarding the role of non-coding RNAs in diabetes

MicroRNAs and diabetes

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Abstract

Diabetes mellitus (DM) is a group of metabolic disorders defined by hyperglycemia induced by insulin resistance (IR), inadequate insulin secretion, or excessive glucagon secretion. In 2021, the global prevalence of diabetes is anticipated to be 10.7% (537 million people). Non-coding RNAs (ncRNAs) appear to have an important role in the initiation and progression of diabetes, according to a growing body of research. The two major groups of ncRNAs implicated in diabetic disorders are microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). MiRNAs are single-stranded, short (17-25 nucleotide), non-coding RNAs that influence gene expression at the post-transcriptional level. Because diabetes has reached epidemic proportions worldwide, it appears that novel diagnostic and therapeutic strategies are required to identify and treat complications associated with these diseases efficiently. MiRNAs are gaining attention as biomarkers for DM diagnosis and potential treatment therapeutics due to their function in maintaining physiological homeostasis *via* gene expression regulation. We shall address the issue of diabetes's DM's gradually expanding global prevalence in this review by presenting a complete and up-to-date synopsis of various regulatory miRNAs involved in these disorders.

Furthermore, we hope this article will spark discussion about ncRNAs as prognostic biomarkers and therapeutic tools for diabetes. We will examine and synthesize the

recent findings of various research that used a novel, high-throughput technologies to uncover ncRNAs involved in diabetes, necessitating a systematic approach to examining and summarizing their roles and possible diagnostic and therapeutic uses in this paper.

Key Words: non-coding RNA; microRNA; diabetes; circulating miRNA biomarkers; non-coding RNA as a therapeutic target; CRISPR/Cas9 system.

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Core Tip: Diabetes mellitus, a chronic endocrinopathy characterized by disrupted glucose, lipid, and amino acid metabolism, is rising and has reached pandemic proportions. A vast body of evidence demonstrates that miRNAs play a key role in diabetic physiopathology. Here, we explore numerous regulatory miRNAs involved in diabetes and discuss their potential diagnostic and therapeutic applications in this paper.

INTRODUCTION

Diabetes mellitus (DM) is a chronic endocrinopathy caused by genetic and environmental factors that lead to deranged carbohydrates, lipids, and amino acid metabolism. If DM treatment is left out of control, the delayed effects of such a metabolic derangement promote systemic, mainly vascular consequences (1-3). The prevalence of DM is increasing rapidly and has reached pandemic proportions (4, 5). According to studies and International Diabetes Federation (IDF), the global prevalence of DM type 2 (T2DM) was 9.3% in 2019, expected to rise to 10.9% by 2045, and affects approximately 629 million people (6, 7).

The pathophysiology of DM is based on hyperglycemia induced by either insulin resistance (IR), insulin deficiency, or both (4). Ineffective glucose utilization favours the

activation of alternative glucose metabolic pathways (i.e. polyol, protein kinase C, and hexosamine), contributing to mitochondrial dysfunction, reactive oxygen species (ROS) generation, and the development of cellular and tissue hypoxia (8). DM can cause endothelial cell destruction and low-grade systemic inflammation, leading to vascular problems, including diabetic foot ulcers, peripheral neuropathy, nephropathy, maculopathy, and retinopathy. (9-11). In DM patients, vascular issues contribute to a two to four-fold increase in myocardial infarction, stroke, and overall mortality (12-14).

There are at least five types of DM, with type 1 (T1DM) and T2DM being the most prevalent in clinical settings (2, 15). T1DM represents 10% of all cases of diabetes and is one of the most frequent chronic diseases of childhood, with a worldwide incidence that is increasing by 3% annually (16-18). Multiple mechanisms, including autoimmunity, genetic susceptibility, and epigenetic modulation, have been implicated in T1DM pathogenesis (19). The presence of autoantibodies against β -cell antigens, such as insulin, decarboxylase, tyrosine phosphatases-2 and -2b, and glutamic acid, have been detected in T1DM patients (19, 20). More than 50 mutations that describe disease susceptibility were discovered at 50 different genetic loci, with human leukocyte antigen class II (HLA) mutations being the most common. In addition, environmental factors such as nutritional habits, viruses, and other epigenetic factors directly affect the expression of insulin genes and genes responsible for autoimmune responses (21).

T2DM is another type of diabetes that is more common in elderly or obese patients and is distinguished by cells and tissues that are resistant to high insulin levels and even high blood sugar levels. T2DM accounts for most (90-95%) of DM cases (2, 7). While genetic predisposition and obesity are the main risk factors for the development of T2DM, autoimmunity plays a pivotal role in the development of T1DM (1, 22).

Basic scientific research continuously aims to identify improved biochemical markers of early or advanced endothelial injury. Besides C-reactive protein, homocysteine, nitric oxide, and inflammatory cytokines, microRNAs (miRNAs) are promising tools for assessing vascular complications risk in DM patients. Furthermore, due to accumulating evidence regarding their role in the onset and progression of DM,

miRNAs are gaining substantial attention as novel diagnostic biomarkers for DM diagnosis and potential therapeutic agents.

In this review, we address the problem of the global prevalence of DM s by providing a systematic and up-to-date summary of various miRNAs involved in the pathogenesis and pathophysiology of this condition. We also summarize the recent findings of numerous studies that used a novel high-throughput methodology to identify miRNAs involved in the physiopathogenesis of DM and their potential diagnostic and therapeutic applications.

ROLES OF MICRORNAS IN DM

The rapid advancement of high-throughput sequencing technologies, such as microarray, deep RNA sequencing, and next-generation sequencing (NGS), revealed novel insights into the structure and function of the human genome and transcriptome. An unexpected finding of such studies is that only ~ 2% of the human genome is transcribed into protein-coding messenger RNA (mRNA) (23, 24), while the remaining ~98% of the genome is transcribed into non-coding RNAs (ncRNAs), including structural RNAs (rRNAs and tRNAs), and regulatory RNAs such as miRNA, and lncRNA (25). MiRNAs are small (17-25 nucleotide) single-stranded non-coding RNA molecules that regulate gene expression post-transcriptionally primarily by binding to complementary cis-elements in the 3'-untranslated region (3'UTR) of target mRNAs (26). They may, however, bind anywhere along the mRNA transcript's length to inhibit translation and regulate mRNA stability and degradation (27, 28). MiRNA interactions with coding areas and the 5' UTR are thought to mute gene expression (29, 30), whereas miRNA interactions with promoter regions are thought to activate transcription (31).

MiRNAs are transcribed by RNA polymerase II as long precursor molecules, which are cleaved by the nuclear RNase III-type endoribonuclease DROSHA to approximately 70 nucleotide long precursor miRNA (pre-miRNA)(32), before being transported into the cytoplasm and further processed by another RNase III enzyme, DICER, to generate mature double-stranded miRNAs(33). The guide strand of mature miRNA associates

with Argonaute (AGO) proteins and is incorporated into the minimal miRNA-induced silencing complex (miRISC) that interacts with complementary sites within the target mRNAs (33) (Figure 1). It has been estimated that around 60% of human transcripts contain potential miRNA-binding sites within their 3'UTRs (34) and that a single miRNA can potentially bind to more than 100 target mRNAs, where several miRNAs may act synergistically to finely tune the expression of the same transcript (35-37).

miRNAs as glucose homeostasis regulators

MiRNAs regulate glucose homeostasis by controlling insulin production, secretion, and cell proliferation. Fine-tuned insulin secretion from pancreatic β -cells is required for blood glucose homeostasis; perturbations in this process can result in hyperglycemia and DM. The tissue-specific expression of miRNAs is an important aspect of their role in the pathophysiology of DM. For instance, miR-9, miR-375, miR-376 and miR-7 were shown to be highly expressed in the human pancreas, where they exhibit an important role in pancreatic islet function (38-40), participating in pancreas development and the regulation of islet mass, as well as β -cell proliferation and insulin secretion. Expanding knowledge of tissue-specific miRNA expression in animal models and human subjects is illustrated by several studies that provide valuable insights into connections between miRNAs and DM. For example, in streptozotocin (STZ)-induced T1D mice, miRNA-microarray profiling revealed 64 up-regulated and 72 downregulated pancreatic miRNAs, and subsequent qRT-PCR analysis validated the decreased expression of let-7, miR-148b-3p, miR-27a-3p, miR-7a-5p, miR-7b-5p, miR-26a-5p, and miR-26b-5p in diabetic mice. (41). Another study confirmed the downregulation of miR-26a-5p in pancreatic mice tissues (42). Regarding T2DM animal models, increased levels of miRNAs belonging to the miR-199 and miR-200 families, as well as miR-34a, miR-132, miR-146, let-7b, and miR-21, were observed in pancreatic islets of diabetic mice (43, 44) whereas miR-30d, miR-184, miR-203, miR-210, miR-338-3p and miR-383 had significantly decreased levels (44-46). MiR-199a-5p and miR-184 were consistently dysregulated in several mice models of obesity and/or IR. For instance, increased expression of miR-199a-5p was also reported in islets of diet-induced obese (DIO) mice

(44, 47), whereas decreased expression of miR-184 was confirmed in an independent study using the islets of mice on a high-fat diet (48). In the non-obese spontaneous Goto-Kakizaki (GK) rat T2DM rat model, global evaluation of miRNAs' expression pattern in pancreatic islets identified thirty dysregulated miRNAs (49). A study by Karolina *et al* performed on pancreatic T2DM rat tissue showed a significant increase of miR-144, miR-150, miR-29a, miR-192, and miR-320a observed, while miR-146a, miR-30d, and miR-182 were highly downregulated (50).

Human studies show a cluster of highly-expressed miRNAs expressed explicitly in human β -cells, such as miR-655, miR-656, miR-127, miR-136, miR-543, miR-369, miR-411, miR-432, miR-487, miR-495, miR-589, is significantly downregulated in islets from T2DM patients (51). Other studies also found increased miR-124a and miR-187 expression in the islet tissue of T2DM patients (52, 53). However, miR-7a, and miR-184, implicated in the regulation of pancreatic β -cell function, showed a significantly decreased expression in human T2DM islets (47) (48). In T1DM patients, significant upregulation of miR-125a-5p compared to healthy controls was observed (54).

miR-375, which has an established role in regulating insulin secretion (Figure 2) (40), was the most highly expressed miRNA in human pancreatic islets (55). The target of miR-375 is myotrophin (Mtpn) which is involved in the cytoskeleton remodeling by depolymerizing actin filaments (40) and mediating exocytosis by enabling the fusion of insulin vesicles on membranes of β -cells (40, 56) (Figure 3). In addition, Mtpn was shown to up-regulate the nuclear transcription factor β (NF- κ B), thus inducing the expression of proteins responsible for targeting insulin vesicles to the membrane (57, 58).

Lovis *et al* found that miR-124a and miR-96 could also operate as transcriptional regulators of proteins involved in insulin exocytosis and secretion. (Figure 3) (59). miR-124a increases the levels of Rab3A, SNAP25, and synapsin-1A while decreasing Noc2 and Rab27A levels (Figure 3). Rab27A is a GTPase that allows vesicles to be transported to the cell membrane. The direct binding of miR-124a to the 3'-untranslated region of Rab27A reduces Rab27A expression (Figure 3). MiR-124a overexpression causes

excessive insulin release at rest and decreases glucose-induced insulin secretion (59). MiR-96 has been shown to reduce glucose-induced insulin release by up-regulating granuphilin, a negative regulator of insulin exocytosis, while suppressing Noc2 expression. (59), a protein that is essential for the normal regulation of endocrine cells' exocytosis (60). MiR-7 has also been found to inhibit glucose-induced insulin release in β -cells. It acts by directly regulating the expression of genes involved in the late stages of insulin granules fusion with the plasma membrane, including the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, which mediates membrane fusion of vesicles with their target cellular compartments (47).

Several miRNAs are implicated in the regulation of insulin production. MiR-375 has been shown to inhibit 3'-phosphoinositide-dependent protein kinase-1 (PDK1), a crucial component of the PI3K cascade (Figure 3) (61). Decreased PDK1 Levels lead to the down-regulation of insulin gene expression in response to glucose stimulation (61). MiR-204 has also been found as a negative regulator of insulin production, whose expression is influenced by TXNIP, a redox state regulator in β -cells. TXNIP levels are increased in DM, resulting in elevated miR-204 expression that mediates increased degradation of the insulin transcription factor MAFA (62). Another target of miR-204 is the Glucagon-like peptide 1 receptor (GLP1R) 3' UTR, and this interaction also downregulates glucose-induced insulin secretion (63).

MiR-375 and several other pancreatic miRNAs, including miR-21, miR-34, and miR-200, have been found to influence β -cell proliferation, survival, and apoptosis (Figure 2). For instance, decreased proliferation and viability are associated with a miR-375 knockdown in mice, resulting in a severely diabetic state (56). MiR-21, whose expression is regulated by the NF- κ B proinflammatory cytokine pathway, has been reported to regulate β -cell number (64, 65). MiR-21 overexpression *in vitro* was associated with decreased β -cell number (64). MiR-34a knockdown increased β -cell quantity and mass, which could be explained by miR-34's role in targeting SIRT1 and, as a result, causing p53-mediated apoptosis (64). MiR-200 also induces expression of pro-apoptotic genes in both T1DM and T2DM by suppressing Xiap, a caspase inhibitor,

and Dnajc3, a β -cell heat shock protein, while activating the tumour suppressor protein Trp53 (66).

miRNAs as mediators of immune system equilibrium in DM

The significance of miRNAs as global cellular regulators of gene expression is evident in their function in immune system balance and regulating immune cell differentiation, maturation, and activation, which is especially relevant in the context of T1DM pathogenesis (Figure 2). Various immune cells, such as CD4⁺, CD8⁺ T cells, natural killer (NK) cells, type B lymphocytes, dendritic cells, and chemokines and cytokines, are characteristic of T1DM-associated β -cell damage (67). B lymphocytes play a protective and defensive role for β -cells preventing T1DM development. Mone *et al.* reported that miR-34a overexpression in diabetic mice leads to reduced activity of B lymphocytes *via* a negative expression of the Foxp1 gene (68) involved in B lymphopoiesis, resulting in a reduced ability of pancreatic islets to defend themselves against damage (68, 69). MiRNAs expression also regulates the production of specific T cells on the other end of the homeostatic chain. MiR-590, miR-98, and miR-23 overexpression has also been demonstrated to influence the production of CD 8⁺ T lymphocytes that target pancreatic islet antigens. The mechanisms of action operate by suppressing the expression of TRAIL and FAS ligands, implying that miRNA-mediated gene silencing may promote autoimmunity and the development of T1DM (70). The three primary antibodies in T1DM occurring long before the disease's onset are autoantibodies against specific antigens of pancreatic islet cells IA, IA2B, and GAD (glutamic acid decarboxylase) and a cluster of 32 miRNAs participate in biosynthetic pathways that modify the expression of T1DM autoantibody sequences (71). Furthermore, miRNA-21a and miR-93 are involved in the processes of inflammation and pathways leading to cell death in T1DM patients' PBMC (72), whereas miR-326, which targets significant immune system modulators such as the vitamin D receptor (VDR), was found to be overexpressed in T1DM patients' PBMC (73).

LONG NON-CODING RNAS ROLES IN DM

Long noncoding RNAs (lncRNAs) are a common type of long (>200 nucleotides) linear transcript that regulates gene expression at the transcriptional and posttranscriptional levels, influencing mRNA stability, pre-mRNA splicing, and translation (74-77). Mechanistically, lncRNAs can act as miRNA sponges, scaffolds for protein complexes, and decoys for regulatory factors (76). The interaction of lncRNAs with transcription factors results in transcription regulation (75, 78). It has also been observed that some lncRNAs may interact with pre-mRNAs to influence splicing (79). LncRNAs can potentially block protein interactions with target mRNAs or change protein catalytic activity, acting as decoys (74, 76). It was also discovered that lncRNAs binding to translating mRNAs change the target mRNA's stability and translation (74).

LncRNAs regulate critical physiological processes such as cell proliferation, growth, differentiation, senescence, aging, and secretion (80, 81). They are also implied in the pathogenesis of several diseases, including cardiovascular disease and DM, and they were also implicated in the pathophysiology of various disorders like cardiovascular disease and DM. In humans, lncRNAs are primarily produced by RNA Polymerase II or III (82) and are characterized as sense- or antisense-overlap, bidirectional, intronic, or intergenic lncRNAs based on their proximity to the next protein-coding gene (83). The functions of lncRNAs are governed by their cellular location, with nuclear lncRNAs modulating transcription and splicing and cytoplasmic lncRNAs regulating posttranscriptional events such as mRNA stability, protein synthesis, and posttranscriptional alterations (76).

Various lncRNAs are expressed in a cell-type specific manner in pancreatic β -cells such as GAS5 (growth arrest-specific transcript 5), PLUTO (PDX1 Locus upstream transcript), TUG1 (taurine upregulated gene 1), MEG3 (maternally expressed gene 3), and β LINC (β -cell long intergenic non-coding RNAs).

GAS5 is a lncRNA that regulates cell development and proliferation. GAS5 levels in diabetic patients' serum are considerably lower than in healthy controls (84), and db/db mice (85), and GAS5 silencing *in vitro* is related to cell cycle arrest and decreased insulin production and secretion. PLUTO is an antisense transcript lncRNA upstream of the

gene that codes for PDX1, a transcription factor involved in β -cell differentiation and pancreatic development. Both *PLUTO* and *PDX1* are significantly downregulated in T2DM patients (86). Reduced *PLUTO* expression is related to chromatin changes that limit the interaction of the PDX1 promoter with its enhancer, resulting in lower PDX1 expression (86), implying that *PLUTO* plays a role in the control of β -cell function. *TUG1* and *MEG3* are extensively expressed in the pancreas and are controlled by glucose levels (87, 88). *TUG1* and *MEG3* knockdown reduces insulin synthesis and secretion and promotes β -cell death (88), supporting their roles in β -cell development and insulin production control. *β LINC1* is a highly conserved lncRNA linked to increased glucose intolerance and aberrant insulin secretion (89). *β LINC2* and *β LINC3* are more abundant in pancreatic islets than other organs. *β LINC2* expression levels correlate favourably with body weight, glycemia, and insulinemia, but *β LINC3* expression correlates negatively with BMI and is considerably lower in T2DM patients compared to healthy controls (90).

FUNCTIONS OF CIRCULAR RNAS IN DM

CircRNAs are abundant, conserved tissue-specific covalently closed loop circular RNAs (91-95) produced by the direct ligation of 5' and 3' ends of linear RNAs as intermediates in RNA processing or generated by backsplicing where a downstream 5' splice donor attacks an upstream 3' splice acceptor site of pre-mRNA forming a covalently closed circRNA lacking the 5' and 3' ends (96, 97). CircRNAs regulate gene expression by acting as miRNA sponges, modulating protein-protein interactions, binding to ribosomes and interfering with translation or modifying transcription (98). CircRNAs are thought to play a role in the aetiology of many diseases, including DM (99). CircRNA *Cdr1as*, for example, regulates insulin production and secretion by acting as a sponge for miR-7, reducing insulin secretion. *Cdr1as* contains about sixty miR-7 binding sites (93), and *Cdr1as* upregulation increases insulin secretion by inhibiting miR-7 activity (47). miR-7 directly targets and inhibits the expression of paired box 6 (Pax 6) and the myosin VIIA and Rab interacting protein (Myrip). Pax 6 is a transcription factor

that interacts with the promoters of the *ins1* and *ins2* genes to stimulate insulin production and secretion, whereas Myrip is involved in secretory granule transport and release. *Cdr1as* expression is downregulated in db/db mouse islets (100), but *Cdr1as* overexpression increases Pax 6 and Myrip expression, resulting in enhanced insulin transcription and secretion in pancreatic islets (101).

CircHIPK3 is abundantly expressed in pancreatic β -cells, and decreased *circHIPK3* levels are associated with reduced proliferation of β -cells (100). *CircHIPK3* silencing decreases insulin mRNA levels and perturbs glucose-stimulated insulin secretion (100).

CircAFF1 (101), another highly expressed circRNA in pancreatic islets, causes β -cell death *in vitro*, implying its role in β -cell growth and function (100).

THE FUNCTION OF MICRORNAS AS ENDOCRINE SIGNALING MOLECULES IN THE REGULATION OF INSULIN PRODUCTION AND FAT METABOLISM

Numerous investigations have shown that miRNAs act as endocrine signaling molecules, regulating insulin production and fat metabolism. Specific miRNAs directly regulated several insulin-signalling components, including insulin receptors (INSR) and several transcription factors. For example, miR-424-5p was found to target the 3'UTR sequence of INSR mRNA, and its overexpression in human hepatocytes HepG2 cell line leads to decreased INSR levels and lipid accumulation (102). Furthermore, treatment of HepG2 cells with saturated fatty acids leads to increased miR-424-5 and a reduced expression of INSR (102). Similarly, miR-15b (103), miR-195 (104), and miR-96 (105) bind to human INSR mRNA, and in the liver of mice on a high-fat diet or in HepG2 cells treated with saturated fatty acids, elevated expression of miR-96 and miR-195 was observed, accompanied by decreased INSR (104, 105). MiR-122, miR-144, and miR-146a were reported to indirectly modulate INSR by controlling the expression of protein tyrosine phosphatases that remove phosphate groups from tyrosine residues of the cytoplasmic domain of INSR and negatively affect insulin signaling (50, 106, 107). In addition, INSR compartmentalization and signal transduction depends on the presence of caveolae, specialized microdomains in plasma membranes composed of caveolin

proteins. It has been discovered that miR-107 and miR-103 bind to the 3'UTR of caveolin-1 mRNA (108) and play a role in IR by increasing liver glucose production. AntagomiRs-mediated silencing of miR-107 and miR-103 in adipocytes of diet-induced obesity (DIO) mice normalized glucose status (108).

MiRNAs also target IR substrates (IRS), altering insulin signaling and cholesterol and fatty acid metabolism. MiR-96, miR-126, and miR-145 regulate IRS1 expression (109-111), whereas IRS2 is targeted by miR33a/b (112, 113). However, it has been reported that different sets of miRNAs regulate IRS in various target tissues. IRS-1 is regulated in skeletal muscle by miR-29a, miR-29c, and miR-128a (104, 114, 115) and IRS2 by miR-135a (116). IRS-1 mRNA contains a binding site for miR-29, which has been shown to activate lipid metabolism genes such as peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (117).

MiR-26, which is significantly downregulated in the livers of overweight people and obese leptin-deficient mice, is another miRNA implicated in regulating insulin sensitivity, glucose and fat metabolism (118). MiR-26a levels correlate positively with body mass index (BMI) and are negatively related to homeostatic model assessment for IR (HOMA-IR). Furthermore, miR-26a overexpression prevented metabolic activity changes associated with obesity (118).

MiRNAs regulate insulin-like growth factor-1 (IGF-1) and its receptor (IGF-1R) expression and secretion, as demonstrated by Ling *et al*, who revealed that miR-320 modulates the insulin signaling pathways by influencing IGF-1 expression and insulin sensitivity in adipocytes (119). This finding implies that specific miRNAs may bind to multiple targets to regulate glucose metabolism, which is supported by the discovery that miR-1 regulates IGF-1 and IGF-1R expression in cardiac and skeletal muscles (120), whereas let-7 may bind to the 3'UTR regions of INSR, IRS-2, and IGF-1R (121). The discovery that miR-143-3p, which regulates IGF-2R receptor expression, is significantly

up-regulated in the serum of T2DM patients and multiple tissues of obese mice (including pancreas, skeletal muscle, and heart), contributing to insulin resistance associated with metabolic syndrome, lends support to the role of IGF signaling in metabolic diseases (122).

SPECIFIC MICRORNA DYSREGULATION CAN CONTRIBUTE TO METABOLIC DISEASES

As previously mentioned, ¹pancreatic β -cells are characterized by a high abundance of miR-375 (40). However, studies of miRNAs' distribution in other organs and tissues, such as liver and adipose tissue, suggest that miRNA expression profiles may serve as signatures of cell identity (123). Furthermore, high-throughput omics studies have found a link between miRNA expression in different tissues, such as the pancreas, liver, and adipose tissue, and conditions like metabolic disease and obesity. (124-126). For instance, 221 out of 1736 genomic loci associated with obesity correspond to miRNAs (124). The role of miRNAs in the pathophysiology of T2DM and associated metabolic disorders was investigated. ¹Specific miRNAs such as miR-27a and miR-222 are up-regulated in adipose tissue, whereas mir-122, miR-103, and miR-195 were found to be enriched in the liver (127), with miR-122 accounting for nearly 70% of the total miRNA expressed in this tissue (128-130). In addition, treating adipocytes with glucose results in increased expression of miR-27a, miR-29a, and miR-222 and ¹downregulation of miR-10b in skeletal muscle. Other studies reported that muscle cells are enriched in miR-133a, miR-133b, miR-1, miR-486, miR-206, miR-208a, miR-208b, and miR-499 (131-133). Several studies show that specific miRNAs are differentially expressed in obese subjects' white adipose tissue compared to non-obese controls (109-112), and visceral adipose tissue miRNAs are more important in metabolic dysregulation than subcutaneous tissue miRNAs (134, 135). Furthermore, a correlation has been found between miRNA expression in adipose tissue and metabolic parameters such as BMI, glycemia, leptinemia, and adipogenesis. (136, 137). For instance, elevated ²miR-21 expression was observed in the white adipose tissue (WAT) of obese human subjects,

and it positively correlated with BMI (138). Interestingly, treatment with a miR-21 inhibitor (locked nucleic acid (LNA)-miR-21) resulted in decreased adipocyte size, significant weight loss, and inhibition of expression of TGF β -receptor 2 (TGFB2) and phosphatase and tensin homolog (PTEN) (139).

Circulating miRNAs exert an additional level of the regulation of metabolic homeostasis, which can mediate communications between various types of cells. Extracellular miRNAs may be utilized for evaluating an individual's metabolic condition because dysregulation of their expression is linked to various metabolic illnesses, including T2DM, obesity, and CVD (140), and correlates with individual lifestyle characteristics such as exercise (141-144), dietary intake (145), and the composition of gut microbiota (146). Adipocytes and adipose tissue macrophages (ATM) can alter insulin-sensitive organs like the liver and muscles by releasing exosomal vesicles carrying miRNAs. (147). MiRNA profiling of ATM exosomes revealed enhanced expression of miR-155, which targets the PPARG gene that encodes for peroxisome proliferator-activated receptor gamma (PPAR γ), which regulates glucose metabolism and fatty acid storage (148). Furthermore, it has been established that adipose tissue is a major source of exosomal miRNAs that regulate gene expression in distant organs (149). Several studies confirmed the link between adipose tissue and miRNA profiles, demonstrating that weight loss was associated with significant changes in circulating miRNA levels (150, 151). Furthermore, Thomou *et al* observed that the liver could take up exosomal miR-99b from adipose tissue, resulting in a decreased expression of hepatic fibroblast growth factor 21 (FGF21) and, as a result, glucose intolerance (149).

Extracellular vesicles (EVs), specifically exosomes, play a vital role in inter-organ communication by carrying lncRNAs and miRNAs that modulate metabolic pathways. EVs are tiny vesicles enclosed by a membrane, originate from the endosome, and are released by cells into the extracellular fluids depending on their cargo (152). According to the Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV 2018) recommendations, EVs are a component of the total secretome released by the cell, and

no specific marker can distinguish EV subtypes and their subcellular origin (153). Exosomes and microvesicles, two forms of EVs released by cells, are distinguished by their manner of synthesis rather than their size. Cells undergo EV biogenesis, a process that includes ⁴inward invagination of the plasma membrane within the cytosol, forming early and late endosomes (LEs). These LEs join together to create multivesicular bodies (MVBs), which invaginate to form intraluminal vesicles (ILVs) (154). Exocytosis occurs when these ILVs fuse with the cell's plasma membrane and release exosomes into the extracellular environment (155). Exosomes are found in various bodily fluids and are released by various cell types, including lymphocytes and pancreatic islets (155). The transfer of nucleic acids by exosomes is enhanced in inflammatory conditions, and miRNAs represent one of the main cargos transported by exosomes (156).

In DM, these molecules target specific tissues regulating their activity. Therefore, to understand the pathogenesis of T1DM and T2DM, it is crucial to investigate the communication between affected organs in response to elevated blood glucose levels. Exosomes act as messengers, linking the immune response to pancreatic damage and adipocyte stimulation, leading to IR in the liver and muscles. Exosomes containing lncRNAs and miRNAs also contribute to cellular communication by altering metabolic and insulin signals, impacting inflammatory processes in pancreatic cells. Exosome-carried miRNAs, particularly, hold great promise as biomarkers or in developing innovative therapeutics for diabetes and its consequences (157).

Islet insulinitis is connected with the transfer of a specific group of miRNAs from lymphocytes to cells ⁴via exosomes, including miR-142-3p, miR-142-5p, and miR-155, leading in the selective death of insulin-secreting cells. Inactivation of these miRNAs protected cells against apoptosis caused by T lymphocyte exosomes *in vitro* and reduced T1D development in NOD mice *in vivo*. As a result, it has been postulated that miRNA transfer mediated by exosomes released by lymphocytes causes β -cell death and may be one of the mechanisms contributing to the development of T1DM (158).

Katayama *et al* used microarrays to evaluate the expression profile of exosomal miRNAs in healthy people and those with T2DM (159). They discovered that miR-20b-5p in exosomes is abundant in people with T2DM. Further *in vitro* studies revealed that this miRNA targets the AKT interacting protein (AKTIP), which modifies AKT protein activity and decreases glycogen buildup in muscles and IR (159).

Downregulation of exosomal lncRNA-p3134 in diabetic mice reduced ²glucose-stimulated insulin production by lowering key regulators (Pdx-1, MafA, GLUT2, and Tcf7 L2) in β -cells. This shows that lncRNA-p3134 regulates insulin secretion and that its downregulation leads to diabetes pathogenesis (160).

On the other hand, exosomal miRNAs (miR-20b-5p, miR-155, miR-450b-3p, miR-151-3p, and miR-29b-3p) were found to be elevated in diabetic mice ²and targeted skeletal muscles *via* insulin signaling regulatory proteins (PPAR, AKT, GLUT4, and FOXO). This contributes to diabetes pathogenesis by influencing insulin signaling and glucose absorption in skeletal muscles (161). Other exosomal miRNAs (miR-122, 192, 27a/b, 155, and ²29b-3p) were upregulated in diabetic models and targeted adipocytes *via* PPAR proteins. This impairs lipid metabolism and contributes to diabetes development (162).

Exosomal miRNAs (miR-142-3p and miR-142-5p) were also found to be enhanced in diabetic mice and target pancreatic cells *via* cytokines that are elevated. This contributes to diabetes pathogenesis by encouraging immune cell recruitment and β -cell death during autoimmune attacks (158). Other exosomal miRNAs that target organs such as astrocytes, retinal tissue, and renal cells ²(miR-106, miR-146a, miR-222, and miR-486) have potential therapeutic roles in protecting pancreatic cells or treating diabetic complications (163).

Exosomal miRNAs and lncRNAs influence diabetes development in various ways, including regulating pancreatic inflammation and regulating metabolic and insulin signaling in target organs. Despite mounting evidence, research on the involvement of exosomes harbouring ncRNAs in diabetes is still in its early stages, but they have promise and significant roles in pathogenesis, diabetic diagnosis, and treatments (164, 165).

Finally, chronic inflammation is a feature of metabolic disorders such as DM and obesity (166), and several miRNAs are associated with regulating the expression of inflammatory markers (167-169). The overexpression of miR-132 in human adipose-derived stem cells results in increased production of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP1) (170), which was also found to be regulated by miR-126 and miR-193b (171). Other studies have found a link between low miR-221 expression and high TNF- α levels in human adipose tissue-derived mesenchymal stem cells from obese female subjects (172). MiR-145, on the other hand, was found to boost TNF- α expression in adipocytes by activating the nuclear transcription factor kappa-B (NF- κ B) signaling pathway (173). The investigation of miRNA expression in leptin-deficient ob/ob mouse adipocytes and TNF- α -treated adipocytes *in vitro* revealed a similar expression pattern (174). In adipocytes isolated from mice, TNF- α increases the expression of a set of miRNAs, including miR-130, miR-150, miR-146a, miR-146b, miR-221, and miR-222, while decreasing miR-143 and miR-103 Levels (174-177). In human preadipocytes, both TNF- α and leptin induce decreased expression of miR-221 and upregulation of miR-335 (177, 178).

CIRCULATING MICRORNAS AS T1DM AND T2DM BIOMARKERS

Increasing evidence supports the use of extracellular circulatory miRNAs as biomarkers for various pathophysiological conditions (123, 179). Highly stable extracellular miRNAs that are protected from degradation due to their enclosure in exosomal vesicles (180) or association with AGO2 proteins (181, 182) and high-density lipoproteins (HDL) (183), are present in various biological fluids, such as serum (184), plasma (181), cerebrospinal fluid (185), saliva (180), urine, and tears (186). Although the concentration of miRNAs in body fluids is in the femtomolar range (187), highly sensitive assays such as qRT-PCR, microarrays, and RNA sequencing (RNA-seq) can detect them in small samples even after prolonged storage (186).

Several studies investigated the expression of miRNAs in the serum/plasma of diabetic patients, and several circulating miRNAs are consistently dysregulated in T1DM

patients compared to controls. In T1DM, eleven circulating miRNAs implicated in immune system function, cell survival and proliferation, and insulin production were dysregulated (188). This set included miR-100-5p, miR-21-5p, miR-150-5p, miR-24-3p, miR-146a-5p, miR-148a-3p, miR-181a-5p, miR-210-5p, miR-342-3p, miR-1275 and miR-375. In a study by Nielsen *et al*, global profiling of serum miRNAs expression in new-onset T1DM in children and age-matched healthy controls revealed a set of twelve up-regulated miRNAs in T1DM patients, including miR-24, miR-152, miR-30a-5p, miR-200, miR-148a, miR-181a, miR-210, miR-27a, miR-29a, miR-26a, miR-27b, and miR-25, some of which are implicated in β -cell function and glycaemic control (189). Erener *et al* studied serum miRNA expression and signaling pathways involved in T1DM development (190). They found six miRNA (miR-222-3p, miR-24-3p, miR-454-3p, miR-144-5p, miR-345-5p, and miR-140-5p) that are specifically dysregulated in new-onset T1DM but not at later stages of DM, and are involved in Wnt, MAPK, and PI3K/Akt signaling pathways (190). The plasma levels of miR-30d, miR29a, miR-21, miR-24, miR-34a, miR148a, miR-126, and miR-146 and were significantly up-regulated in adult T1DM patients (191), and higher levels of miR-210, miR-21, and miR-181a in T1DM were also confirmed in other studies (192, 193). More than one independent study found upregulation of miR-21, miR-148a, miR-24, miR-210 and miR-181a-5p in T1DM, indicating their potential use as circulating biomarkers of T1DM.

Global profiling of circulating miRNAs in blood samples of T2DM patients revealed approximately seventy up-regulated miRNAs and around a hundred downregulated miRNAs in T2DM patients (50). A subsequent meta-analysis suggested that plasma levels of miR-103, miR-29a, miR-107, miR34a, miR-142-3p, miR-132, miR-375 and miR-144 may potentially serve as biomarkers for T2DM (194). miR-126a was the most significantly downregulated circulating miRNA in T2DM patients (195-199). Furthermore, a comparison of plasma miR-126 Levels with healthy controls, T2DM-susceptible, and T2DM patients demonstrated significant miRNA-126 downregulation in both T2DM-susceptible individuals and T2DM subjects, suggesting a close association of miR-126 with the T2DM manifestation, thus making this miRNA a

potential biomarker for the early identification of susceptibility to T2DM (199). In addition, numerous studies reported an association of other serum miRNAs with T2DM (197, 200-208). For example, miRNA profiles comparing T2DM patients to a control group with normal glucose tolerance revealed significantly lower expression levels of miR-486, miR-96, miR-23a, miR-191, miR-186, miR-192, and let-7 (202) whereas increased levels of miR-9, miR-34a, miR-27a, miR-15b, miR-29a, miR-124a, miR-30d, miR-192, miR-150, miR-375, miR-146b, miR-320a, miR-571, miR-486, miR-661, miR-1303, miR-770, and miR-892b were observed (203-206). Whole blood evaluations demonstrated upregulation of miR-320, miR-144, miR-29a, miR-192 and miR-150, downregulation of miR-30d, miR-15a and miR-182 (50, 207), and decreased miR-103b expression in platelets of T2DM patients (208).

Regarding the utility of miRNAs as potential biomarkers of metabolic diseases, circulatory miRNAs miR-17-5p, miR-15a-5p, miR-221, and let-7g were reported as reliable predictive biomarkers of metabolic syndrome (MetS) (209). These miRNAs have a well-documented role in regulating IR, β -cell apoptosis, and central obesity (210, 211). Circulating miR-192 and miR-194 have been suggested as prospective DM risk biomarkers (212), whereas plasma levels of miR-29a, miR-9, miR-28-3p, miR-30a-5p, miR-103, and miR-150 are reliable predictive biomarkers that distinguish between incident-T2DM and non-T2DM patients (145). It should be emphasized that this group of miRNAs is linked to cell proliferation, insulin sensitivity, and secretion and that alterations in their levels can occur up to three years before T2DM development (123). However, more controlled studies on large patient cohorts are required to fully establish the potential of many proposed miRNA-based biomarkers for T1DM, T2DM, and other metabolic diseases.

MICRORNAS AND NANOTECHNOLOGY

In recent years, bio-nanomedicine has turned its attention to EVs as a novel disease treatment approach. One of the most promising applications is the delivery of tolerogenic nanoparticles (TNps) to combat autoimmune diseases like T1DM. EVs and

nanoparticles, as opposed to traditional medicines, provide advantages such as tailored delivery, lower toxicity, and enhanced stability. TNps can induce immunological tolerance in T1DM patients by regulating the immune response *via* various mechanisms (213). On the other hand, EVs can deliver cargo such as cytokines, growth factors, and miRNAs to recipient cells, influencing immune responses *via* a paracrine impact and during the development of the immunological synapse (214).

A recent study has focused on developing EVs to contain TNps to treat T1DM. For example, immunomodulatory nanoparticles containing antisense oligonucleotides to CD40, CD80, and CD86 have been utilized to prevent T1DM in mice by increasing Foxp3⁺ Treg cells (215). Another study found that the ⁴co-culture of islets and bone marrow stem cells enhanced islet-cell survival and functionality in mice, mediated by exosomes *via* a paracrine action (216). Furthermore, clinical studies (217) have shown that exosomes derived from mesenchymal stem cells can suppress immune targeting in allogeneic grafts. These findings imply that EVs have regenerative, anti-apoptotic, immunomodulatory, and angiogenic capabilities, making them a prospective tool for restoring islet-cell function and treating autoimmune disorders (218).

Nanotechnology has gained prominence in diabetes research by leveraging nanomaterials, nanostructures, and nanoparticle design to obtain more exact information on diabetes mellitus diagnosis. Nanoparticles can be used to deliver RNA and proteins to identify and monitor illness progression (219). A recent study aimed to identify critical miRNAs that are dysregulated in pancreatic islets during T1DM progression and to create a theranostic strategy to modulate their expression using an MRI-based nano-drug. Iron oxide nanoparticles combined with miRNA-targeting oligonucleotides were used to treat a mouse model of T1DM (157, 220).

MICRORNAS AS POTENTIAL THERAPEUTIC TARGETS IN DIABETES TREATMENT

RNA-based therapeutic approaches have several important advantages compared to other drugs. Theoretically, miRNAs can simultaneously target several mRNAs, and fine-tuning miRNAs expression may restore physiological homeostasis (221). The mechanism of action of RNA molecules may be elucidated from various available bioinformatics tools, such as *in silico* analysis and RNA structure prediction. Furthermore, ncRNAs are not associated with the development of drug resistance (222). Thus, miRNAs are intriguing pharmacological targets that can potentially treat various complex diseases such as DM, cardiovascular disease, and cancer at the molecular level (222-225). In miRNA-based therapies, the ultimate goal is to restore specific miRNA functions to normal levels, achieved by either restoring the expression of downregulated miRNAs using miRNA mimics or inhibiting the activity of up-regulated miRNAs with a miRNA inhibitor.

MiRNA mimics are double-stranded (dsRNAs) RNAs with the same sequence as a specific endogenous miRNA (226). miRNA mimics have been employed *in vitro* to stimulate the regeneration of insulin-producing cells from induced pluripotent stem cells (iPSCs) (227, 228) (229, 230). The approach is based on discovering that different miRNA clusters control human iPSC reprogramming (231, 232). However, this approach is not used *in vivo* since it may be associated with unwanted side effects (233). Antisense oligonucleotides (ASOs) containing a complementary sequence to the target miRNA are used as miRNA inhibitors. Since most miRNAs repress target gene expression, binding a miRNA inhibitor to the mature target miRNA typically activates target gene expression. Locked nucleic acid (LNA) anti-miRs and antagomiRs are *in vivo* efficient modified miRNA inhibitors. (234, 235). AntimiRs are ASOs fully or partially complementary to an endogenous miRNA and act by preventing its interaction with target genes. AntagomiRs are cholesterol-conjugated antimiRs with improved intracellular delivery (236). At present, antimiRs are the most commonly used miRNA-based therapeutic tool (237, 238). LNA anti-miR-122 treatment has been tested in mice and non-human primates showing reduced plasma cholesterol, improved liver steatosis, and no indication of hepatic toxicity (239, 240). Several other miRNA-targeting

therapeutics are in different phases of preclinical and clinical studies. Regarding the potential treatment of T2DM and IR, ASOs ¹ were used to inhibit miR-103 and miR-107 in the liver and adipose tissue of obese mice resulting in improved insulin sensitivity and glucose homeostasis (108). Modified GalNAc-conjugated oligonucleotides RG-125 (AZD4076) ¹ developed by Regulus Therapeutics, which targets miR-103/mir-107, was tested in phase I and IIa clinical trials to assess their effect on insulin sensitivity and liver fat content in patients with T2DM and nonalcoholic steatohepatitis (NASH) (241). Another anti-miR antagomiR developed by Regulus Therapeutics for treating metabolic illnesses is 2'fluoro/methoxyethyl (2'F/MOE) modified, phosphorothioate backbone modified anti-miR-33, which has been proven to reduce atherosclerotic plaque in T2DM patients (242, 243). A locked nucleic acid (LNA)-modified antisense oligonucleotide targeting miR-208A (MGN-9103) was developed by Viridian Therapeutics, and it demonstrated the potential to ameliorate insulin sensitivity and systemic glucose tolerance in MetS ¹ (244). Several candidate miRNA molecules, such as mir-21 and miR-181a, were suggested as potential therapeutic targets for metabolic disease treatment. MiR-21 suppression with LNA-modified anti-miR-21 in adipose tissue of db/db mice led to a significant decrease in body weight (139), whereas oligonucleotide-mediated downregulation of miR-181 in DIO mice increased levels of protein sirtuin 1 (SIRT1) and ameliorated insulin sensitivity and glucose homeostasis ¹ (245). It is also worth noting that dietary substances like CLA, polyphenols, and long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) indirectly influence miRNA expression. (246-249). For instance, CLA treatment altered the expression of miRNAs related to adipocyte differentiation, lipid metabolism, and obesity, such as miR-143, miR-107, miR-222, miR-103 and miR-221 (246). Furthermore, dietary polyphenols inhibited the increased expression of miR-103 and miR-107, which is associated with a high-fat diet (247), whereas n-3 PUFA consumption alters the expression of miRNAs involved in lipid metabolism and inflammation. (249).

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Gene editing based on Clustered Regularly Interspaced Short Palindromic Repeats/associated protein-9 nuclease (CRISPR/Cas9) has recently emerged as a

technology with broad applications in the research and treatment of various diseases. The CRISPR/Cas9 RNA-guided editing technology could be used for genome editing. It is composed of a Cas9 nuclease that binds to a conserved three-nucleotide sequence known as the proto-adjacent motif (PAM) and creates a double-stranded DNA break. CRISPR RNA (crRNA) is a guide for Cas9 and an adapter trans-activating RNA (tracrRNA). The crRNA and tracrRNA can be combined to create a single-guide RNA capable of directing Cas9 to any target within the PAM sequence (250-252). The CRISPR/Cas9 platform has been used to target the expression of miRNAs implied in various pathophysiological conditions (253, 254). An innovative example of CRISPR/Cas9 use in the context of the development of antidiabetic therapeutics is a recent development of a therapeutic candidate VCTX210. In 2021, CRISPR Therapeutics and ViaCyt companies jointly developed CRISPR-edited stem cell therapy candidate VCTX210 for potentially treating T1DM and T2DM, which has been approved for a clinical trial in Canada. VCTX210 is a stem cell-derived therapy edited with CRISPR-Cas9 intended to replace the β -cells lost in diabetes. Nonetheless, the use of gene editing for therapeutic purposes in the future will necessitate precise delivery of CRISPR to specific target tissues as well as strict control of potential off-target effects (255).

CONCLUSION

Although most current animal and human studies focus on the detection rather than the functional analysis of various miRNAs associated with cell physiology and pathology, many highly specific miRNAs have recently been identified as potential prognostic markers and therapeutic tools in diabetes patients. MiRNAs are involved in the regulation of gene expression in glucose homeostasis, lipid metabolism, and immune system balance. Increasing evidence shows that miRNAs can be a biomarker to predict type 1 and type 2 diabetes. Future studies must extensively investigate the roles of identified miRNAs to find those with the most reliable prognostic and therapeutic potential. Developing highly precise miRNA-based therapies designed for clinical usage will improve the prediction of vascular risk and end-organ vascular damage.

With further advancements in high-throughput methodologies, such as whole genome and transcriptome profiling, and the associated proteomic and metabolomic analyses, a more profound link between various miRNAs and the physiology and pathophysiology of glucose homeostasis and fat metabolism will be more firmly established.

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