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REVIEW

Role of miRNAs in the pathogenesis and susceptibility of diabetes mellitus

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MicroRNAs (miRNAs) are noncoding RNAs of ~22 nucleotides that regulate gene expression post-transcriptionally by binding to the 3′ untranslated region of messenger RNA (mRNAs), resulting in inhibition of translation or mRNA degradation. miRNAs have a key role in fine-tuning cellular functions such as proliferation, differentiation and apoptosis, and they are involved in carcinogenesis, glucose homeostasis, inflammation and other biological processes. In this review, we focus on the role of miRNAs in the pathophysiology of the metabolic disease and diabetes mellitus, the hallmark of which is hyperglycemia caused by defective insulin secretion and/or action. A growing number of studies have revealed the association between miRNAs and the processes of insulin production and secretion in pancreatic β cells. In addition, aberrant expression of miRNAs in skeletal muscle, adipose tissue and liver has also been reported. Intriguingly, the tumor suppressor p53 has been implicated in the pathogenesis of diabetes in association with a number of miRNAs, suggesting that a p53/miRNA pathway might be a therapeutic target. Moreover, data from genome-wide association studies have revealed that several miRNA target sequences overlap type 2 diabetes susceptibility loci. Finally, the recent discovery of circulating miRNAs associated with diabetes onset/progression suggests the potential use of miRNAs as biomarkers.
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INTRODUCTION

Diabetes mellitus is a group of diseases characterized by chronic hyperglycemia owing to deficiency of insulin action. It is a multifactorial disorder stemming from a complex combination of factors including genetic background, aging and environment that cause an increase in blood glucose, resulting in a deficiency of insulin secretion from pancreatic β cells and decreased insulin sensitivity in skeletal muscle, liver and adipose tissue. In type 1 diabetes, insulin deficiency is caused primarily by loss of the insulin-producing pancreatic β cells from the islets of langerhans. Type 2 diabetes develops dependent on genetic and environmental/dietary factors, including those that cause insulin resistance and reduced insulin secretion.[1]

The number of people with diabetes is increasing explosively worldwide. In fact, in 2015, the International Diabetes Federation reported that 415 million adults globally have diabetes, with a prevalence of 8.8%.[2] Accordingly, ~5 million people between the ages of 20 and 79 years died from diabetes and its complications in 2015.[2] Aside from administering insulin, several different types of diabetes medications have been developed such as biguanide, sulfonylureas, α-glucosidase inhibitor, prandial glucose regulators, thiazolidinediones, incretin mimetics, DPP-4 inhibitors and SGLT2 inhibitors. They have remarkably improved length and quality of life for diabetes patients. Although additional new medicines have been developed in recent years, microvascular complications such as neuropathy, retinopathy, and nephropathy and diabetes-related atherosclerotic diseases such as ischemic heart disease, cerebrovascular disease and arteriosclerosis obliterans are becoming a greater problem. Diabetes and its complications are a major cause of disability, reduced quality of life and premature death.

Glucose is a strong regulator of insulin secretion. Insulin is synthesized and secreted by pancreatic β cells. Following oral glucose administration, the associated increase in blood glucose triggers glucose uptake by the β cells through glucose transporters, expression of which is increased by chronic exposure to high glucose levels. Electrical excitation of β-cell membranes stimulates insulin release from the cells. Insulin secretion, which in turn mediates subsequent metabolism of glucose, leads to production of cellular ATP, increases the ATP:ADP ratio and closes ATP-dependent potassium channels in the β-cell membrane, resulting in membrane depolarization and calcium influx into the cell. With the increase in intracellular-free calcium, secretory granules become marginated, fuse with the plasma membrane and release their contents extracellularly.[3]

Approximately 90% of the mammalian genome comprises noncoding sequence, and 70% of this sequence is transcribed.[4,5] Actually, high-throughput RNA sequencing technologies have identified many noncoding transcripts.[4,5] Among these, microRNAs (miRNAs) are small noncoding RNAs that range in length from 19 to 23 nucleotides. miRNAs bind to the 3′ untranslated region of target

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messenger RNA (mRNAs) and guide mRNA degradation or repression of translation. miRNAs typically target mRNAs transcribed from gene clusters rather than single genes,[6] a property that facilitates the critical roles of miRNAs in diverse biological processes, including cell proliferation, differentiation, apoptosis and carcinogenesis.[7] miRNAs are important regulators of multiple processes in development, physiology and pathology, including a recently identified role in metabolic homeostasis. It was recently revealed that miRNAs participate in insulin signaling and glucose homeostasis and that aberrant miRNA expression has a potential pathological role in diabetes.[8]

Following the notion that the miRNA-375 (miR-375) directly regulates insulin secretion,[9] multiple miRNAs that regulate insulin secretion or resistance have been reported. Because miRNAs act at several points in the distinct pathways that lead to insulin secretion or resistance,[10] they could be potential therapeutic targets. Hence, understanding the role of miRNAs in the molecular pathogenesis of diabetes will provide insights to guide the development of targeted therapeutics.

There is growing evidence that miRNAs are secreted in body fluids, such as blood and urine, and that concentrations of specific miRNAs might serve as biomarkers of pathophysiological conditions.[11] Recent advances in genome-wide association studies (GWAS) have contributed to the identification of more than 80 susceptibility loci for type 2 diabetes.[12] Several loci are reported to be targets of islet-expressed miRNAs, suggesting the involvement of miRNAs in the pathophysiology of diabetes.[13] In this review, we summarize the current knowledge of miRNAs associated with diabetes.

ROLE OF miRNAs IN β CELLS

In 2004, miR-375 became the first miRNA reported to directly regulate insulin secretion.[9] Although recent studies have demonstrated that dysfunction or misexpression of this miRNA is involved in various cancers,[14] miR-375 was originally reported as a pancreatic islet-specific miRNA.[9] Recently, however, miR-375 was shown to suppress glucose-induced insulin secretion by targeting myotrophin mRNA (*Mtpn*), which participates in fusion of secretory granules with the plasma membrane through actin depolymerization.[9] Glucose activates the insulin gene promoter, and subsequent upregulated insulin expression modulates the downstream phosphatidylinositol 3-kinase (PI3K) pathway.[15] miR-375 decreases insulin secretion by directly targeting 3′-phosphoinositide-dependent kinase 1 mRNA,[15] which is a key molecule in the PI3-kinase pathway; a reduction in miR-375 level promotes insulin secretion by abolishing the suppression of its target genes. Elevated glucose level decreases miR-375 expression.[15] Notably, the transcription factors pancreatic and duodenal homeobox 1 and neuronal differentiation 1 bind to the miR-375 gene promoter and regulate its expression.[16] Intriguingly, miR-375 knockout mice exhibit hyperglycemia accompanied by reduced β-cell mass.[17] In contrast, elevated miR-375 expression is observed in the diabetic *ob/ob* mice[17] and in pancreatic islets of patients with type 2 diabetes.[18] These findings indicate that miR-375 increases compensatory β-cell proliferation. Moreover, experiments using miRNA knockdown strategies as well as β-pancreatic differentiation in human-induced pluripotent stem cells revealed that miR-375 is essential for the formation of insulin-secreting pancreatic islets.[19,20] Together, these data show that miR-375 regulates not only glucose homeostasis (for example, insulin gene expression) and insulin secretion (through its effect on exocytosis) but also the development, maintenance and survival of pancreatic β-cell mass.

miR-9 negatively regulates insulin secretion by targeting one cut homeobox 2 (Onecut2), thereby increasing the level of granuphilin,[21] which is the Rab3/Rab27 GTPase effector that docks β-cell secretory granules at the plasma membrane and suppresses insulin secretion.[22] The nuclear deacetylase Sirt1 was identified as an miR-9 target in the β-cell line β-TC-6.[23] Sirt1 deacetylates multiple proteins, including transcription factors and histones, in an NAD-dependent manner. Sirt1 also represses expression of the gene encoding mitochondrial uncoupling protein UCP2,[24] which has the effect of enhancing glucose-stimulated insulin secretion.[25]

miR-124a negatively regulates glucose-induced insulin secretion. The expression of miR-124a is upregulated in human type 2 diabetic pancreatic islets,[26] and among its three isoforms, the level of miR-124a2 is increased during pancreatic development in mouse embryos.[27] Like miR-375, miR-124a2 decreases insulin secretion by targeting *Mtpn*.[28] miR-124a2 also directly targets *Foxa2* mRNA,[26] a master regulator of pancreatic development, and thereby affects its downstream targets pancreatic and duodenal homeobox 1 and genes related to insulin secretion and glucose metabolism, including two ATP-dependent potassium channel subunits (Sur-1 and Kir6.2), in the pancreatic β-cell line MIN6.[27] In addition, Rab27A, which is associated with insulin exocytosis, was identified as another target of miR-124a in MIN6 cells.[29] Studies concerning the upstream regulation of miR-124a

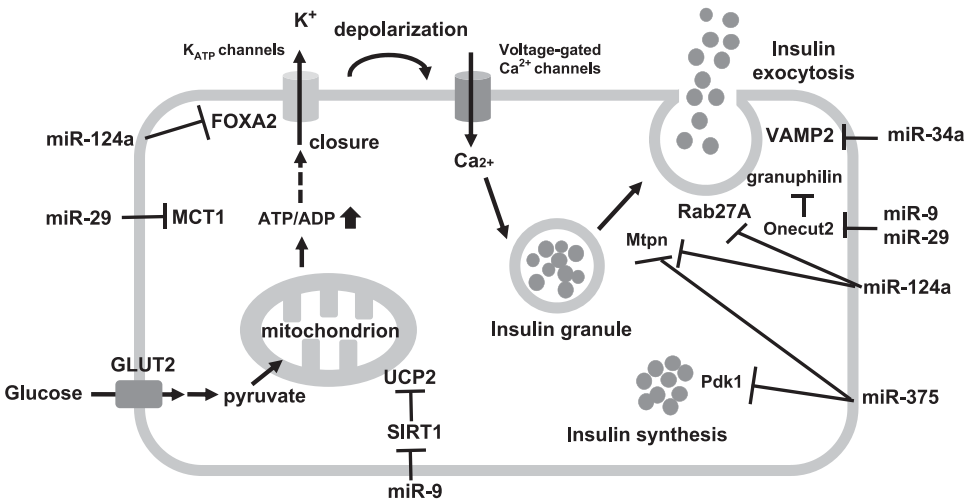

Figure 1 Model for the regulation of insulin secretion by micro RNA (miRNAs) in pancreatic β cells.

Table 1 miRNAs associated with β-cell function

miRNA	*Description*	*References*
let-7	Suppresses *Irs2* and insulin signaling	103
miR-7	Inhibits endocrine pancreas development by targeting *Pax6*; also targets mTOR signaling, negatively regulates GSIS	104–106
miR-9	Negatively regulates insulin secretion by targeting *Onecut2*, which is associated with insulin exocytosis, and *Sirt1*	21,23
miR-15a/b	Targets *Ngn3* to induce regeneration, also inhibits *Ucp2*; increases ATP/ADP ratio by suppressing *Ucp2*	107,108
miR-16	Targets *Ngn3*	107
miR-19	Targets *Neurod1* and decreases insulin mRNA level	109
miR-21	Negatively regulates insulin exocytosis in response to cytokines	110
miR-24	Targets *MEN1*, *Sox6*, *Neurod1* and *Hnf1a*	111–113
miR-26	Increases insulin expression	112
miR-29	Decreases GSIS by targeting *Mct1* and *Onecut2*; overexpression promotes β-cell apoptosis by suppressing *Mcl1*	33,34,114
miR-30	miR-30a-5p is upregulated in glucotoxicity and suppresses GSIS by targeting *Neurod1*; miR-30d overexpression increases insulin gene expression and targets *Map4k4*	115,116
miR-33a	Targets *Abca1*, resulting in β-cell cholesterol accumulation, thereby decreasing GSIS	117
miR-34a	Decreases insulin secretion by targeting *Vamp2*; also inhibits *SIRT1* and promotes β-cell apoptosis by targeting *Bcl2*	44,47,48
miR-124a	Decreases insulin secretion by targeting *Mtpn, Foxa2* and *Rab27A*	27,29,118
miR-130a	Positively regulates GSIS	119
miR-132	Enhances GSIS and contributes to compensatory β-cell mass expansion	120
miR-143	Impairs insulin-stimulated Akt activation and glucose homeostasis	65
miR-145	Targets *Abca1*, resulting in β-cell cholesterol accumulation, thereby decreasing GSIS	121
miR-146a	Induced by IL-1β and NFκB; anti-miR-146 treatment prevents the reduction of GSIS	110
miR-148	Targets *ABCA1* and *Sox6*; knockdown downregulates insulin mRNA level	112,121
miR-182	Knockdown downregulates insulin mRNA level	112
miR-184	Inhibits compensatory β-cell expansion by targeting *Ago2*	122
miR-185	Enhances insulin secretion and promotes β-cell proliferation by targeting *SOCS3*	123
miR-187	Decreases GSIS by targeting *HIPK3*	124
miR-195	Targets *Ngn3*	107
miR-199a-5p	Negatively regulates GSIS	120
miR-200	Promotes β-cell apoptosis by targeting *Ypel2, Dnajc3, Jazf1, Rps6kb1 and Xiap* and activates p53 pathway; also targets *Zeb1, cMaf* and *Zfpm2*	40–42
miR-203	Decreases β-cell apoptosis	120
miR-204	Decreases insulin production by targeting *MafA*	125
miR-210	Decreases β-cell apoptosis	120
miR-335	Impairs GSIS by targeting *Stxbp1*	126
miR-338-3p	Downregulation contributes to compensatory expansion of β-cell mass	120
miR-375	Negatively regulates insulin secretion by targeting *Mtpn* and *Pdk1*; also contributes to development, maintenance and survival of pancreatic β-cell mass	9,17,15
miR-410	Positively regulates GSIS	119

Abbreviations: Ago2, argonaute-2; Abca1, ATP-binding cassette (ABC) transporters; Bcl2, B-cell lymphoma 2; cMaf, avian musculoaponeurotic fibrosarcoma oncogene homolog; Dnajc3, DnaJ heat-shock protein family (Hsp40) member C3; Foxa2, forkhead box A2; HIPK3, homeodomain-interacting protein kinase-3; Hnf1a, hepatic nuclear factor 1 homeobox A; Irs2, insulin receptor substrate 2; Jazf1, JAZF zinc-finger 1; MafA, v-maf musculoaponeurotic fibrosarcoma oncogene family, protein A; Map4k4, Mapkkkk4; Mcl1, myeloid cell leukemia 1; Mct1, monocarboxylate transporter 1; MEN1, multiple endocrine neoplasia 1; miRNA, microRNA; mTOR, mechanistic target of rapamycin; Mtpn, myotrophin; NFκB, nuclear factor kappa B; Ngn3, neurogenin 3; Onecut2, one cut homeobox 2; Pax6, paired box 6; Pdk1, phosphoinositide-dependent protein kinase 1; Rab27A, member RAS oncogene family; Rps6kb1, ribosomal protein S6 kinase polypeptide 1; Sirt1, sirtuin (silent mating type information regulation 2 homolog); SOCS3, suppressor of cytokine signaling 3; Sox6, Sry-related HMG box 6; Stxbp1, syntaxin-binding protein 1; Ucp2, uncoupling protein 2; Vamp2, vesicle-associated membrane protein 2; Xiap, X-linked inhibitor of apoptosis; Ypel2, yippee-like 2; Zeb1, zinc-finger E-box binding homeobox 1; Zfpm2, zinc-finger protein friend of GATA family member 2.

in β cells have reported that thioredoxin-interacting protein (TXNIP), a proapoptotic factor induced by glucose,[30,31] decreases miR-124a expression. TXNIP has a critical role in inflammation, glucotoxicity and β-cell apoptosis. TXNIP induces islet amyloid polypeptide expression, and the consequent elevated level of islet amyloid polypeptide mediates β-cell apoptosis, resulting in diabetes. Given that miR-124a inhibits islet amyloid polypeptide transcription by targeting *Foxa2* mRNA, TXNIP regulates islet amyloid polypeptide-associated glucose homeostasis in an miR-124a-dependent manner.[32]

A study of mouse islets showed that miR-29 targets monocarboxylate transporter 1, thereby affecting insulin release.[33] miR-29 isoforms decrease expression of Onecut2, followed by enhanced expression of granuphilin, which inhibits insulin release in MIN6 and islet cells.[34] Furthermore, miR-29 promotes apoptosis by suppressing the antiapoptotic protein Mcl1.[34] Thus, miR-29 negatively regulates glucose-stimulated insulin secretion (Figure 1). Table 1 lists miRNAs that are associated with β-cell function.[3,8,10,35,36]

p53 AND miRNAs

The tumor suppressor p53 is activated in response to various cellular stresses,[37,38] and its role in cellular metabolism is becoming increasingly apparent. It has been reported that p53 activation in adipose tissue causes inflammation and insulin resistance.[39]

The miR-200 family consists of miR-200a, miR-200b, miR-200c, miR-141 and miR-429. Tissue analysis has shown that miR-200 family members are highly expressed in pancreatic islets in diabetic *ob/ob* mice.[40] TXNIP induces expression of miR-200 family members in rat INS-1 β cells. miR-200 targets zinc-finger E-box-binding homeobox 1 (Zeb1) mRNA and promotes β-cell apoptosis. Suppression of Zeb1 by miR-200 results in inhibition of the epithelial-to-mesenchymal

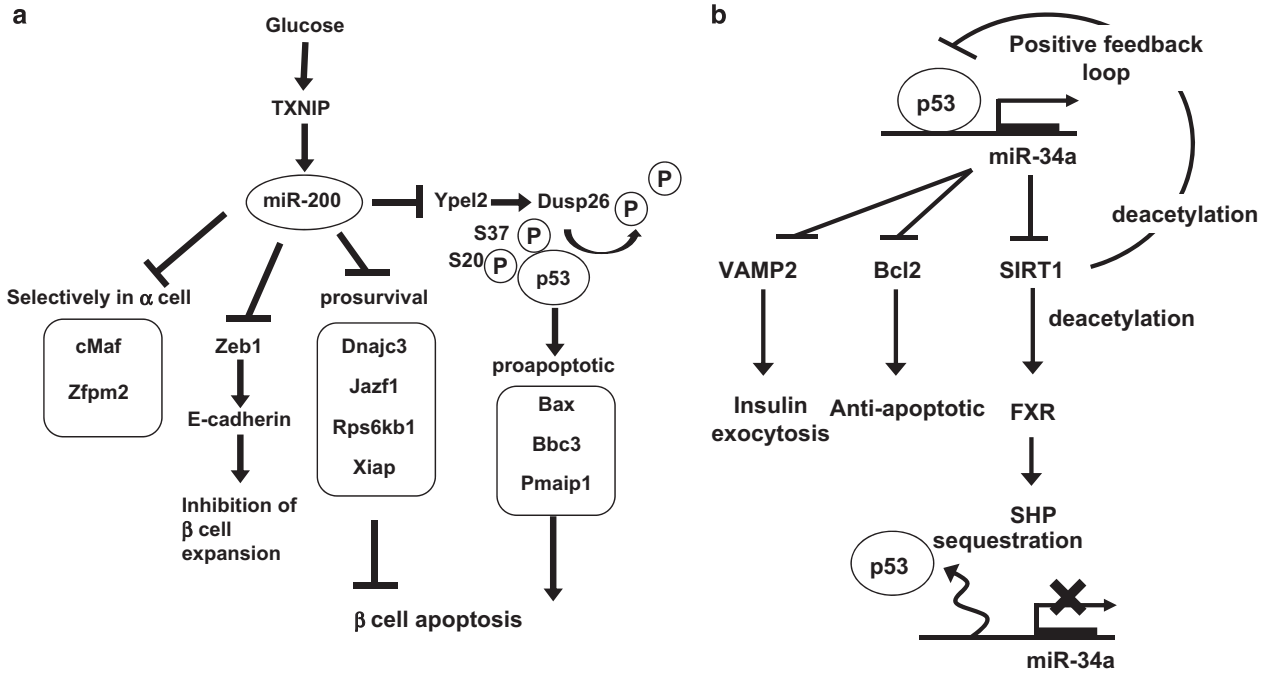

Figure 2 Signaling molecules that are associated with the microRNA (miRNA)/p53 pathway. (**a**) When miR-200 is induced by glucose or by diabetes, it targets *Ypel2*, which induces *Dusp26*. Downregulation of *Dusp26* results in phosphorylation and activation of p53, which then targets genes and facilitates β-cell apoptosis. Induced by TXNIP, miR-200 targets *Zeb1*, subsequently inhibits β-cell expansion via upregulation of E-cadherin. miR-200 also targets prosurvival genes as well as α-cell-specific transcription factors such as *cMaf* and *Zfpm2*. (**b**) p53 directly activates expression of miR-34a. miR-34a targets messenger RNA (mRNAs) encoding vesicle-associated membrane protein 2 (VAMP2), B-cell lymphoma 2 (Bcl2) and sirtuin (silent mating type information regulation 2 homolog) (SIRT1). SIRT1 binds to and deacetylates/inactivates p53, consequently miR-34a expression increases p53 acetylation. SIRT1 also deacetylates and activates FXR, resulting in activation of SHP mRNA, and SHP subsequently sequesters p53 from the miR-34a promoter.

transition via upregulation of E-cadherin.[40] The avian musculoaponeurotic fibrosarcoma oncogene homolog is a transcription factor that regulates glucagon expression, and the zinc-finger protein, multitype 2, is a transcription factor that inhibits PI3K signaling. Both are targets of miR-200c,[41] and exclusively expressed in pancreatic islet α cells. In addition, Belgardt *et al.*[42] demonstrated that miR-200c enhances the activity of Trp53 (p53), further promoting apoptosis. miR-200-induced type 2 diabetes is suppressed by interfering with signaling between Trp53 and its proapoptotic target gene, *Bax*. Loss of miR-200 in mice attenuates β-cell apoptosis and improves type 2 diabetes.[42] miR-200 targets the mRNAs encoding DnaJ heat-shock protein family (Hsp40) member C3 as well as JAZF zinc-finger 1 (namely *Jazf1*), ribosomal protein S6 kinase polypeptide 1 and X-linked inhibitor of apoptosis, all of which are antiapoptotic.[35] miR-200 modulates Trp53 activity by regulating several direct target genes, particularly *Ypel2*, which is necessary for full expression of dual-specificity phosphatase 26 (putative, *Dusp26*), encoding a phosphatase that, in turn, controls Trp53 activity.[43]

The level of miR-34a is elevated in islets of diabetic *db/db* mice.[44] miR-34a is a direct transcriptional target of p53 that induces apoptosis by both p53-dependent and -independent means.[45,46] Inhibition of miR-34a partially protects palmitate-treated MIN6B1 cells (another pancreatic β-cell line) from apoptosis.[44] miR-34a directly targets B-cell leukemia/lymphoma 2 (*Bcl2*); thus, miR-34a-induced suppression of *Bcl2* accounts for the palmitate-induced increase in apoptotic rate in pancreatic β cells.[47] miR-34a negatively regulates insulin secretion by targeting Vamp2, a protein that has an important role in insulin exocytosis.[44] miR-34a inhibits SIRT1 expression and, because SIRT1 can deacetylate/inactivate p53, miR-34a expression increases p53 acetylation. This further induces miR-34a, creating a positive feedback loop.[48] SIRT1 also deacetylates farnesoid X receptor (Fxr; Nr1h4), which is also known as nuclear bile acid receptor, and increases its activity.[49] FXR inhibits miR-34a expression in the liver.[50] FXR induces expression of Shp (orphan nuclear receptor and transcriptional corepressor small heterodimer partner),[51,52] which inhibits miR-34a expression by blocking p53 binding to the miR-34a promoter.[50] (Figure 2).

In addition, miR-375 is a negative regulator of p53, downregulating p53 and hence its target genes in cancer cells.[53] miR-29 upregulates p53 and induces apoptosis in a p53-dependent manner. miR-29 directly targets the p85α regulatory subunit of PI3K and cell division control protein 42, both of which negatively regulate p53.[54]

miRNAs AND INSULIN RESISTANCE IN TARGET TISSUES

Several lines of evidence indicate the involvement of miRNAs in altering gene expression that takes place in adipocytes of obese subjects. Expression of miR-29 is increased in skeletal muscle, liver and fat tissues of Goto–Kakizaki rats, an established model of type 2 diabetes.[55] Expression of miR-29 is induced with hyperglycemia or hyperinsulinemia in adipocyte-derived 3T3-L1 cells.[56] miR-29 expression is regulated in part by FOXA2, and miR-29 modulates FOXA2-mediated regulation of lipid-metabolism genes such as *PPARGC1A*, *HMGCS2* and *ABHD5*.[57] miR-29 overexpression in 3T3-L1 cells impairs insulin-induced glucose uptake, resulting in insulin resistance.[55] miR-29a and miR-29c are important negative regulators of insulin signaling in the liver through regulation of PI3K mRNA level.[58] miR-29a prevents insulin-mediated inhibition of the gene encoding phosphoenolpyruvate carboxykinase by directly targeting the mRNA encoding PI3K p85α subunit.[59] Expression of miR-320 is significantly upregulated in insulin-resistant 3T3-L1

adipocytes, and insulin resistance is ameliorated by treatment with an anti-miR-320 oligo.[60] miR-320 also contributes to changes in insulin sensitivity and targets p85, which is involved in the phosphorylation of the protein kinase Akt.

miR-27 is downregulated during adipocyte differentiation. miR-27a and miR-27b target peroxisome proliferator-activated receptor γ, which is a key regulator of adipocyte differentiation and adipogenesis.[61,62] miR-27 might have an important role in inducing insulin resistance along with hypertrophy of adipocytes or qualitative and/or quantitative alteration of adipokine expression in obesity.

miR-103 and miR-107 are expressed at high levels in obese mice, and silencing of these miRNAs improves insulin resistance in adipose tissue and liver. miR-103/107 expression in either fat or liver causes insulin resistance by targeting caveolin-1, an essential regulator of the insulin receptor (Insr).[63] miR-143 is upregulated in the mesenteric fat tissue of high-fat diet-induced obese mice and in the liver of *db/db* mice,[64,65] which are obese and diabetic as a result of mutations in the leptin receptor gene.[66,67]

Oxysterol-binding protein-related protein 8 is involved in AKT activation and is an miR-143 target regulated at the level of translation.[65] Insulin-stimulated AKT activation and consistent phosphorylation of the AKT substrate glycogen synthase kinase-3β were found to be reduced in oxysterol-binding protein-related protein 8 knockdown cells, demonstrating that miR-143 contributes to reduction of insulin sensitivity.[65]

In skeletal muscle, molecules in the insulin-PI3K-mTOR signaling pathways, such as *Insr*, insulin-like growth factor 1 receptor (*Igf1r*), *Irs2*, *Pik3ip1*, *Akt2*, *Tsc1* and *Rictor*, are downregulated by the miRNA let-7. Mice deficient for the muscle-specific RNA-binding protein Lin28a and inducible let-7 transgenic mice exhibit glucose intolerance.[68] Moreover, global knockdown of the let-7 family ameliorates insulin sensitivity in liver and muscle, in part by restoring Insr and Irs2 expression levels.[69,70] miR-1 and miR-133a are expressed specifically in muscle and regulated by insulin through sterol regulatory element-binding protein-1c and myocyte enhancer factor 2C.[71] In a set of experiments using human skeletal muscle, sterol regulatory element-binding protein-1c was found to be activated by insulin, with consequent downregulation of miR-1 and miR-133 by inhibiting myocyte enhancer factor 2C. Further, the response of miR-1 and miR-133a to insulin is impaired in skeletal muscle in type 2 diabetes, possibly owing to altered activation of sterol regulatory element-binding protein-1 c.[71,72]

miR-122 is one of the liver-specific miRNAs.[73] Inhibition of miR-122 with antisense oligonucleotides in mice fed a high-fat diet resulted in a reduction in hepatic steatosis accompanied by lowered plasma cholesterol level, increased hepatic fatty acid oxidation and decreased synthesis of hepatic fatty acids and cholesterol.[74,75] Esau *et al.*[74] demonstrated that miR-122 targets mRNAs encoding Gys1, Aldoa, Ccng1, P4ha1 and Slc7a1/Cat1. AMP kinase is also activated by miR-122, possibly through an indirect pathway.[74] In contrast, decreased miR-122 expression was observed in the liver of both *ob/ob* mice and streptozotocin-induced diabetic mice.[76] Protein tyrosine phosphatase 1B, which inhibits hepatic insulin signaling via dephosphorylating tyrosine residues in both Insr and Insr substrate, is another direct target of miR-122.[77] The reduction of miR-122 in nonalcoholic fatty liver disease and/or diabetic liver may partly contribute to the self-protection mechanism of liver cells in response to nutrient overload with lipids or glucose.[76] Taken together, these

Table 2 miRNAs associated with insulin resistance

miRNA	*Organ/tissue*	*Description*	*References*
let-7	Adipose/muscle	Targets *Insr*, *Igf1r*, *Irs2*, *Pik3ip1*, *Akt2*, *Tsc1* and *Rictor*; knockdown ameliorates insulin sensitivity	69
miR-1/133a	Muscle	Negatively regulated by insulin through SREBP1c and MEF2C	71
miR-21	Adipose	Inhibits *PTEN*, thereby leading to insulin resistance and steatosis	78
miR-27	Adipose	miR-27a and miR-27b inhibit adipogenesis by targeting *PPARγ*	61,62
miR-29	Liver/adipose/muscle	Promotes insulin resistance	55,56
miR-33a/b	Liver	Targets *CROT, CPT1a, HADHB, AMPKα, SIRT6* and *IRS2*	127
miR-34a	Liver	Contributes to insulin resistance by targeting *SIRT1*	50
miR-93	Adipose	Contributes to insulin resistance by targeting *GLUT4*	128
miR-96	Liver	Targets *IRS-1* and impairs insulin signaling	129
miR-103/107	Liver/adipose/muscle	Causes insulin resistance by targeting *Cav1*	63
miR-122	Liver	miR-122 inhibition reduces liver steatosis; targets *Gys1, Aldoa, Ccng1, P4ha1, Slc7a1/Cat1* and *PTP1B*	74,77
miR-126	Liver	Targets *IRS-1* and impairs insulin signaling	130
miR-130	Adipose	Represses adipogenesis by targeting *PPARγ*	131
miR-143	Liver/adipose	Reduces insulin sensitivity by targeting *Orp8*	65
miR-181a	Liver	Inhibition of miR-181a upregulates *SIRT1* and improves insulin sensitivity	132
miR-200	Liver	Downregulated by IL-6; impairs the PI3K/AKT/GSK pathway and glycogenesis	133
miR-221	Adipose	Develops insulin resistance by affecting PPARα and PPARγ signaling and by targeting *ADIPOR1*	134
miR-223	Muscle	Overexpression increases glucose uptake via inducing Glut4 expression	135
miR-320	Adipose	Promotes insulin resistance	60
miR-335	Liver/adipose	Induced by leptin, resistin, TNF-α and IL-6; functionally implicated in both fatty acid metabolism and lipogenesis	136
miR-378	Adipose	Induced by TNF-α; directly regulates adiponectin	137
miR-494	Muscle	Exacerbates insulin resistance by downregulating *Slc2A4*	138
miR-802	Liver	Attenuates insulin sensitivity by targeting *Hnf1b*	139

Abbreviations: ADIPOR1, adiponectin receptor 1; Aldoa, aldolase A, fructose-bisphosphate; AMPKα, AMP kinase subunit-α; Cav1, caveolin-1; Ccng1, cyclin G1; CPT1a, carnitine palmitoyltransferase 1 A; CROT, carnitine *O*-octaniltransferase; GLUT4, glucose transporter type 4; GSK, glycogen synthase kinase; Gys1, glycogen synthase 1; HADHB, hydroxyacyl-CoA-dehydrogenase; Hnf1b, hepatocyte nuclear factor 1β; Igf1r, insulin-like growth factor 1 receptor; IL-6, interleukin-6; Insr, insulin receptor; IRS-1, insulin receptor substrate 1; Irs2, insulin receptor substrate 2; MEF2C, myocyte enhancer factor 2C; miRNA, microRNA; Orp8, oxysterol-binding protein-related proteins; P4ha1, procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha 1 polypeptide; PI3K, phosphoinositide 3-kinase; Pik3ip1, PI3K-interacting protein 1; PPARγ, peroxisome proliferator-activated receptor γ; PTEN, phosphatase and tensin homolog; PTP1B, protein tyrosine phosphatase 1B; Rictor, RPTOR independent companion of MTOR complex 2; Slc2A4, solute carrier family 2 member 4; Slc7a1/Cat1, solute carrier family 7 (cationic amino acid transporter, y+ system), member 1; SIRT1, sirtuin 1; SIRT6, sirtuin 6; SREBP1c, sterol regulatory element-binding protein-1c; TNF-α, tumor necrosis factor α; Tsc1, tuberous sclerosis complex 1.

data suggest that miR-122 primarily functions in the regulation of glucose metabolism, possibly through multiple pathways.

miR-21 also has an important role in liver.[72] Unsaturated fatty acids promote nuclear factor-κB-mediated induction of miR-21. miR-21 downregulates phosphatase and tensin homolog, which represses Akt phosphorylation in hepatocytes. Liver-specific phosphatase and tensin homolog knockout in mice leads to insulin resistance and steatosis.[78] miR-21 is upregulated in liver of rats fed a high-fat diet and in liver biopsies of obese patients, consistent with downregulation of phosphatase and tensin homolog.[78] Table 2 lists the miRNAs associated with insulin resistance.[8,10]

Thus, considering that many miRNAs are tightly associated with the regulation of insulin resistance through various organs in a number of other human conditions that are affected by nutrient, metabolic and inflammatory status, the clinical development of potential miRNA-targeting therapeutics such as small molecules to manipulate as well as specifically inhibit miRNA expression and functions or challenges to ensure the specificity, efficacy and delivery strategies of therapeutic oligonucleotides *in vivo*, would provide novel insights into the therapeutic modalities and application for many diseases, particularly type 2 diabetes and obesity.

Many miRNAs are involved in regulating insulin resistance in various organs in a number of other human conditions that are affected by nutrient, metabolic and inflammatory status. Hence, the clinical development of potential therapeutics that target miRNAs would provide novel insights into therapeutic modalities and applications for many diseases, particularly type 2 diabetes and obesity. Such therapeutics could manifest as small molecules that modulate or specifically inhibit miRNA expression and/or functions. With respect to potential therapeutic oligonucleotides, studies are needed to address the challenges involved in ensuring the specificity, efficacy and delivery strategies of the oligonucleotides *in vivo*.

CIRCULATING miRNAs AS POTENTIAL BIOMARKERS

In the biogenesis of miRNAs, each primary miRNA is transcribed by RNA polymerase II and processed by the endonuclease Drosha to yield a precursor miRNA (pre-miRNA) hairpin of ~70 nucleotides. Subsequently, exportin-5 mediates transport of pre-miRNAs to the cytoplasm where they are further processed by the endoribonuclease Dicer to yield 20- to 22-nucleotide mature miRNA duplexes. Following strand separation, each miRNA is loaded into the RNA-induced silencing complex and binds to the 3′-untranslated region of a target mRNA, which guides mRNA degradation or repression of translation. Circulating miRNAs have been studied primarily as potential blood biomarkers of cancer.[79]

In addition to functioning intracellularly, miRNAs are associated with lipoprotein or bound to Argonaute-2 (the catalytic component of the RNA-induced silencing complex) and secreted by cells in exosomes.[80] Several circulating miRNAs are protected from endogenous RNase degradation,[79] are relatively stable in blood, and are taken up by cells. Circulating miRNAs are stable for up to ~24 h when plasma is left at room temperature, whereas miRNAs expressed exogenously in blood are rapidly degraded.[79] Circulating miRNAs are also stable when plasma is subjected to multiple freeze-thaw cycles.[79] Therefore, it has been suggested that miRNAs also function as intercellular signal transmitters.

Zampetaki *et al.*[81] reported altered expression of plasma miR-15a, miR-28-3p, miR-29b, miR-126 and miR-223 in patients several years before manifestation of type 2 diabetes. Plasma samples were obtained from a prospective population-based survey initially designed to investigate atherosclerosis in Italy.[81] Because circulating miRNAs are stable to freezing and thawing, expression levels in blood serve as biomarkers for predicting onset of type 2 diabetes. Another study analyzed blood samples of 265 individuals that included patients with metabolic syndrome, type 2 diabetes, hypercholesterolemia and hypertension, and observed upregulation of miR-150, miR-192, miR-27a, miR-320a and miR-375 in type 2 diabetes patients. Of these, levels of miR-27a and miR-320a correlated strongly with fasting glucose level.[82]

Nielsen *et al.*[83] reported the first comparison of miRNA levels in serum samples from children with or without type 1 diabetes. Those investigators identified 12 miRNAs (miR-152, miR-30a-5p, miR-181a, miR-24, miR-148a, miR-210, miR-27a, miR-29a, miR-26a, miR-27b, miR-25 and miR-200a) that were differentially expressed in patients with type 1 diabetes. Interestingly, some of the miRNAs are associated with apoptosis (miR-24, miR-25, miR-26a, miR-181a and miR-210)[84–86] and regulation of pancreatic β cells (miR-24, miR-29a, miR-148a and miR-200a).[83]

Osipova *et al.*[87] measured miRNA levels in plasma and urine of patients with type 1 diabetes and observed upregulation of miR-21 and miR-210 in both fluids. In contrast, miR-126 level was reduced in urine; notably, miR-21 has been reported to promote kidney fibrosis.[88,89] miR-126 also promotes angiogenesis by targeting Spred-1 via modulation of MAP kinase signaling.[90,91] These circulating miRNAs may be valuable for identifying patients at high risk of developing diabetic complications as well as being potential therapeutic targets.

INVOLVEMENT OF miRNAs IN REGULATING GENES ASSOCIATED WITH SUSCEPTIBILITY TO TYPE 2 DIABETES

Technology to identify genetic determinants of type 2 diabetes and other diseases has evolved dramatically in recent years. Precise information about single-nucleotide polymorphisms (SNPs) is now available owing to advances in high-throughput genomic sequencing technology and the International HapMap project (URL https://hapmap.ncbi.nlm.nih.gov). In recent years, GWAS, which examine SNPs across the genome, have identified more than 80 genes associated with susceptibility to type 2 diabetes.[12]

Many polymorphisms implicated in type 2 diabetes susceptibility have been reported in the noncoding regions of these susceptibility genes.[92–94] Although many genes or polymorphisms associated with type 2 diabetes susceptibility have been identified, the mechanism(s) by which they promote susceptibility remains unknown. Genetic polymorphisms in the 3′-untranslated regions of mRNAs targeted by miRNAs may destroy or create miRNA-binding sites, potentially resulting in susceptibility to type 2 diabetes and disease development.[92]

On the basis of GWAS, Van de Bunt *et al.*[13] identified miRNAs that regulate type 2 diabetes susceptibility genes and, therefore, may constitute part of the genetic basis for type 2 diabetes. The primary pathophysiological feature of diabetes is insufficient insulin secretion, and many of the identified susceptibility genes are associated with this process. miRNAs that are typically expressed in pancreatic β cells have been identified by RNA sequencing and compared with expression profiles of pancreas-specific miRNAs in type 2 diabetes.[13] Total RNA was extracted from islets of six donors and analyzed by next-generation sequencing, and 384 miRNAs were identified. Comparison of the β-cell miRNA profile with those of other human tissues revealed 40 miRNAs that are expressed at high levels specifically in β cells. These include miR-375, a direct regulator of insulin secretion, miR-27b-3p and miR-192-5p, whose association with diabetes had not been reported. Intriguingly, a large number of the genes that were identified as potential targets of these β-cell-specific miRNAs

(based on miRNA target prediction or an algorithm) were among those previously identified as type 2 diabetes susceptibility genes in GWAS. Actually, GWAS data have been intensively analysed using the data of the diabetes genetics replication and meta-analysis (DIAGRAM) consortium.[95] Indeed, several type 2 diabetes susceptibility loci identified through DIAGRAM overlapped with the genomic region encoding islet-expressed pre-miRNAs. Although maximum accuracy has not been attained at this point, much effort has been expended to identify sequences recognized by miRNAs and sequences to which miRNAs bind using algorithms such as TargetScan, miRanda, and miRDB to predict binding sites.[96–98] Most notably, pancreatic β-cell-specific miRNA target sites were identified in type 2 diabetes susceptibility genes, such as AP3S2, CNK16, NOTCH2, SCL30A8, VPS26A and WFS1, whose polymorphisms are associated with decreased insulin secretion.[13]

SNPs occur relatively rarely in pre-miRNA sequences, having been documented in only 10% of human pre-miRNAs, and SNPs in the functional seed region have been identified in $<1\%$ of mature miRNAs.[99] Polymorphisms in primary miRNAs may alter secondary structure and affect miRNA processing efficiency, which may contribute to pathogenesis of type 2 diabetes.[92] Ciccacci *et al.*[100] reported two polymorphisms in a miRNA associated with type 2 diabetes susceptibility. The G allele of rs895819 in hsa-mir-27a exhibits a significant protective effect, whereas the G allele of rs531564 in hsa-mir-124a appears to be a risk allele[100] that alters the secondary structure of pri-miR-124. The latter polymorphism might alter primary miRNA stability, efficiency of primary miRNA processing to pre-miRNA. Furthermore, the G allele is associated with an elevated level of mature hsa-miR-124. Finally, the rare SNP rs72631823 in pre-miR-34a is associated with an elevated level of mature miR-34a in pancreatic β cells, which is associated with β-cell apoptosis.[101]

These data support the relevance of polymorphisms in miRNAs or miRNA-binding sites on target mRNAs to diabetes susceptibility. Moreover, these findings suggest that miRNAs contribute, at least in part, to the pathophysiology of diabetes. As DIAGRAM previously focused primarily on samples from persons of European descent, future research using samples from ethnically diverse populations will be necessary to demonstrate the reproducibility of these results and determine whether the findings can be applied across ethnic groups.

FUTURE PERSPECTIVES ON miRNAs IN DIABETES

Although many diagnostic markers for diabetes have been identified and new medicines developed, the number of diabetes patients is increasing worldwide. The number of diabetes patients continues to increase worldwide, and hence many diagnostic markers for diabetes have been identified and new medicines developed. Still, effective therapeutic agents are needed for those who do not respond adequately to current therapies. The basic approach for treatment of diabetes with a miRNA is to normalize aberrant miRNA expression. For example, antisense oligonucleotides can be utilized to specifically bind miRNA sequences to prevent binding to the target; alternatively, functionally inert miRNA-mimics can also be used that comprise the same nucleotide sequences as the endogenous miRNA.[10] The best known therapeutic that targets a specific miRNA is miravirsen, an antisense inhibitor of miR-122 produced to reduce hepatitis C viral RNA levels in patients with chronic hepatitis C infection.[102] Controlling pathological upregulation of miRNAs using antisense oligonucleotides may also restore metabolic homeostasis. Inhibition of miR-122 has been associated with improved steatosis in a mouse model of diet-induced obesity, suggesting that miR-122 antagonism may be useful in treating nonalcoholic fatty liver disease.[74] Conventional drugs can also correct dysregulated miRNA expression. For example, miR-29 is upregulated in liver of mice with diet-induced insulin resistance, and the insulin-sensitizing drug pioglitazone reverses miR-29 upregulation in the Zucker diabetic fatty rat model of diabetes.[57]

CONCLUDING REMARKS

Numerous observations have deciphered a wide variety of the functional roles of miRNAs in the pathophysiology of the metabolic disease and diabetes mellitus, as well as in the process of establishing and/or maintaining β-cell identity and its functions. Although thousands of miRNAs have been identified in both mouse and humans and a few dozen miRNAs, including even not mentioned in this review, have been reported to be associated with diabetes, the information concerning their precise roles is still largely veiled and thereby should be revealed in the future. Identification of miRNA targets is one of the most challenging tasks because a single miRNA has hundreds of potential mRNA targets. Conversely, several different miRNAs can cooperatively or differentially control a single mRNA target. To render the situation even more multifaceted, it is now established that miRNAs can have both positive and negative roles in the regulation of gene expression and that their area of intervention on gene expression is not limited to the cytosol, but expands to the nucleus. Furthermore, circulating miRNAs *in vivo* have recently emerged as potential biomarkers for the degree of pathophysiological conditions such as type 1 and type 2 diabetes, regulation of pancreatic β cells and severity of complications. These thought-provoking features of the miRNA world are adding new layers of complexity to mechanistic insights into the underlying pathogenesis and susceptibility of diabetes mellitus, particularly roles in β-cell function and insulin resistance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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