Published in final edited form as:

Pharmacol Res. 2013 September ; 75: 37–47. doi:10.1016/j.phrs.2013.06.005.

MicroRNAs as Pharmacological Targets in Diabetes

Yiping Mao, Ramkumar Mohan, Shungang Zhang, and Xiaoqing Tang

Dept. of Biological Sciences, Michigan Technological University, Houghton, MI 49931

Abstract

Diabetes is characterized by high levels of blood glucose due to either the loss of insulin-producing beta-cells in the pancreas, leading to a deficiency of insulin in type 1 diabetes, or due to increased insulin resistance, leading to reduced insulin sensitivity and productivity in type 2 diabetes. There is an increasing need for new options to treat diabetes, especially type 2 diabetes at its early stages due to the ineffective control of its development in patients. Recently, a novel class of small noncoding RNAs, termed microRNAs (miRNAs), found to play a key role as important transcriptional and posttranscriptional inhibitors of gene expression in fine-tuning the target messenger RNAs (mRNAs). miRNAs are implicated in the pathogenesis of diabetes and have become an intriguing target for therapeutic intervention. This review focuses on the dysregulated miRNAs discovered in various diabetic models and addresses the potential for miRNAs to be therapeutic targets in the treatment of diabetes.

Keywords

microRNAs; diabetes; insulin resistance; miRNA therapeutics

1. Introduction

MicroRNAs (miRNAs) are 21–23 nt non-coding RNAs that directly bind to the 3' untranslated region (3'-UTR) of complementary mRNA targets and consequently results in an inhibition of the targeted gene expression [1, 2]. More than 2000 miRNA genes have been reported in the human genome. Many microRNAs are evolutionarily conserved and ubiquitously expressed, but some of them are restricted to specific tissues in which they play specific roles. In animals, a single miRNA can target multiple functionally related or different mRNAs in different physiological processes or cell types [3]. Likewise, a single mRNA may contain multiple miRNA binding sites at its 3' UTR, allowing a coordinated regulation by various miRNAs under different physiological conditions. In contrast to the conventional drug that therapeutically modulates a single target gene, miRNAs can simultaneously modulate multiple genes at multiple levels of a pathological process, giving a bigger potential to be pharmacological targets for intervention against complex diseases like diabetes.

Diabetes mellitus is a disorder of dysregulated glucose homeostasis [4]. Glucose homeostasis requires coordinated metabolic regulation among pancreas and insulin targeted organs (adipose, muscle, liver and brain) (Fig. 1) [5]. In pancreas, β -cells and α -cells secrete insulin and glucagon, respectively in response to the change of blood glucose. While high

Correspondence to: Xiaoqing Tang.

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glucose-stimulated insulin increases glucose uptake, utilization and storage in fat and muscle, low glucose-stimulated glucagon acts in liver to promote hepatic glucose production to raise blood glucose level [6]. Adipose fat cells release adipokines (leptin, adiponectin and cytokines) and free fatty acids (FFAs), which regulate food intake, insulin secretion and insulin sensitivity [7]. In addition, liver can produce signals (such as *Betatrophin*) to regulate β -cell proliferation under certain conditions [8–12]. Thus, crosstalk among these organs/tissues is extremely important and perturbation of this control system may lead to the development of diabetes. In both type 1 and type 2 diabetes, the patients develop common progressive complications such as heart attacks and strokes, blindness, diabetic nephropathy and kidney failure [13–16]. Therefore, prevention and treatment of diabetes, especially type 2 diabetes, in early stages are required.

To date, drug therapy remains the most powerful way of slowing the progression of type 2 diabetes [17, 18]. The anti-diabetic drugs may target adipose and muscle tissue to reduce insulin resistance, act on liver to inhibit glucose production, or stimulate the pancreas to release insulin [19]. However, these drugs can help diminish the effects of diabetes, but can't completely halt the progression of the disease. The need for new diabetes drugs remains acute. The function of miRNAs that target multiple genes to release insulin resistance and to improve pancreatic beta cell function has good potential to be developed into pharmacological targets to treat diabetes.

Recent reports indicate that miRNAs play important roles in control of insulin biosynthesis and release [20], pancreatic β -cell development and survival [21, 22], glucose and lipid metabolism [23] and their involvement in secondary complications associated with diabetes [24, 25]. miRNA dysregulation has been observed in diabetic subjects in both human and animal, implicating a role of miRNAs in diabetes pathogenesis [20, 26–32]. In this review, we outline important dysregulated miRNAs that have been observed in β -cells, adipose, muscle and liver of various diabetic models for discussions regarding their potential roles and therapeutic interventions in diabetes (Fig. 1).

2. miRNAs in regulating insulin biosynthesis, insulin secretion and β -cell survival in pancreatic β -cells

β -cell loss and dysfunction are hallmark for both type 1 and type 2 diabetes. In β -cells, insulin biosynthesis and secretion is tightly regulated by a network of transcriptional activators and repressors that maintain the fate of β -cells and activate genes in response to the change of plasma glucose [33]. The discovery of miRNAs has added a novel regulatory layer on modulating the level of key components involved in β -cell differentiation and proliferation, insulin biosynthesis and secretion, and β -cell survival and regeneration [34–37]. The major miRNAs that have been reported to play a role in pancreatic beta cells are outlined and discussed below (Table 1).

miR-375

miR-375 is the most abundant miRNA in β -cells and was one of the first miRNAs elucidated as a key factor regulating insulin secretion [20]. Overexpression of miR-375 not only reduces insulin secretion by targeting Myotrophin (MTPN) [20], but also represses 3-phosphoinositide-dependent protein kinase-1 (PDK1) and attenuates insulin gene transcription [38]. Indeed, elevated miR-375 level was revealed in islets of obese diabetic mice (*ob/ob*) [26] and human subjects with type 2 diabetes [31]. However, El Ouaamari reported that miR-375 expression is decreased in the islets of diabetic Goto-Kakizaki (GK) rats which is contradictory and needs further verification [38].

While miR-375 negatively regulates insulin secretion and transcription, miR-375 is required for maintaining β -cell mass [26]. Mice lacking miR-375 are hyperglycemic, exhibit increased pancreatic β -cell mass, plasma glucagon levels, and decreased α -cell mass, indicating miR-375 is required to be optimal for normal glucose homeostasis by maintaining normal β -cell and α -cell mass ratio [26]. When miR-375 is deleted in *ob/ob* mice, they develop a marked decrease in β -cell mass, which induces severe insulin-deficient diabetes not found normally in *ob/ob* mice [26].

In addition, miR-375 plays an important role in embryonic pancreas development. First, miR-375 is highly and specifically up-regulated in the later stages of pancreatic development [21]. Second, the promoter elements (E-boxes and TATA sequences) of miR-375 gene have a role in the selective expression of miR-375 in β - and α -cells, but not in other types of endocrine or non-pancreatic cells [39, 40]. Finally, expression of miR-375 is also under the control of Pdx-1 and NeuroD/Beta2, two critical transcription factors for the development of the endocrine pancreas and the production of insulin [41]. Accordingly, targeted inhibition of miR-375 in zebrafish resulted in major defects in pancreatic islet development [21]. miR-375 is expressed at high levels during human pancreatic islet development although non-beta cells seems contain higher levels of miR-375 too [42]. Roles of miR-375 in regulating pancreas development suggest that miR-375 is likely to play important roles in β -cell regeneration. Indeed, a new study reports that miR-375 expression was critical for differentiation of human embryonic stem cells into insulin-producing cells (IPCs) [43].

Interestingly, miR-375 has recently been detected in the plasma and the increase of circulating miR-375 is strongly associates with the onset of hyperglycemia, which will be discussed more below [30, 44]. Taken together, islet enriched miR-375 have multiple functions in β -cells, including insulin transcription and secretion, β -cell proliferation and regeneration. Therefore, miR-375 is likely the most promising pharmacological target for diabetes treatment.

miR-7

While miR-375 is the most abundant islet miRNA, miR-7 is the most abundant endocrine miRNA [45–47]. miR-7 controls the differentiation and maturation of pancreas by targeting PAX6, a key transcription factor for the development of pancreas [48]. Silencing of miR-7 increases Pax6 expression and promotes β -cell and α -cell differentiation [48]. In addition, the endocrine specificity of miR-7 is governed by a network of pancreatic transcription factors including Neurogenin-3 (Ngn3) and NeuroD/Beta2 [47], implicating miR-7 as a key target in fine-tuning the β -cell development and could be utilized for β -cell regeneration. β -cell can be regenerated from various cells such as embryonic stem cells, α -cells and ductal precursor cells [49, 50], or reduplicated from pre-existing β -cells [51]. Indeed, both miR-7 and miR-375 display dynamic expression pattern during the differentiation of human embryonic stem cells into insulin-producing cells [43].

miR-7 is not only important in pancreas development, but also acts as a brake on β -cell proliferation. Wang and colleagues recently revealed that miR-7 inhibit adult β -cell replication by targeting multiple components related to the mammalian target of rapamycin (mTOR). In this study, the authors tested and validated five miR-7 direct targets including p70S6 kinase (p70S6K), eukaryotic translation initiation factor 4E (eIF4E), MAPK-interacting kinases MAP kinase-interacting serine/threonine-protein kinase 1 and 2 (MKNK1 and MKNK2), and mitogen-activated protein kinase 2-associated protein 1 (Mapkap1) [52]. All of these targets are suppressed by miR-7 at the protein translational levels. Most importantly, this miR-7-mTOR proliferation axis is conserved in primary human β -cells, suggesting miR-7 could be as a therapeutic target for diabetes. The

highlighted importance of miR-7 suggests that modulation of miR-7a expression could be utilized in the control of the development of diabetes.

miR-29a/b/c

miR-29a/b/c expression is increased in the islets isolated from prediabetic non-obese diabetic (NOD) mice [27]. Proinflammatory cytokines or high glucose can trigger the increase of miR-29a/b/c in islets and MIN6 cells [27, 53]. Nevertheless, miR-29a promoter contains potential binding sites for nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), a transcription factor known to contribute to cytokine-mediated β -cell dysfunction and to the development of type 1 diabetes [54], and that may be responsible for the induction of miR-29 family in NOD mice.

During the initial phases of type 1 diabetes, the increased miR-29a/b/c promotes cytokine-mediated β -cell apoptosis by targeting antiapoptotic myeloid leukemia cell differentiation protein (MCL1) mRNA [27, 55]. Accordingly, selectively silencing of miR-29 protects β -cells against cytokine-induced apoptosis [27]. miR-29a/b/c overexpression also downregulates insulin secretion by targeting the transcription factor one cut domain family member 2 (Onecut-2), which, in turn, activates granuphilin, a known inhibitor of insulin exocytosis [27].

However, the normal expression of miR-29a/b/c in the β -cell is required for glucose-stimulated insulin secretion by silencing membrane monocarboxylate transporter (MCT1) [53]. MCT1 is widely expressed in other tissues but repressed (disallowed) in β -cells [56]. Inappropriate overexpression of MCT1 causes inappropriate insulin release (hyperinsulinism), which is observed in the rare genetic disorder physical exercise-induced hypoglycemia [56]. Most importantly, miR-29a/b/c selectively target both human and mouse MCT1, demonstrating that miR-29a/b/c contribute to the β -cell-specific silencing of the MCT1 transporter and may provide a new therapeutic strategy for some forms of type 2 diabetes.

miR-21

Similar to miR-29 family, proinflammatory cytokines also trigger the activation of miR-21 [28]. The level of miR-21 is abnormally elevated in the islets isolated from NOD and *db/db* mice [28, 57, 58]. Blocking miR-21 using antisense molecules prevents the cytokine-reduced insulin secretion and protects islets from cytokine-triggered cell death [28]. However, the molecular mechanisms of miR-21 in β -cell function have not been fully elucidated. On one hand, the cytokine-induced miR-21 impairs insulin secretion by blocks the expression of Piccolo (PICO), which is a Ca²⁺ sensor involved in insulin secretion in islets [59]. On the other hand, the increase of miR-21 prevents cytokine-mediated β -cell death by decreasing its target, programmed cell death protein 4 (PDCD4) [60]. PDCD4 induces β -cell death and *Pdcd4* deficiency prevents Streptozotocin (STZ) induced β -cell loss [60]. These data suggest that miR-21 targets both PICO and PDCD4 that might have divergent effects to β -cells.

Most importantly, miR-21 is one of the most upregulated miRNA in response to the generated inflammation during islet transplantation [58]. Inflammation contributes to immune rejection in islet transplantation and causes β -cell dysfunction and death [61]. The discovery of miR-21 involved in immune rejection in transplanted islets will be important for developing new molecular therapies to enhance β -cell function and survival after transplantation.

Additionally, miR-21 has been validated as an important therapeutic target in cancer and cardiovascular disease [62]. miR-21 upregulation is associated with the development of

heart disease and heart failure [63–65]. miR-21 is also an “oncomiR” and highly enriched in many human cancers and can be detected in serum samples from cancer patients [62]. Therefore, miR-21 can serve as a biomarker for cancer diagnosis and prognosis. Low plasma levels of miR-21 have also been detected in patients with type 2 diabetes [66], which is not in agreement with the increased miR-21 in the islets of *db/db* mice and therefore requires further investigation.

miR-9

Like miR-29, miR-9 also negatively regulates glucose-induced insulin secretion in β -cells by directly targeting Onecut-2, a transcription repressor of the negative insulin release controlling factor granuphilin [67]. More interestingly, the change of miR-9 expression level is in a perfect coordination with the level of insulin release in response to glucose challenge in mice [68]. After intraperitoneally administration of glucose in mice to stimulate insulin secretion, miR-9 level in isolated islets was increased significantly by 60 min post-glucose injection, coinciding with the time point at which insulin levels started to decline [68]. miR-9 controls glucose-challenged insulin secretion by directly targeting and down-regulating Sirtuin 1 (SIRT1) expression [68]. Sirt1 is an NAD-dependent protein deacetylase and has been well known to play a crucial role in insulin secretion in response to glucose challenge [69, 70].

miR-124a

miR-124a is preferentially expressed in brain and pancreas, which is another one of important miRNAs in regulating pancreas development [22, 71, 72]. miR-124a expression is strikingly increased at e18.5 compared with e14.5, two key stages of mouse embryonic pancreas development [22]. miR-124a targets gene encoding forkhead box protein A2 (Foxa2), which is essential in β -cell differentiation and pancreas development [22]. However, miR-124 was not detectably expressed in mature mouse islets [53].

In pancreatic β -cell line MIN6 and INS-1 cells, miR-124a is a glucose-induced miRNA. Overexpression of miR-124a downregulates glucose-stimulated insulin secretion by repressing Foxa2, which in turn decreases levels of Sur-1 and Kir6.2, ATP-sensitive K(+) channel subunits [22]. Thus, impeding the glucose-stimulated miR-124a circuit disrupts the auto-regulatory machinery of insulin secretion. Accordingly, miR-124a was reported to target Ras-related protein Rab-27A (Rab27a), a well-known exocytotic Rab protein involved in insulin exocytosis machinery for insulin secretion [73].

miR-30d

Different from the various miRNAs involved in insulin secretion, only a few miRNAs have been reported to regulate insulin biosynthesis [74, 75]. Melkman-Zehavi reported that a set of miRNAs including miR-24, miR-26, or miR-148 stimulate insulin promoter activity and insulin mRNA levels by targeting transcriptional repressors Bhlhe22 and SOX6 in cultured β -cells or in isolated primary islets [74]. On the other hand, miR-30d stimulates insulin production through downregulating mitogen-activated protein 4 kinase 4 (MAP4K4), and in turn activating β -cell specific transcription factor v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) [29]. MAP4K4 is a cytokine-inducible kinase and activated MAP4K4 suppresses insulin production and secretion [29, 76]. Genetic variation of MAP4K4 is associated with insulin resistance and β -cell failure in human prediabetic patients [77].

The miR-30d is decreased in the islets of diabetic *db/db* mice [29], implying a role of miR-30d in diabetes pathogenesis. Indeed, miR-30d overexpression partially restores MafA protein level and insulin signaling inhibited by tumor necrosis factor- α (TNF- α) in

pancreatic β -cells [29, 75]. Different from other insulin transcription factors, MafA is significantly degraded at the early stage of diabetes, which results in a serious disruption of β -cell function in the development of type 2 diabetes [78, 79]. Thus, miR-30d is a good target for therapeutic interventions in term of the impact in preventing MafA degradation and restoring β -cell function.

In addition, miR-30 family members (including miR-30a, b, c, d and e) are responsible for epithelial-to-mesenchymal transition of primary cultures of human pancreatic epithelial cells, an important event in development of pancreatic islets [80, 81]. miR-30 family is required for maintaining the epithelial phenotype and forced deletion leads to mesenchymal transition. On the other hand, the expression of miR-30 family increase during differentiation of pancreatic islet-derived mesenchymal cells into hormone-producing islet-like cell aggregates [80, 81]. Together, the data suggest that manipulation of miR-30 levels may provide novel strategies to generate insulin-producing cells from pancreatic progenitors or stem cells for diabetes therapy.

miR-338-3p

Pregnancy and obesity are frequently associated with β -cell mass expansion. miR-338-3p expression is strongly downregulated during pregnancy in rats and correlated with β -cell mass expansion [82]. Additionally, expression of miR-338-3p is also reduced in young prediabetic *db/db* mice and in high-fat diet fed mice, suggesting a reduction of miR-338-3p is an important requirement in compensatory β -cell mass expansion occurring under insulin resistant states [82].

miR-338-3p targets the G protein-coupled estrogen receptor 30 (GPR30) and the glucagon-like peptide 1 (GLP1) receptor, which are known to promote β -cell proliferation and protect β -cells against apoptosis [82]. Inhibition of miR-338-3p in beta cells using specific anti-miR molecules activates both GPR30 and GLP1, and therefore increases β -cell proliferation and survival [82]. Interestingly, the GLP1 analog exendin-4 significantly downregulates the level of miR-338-3p, suggesting an involvement of miR-338-3p in incretins-mediated β -cell proliferation [82]. Taken together, miR-338-3p is a good target for therapeutic interventions in protecting β -cells against apoptosis. More evidence is required to confirm the change of miR-338-3p in human pregnancy subject.

Taken together, differential expression of miRNAs is necessary for β -cells to adjust to metabolic changes caused by hyperglycaemia, hyperinsulinemia, or obesity. Future studies should address how these microRNAs cooperate with each other to maintain the essential functions of β -cells.

3. miRNAs in regulating insulin sensitivity in skeletal muscle and adipose tissue

Insulin markedly stimulates glucose utilization in skeletal muscle and adipose tissue to remove glucose from the blood. While adipose tissue accounts for 10% of insulin-stimulated glucose uptake, skeletal muscle has been reported to account for 70–75% of insulin-stimulated glucose disposal. Disordered fat storage and metabolism in skeletal muscle and adipose tissues disrupts important signaling pathways and reduces insulin sensitivity, which directly contribute to the development of insulin resistance and obesity [5]. miRNAs can accelerate or inhibit the proliferation and differentiation of adipocyte and muscle [83–85]. In addition, miRNAs may regulate adipogenic lineage commitment in multipotent stem cells and hence govern fat cell numbers [86]. Abnormal expression of certain miRNAs in skeletal muscle and adipose tissues is associated with the development of obesity [84, 87]. Therefore, understanding the role of miRNAs in the development of adipocytes and muscle

could investigate new therapeutic targets for anti-diabetic drugs. In addition, identifying dysregulated miRNAs during the development of obesity could provide early obesity biomarkers for clinical diagnosis.

miR-143

miR-143 has been shown to accelerate adipogenesis and regulates lipid metabolism in both human and rodents [83, 88, 89]. Overexpression of miR-143 in preadipocytes increases the expression of adipocyte differentiation markers such as CCAAT/enhancer binding protein (C/EBP)- β , peroxisome proliferator activated receptor- γ (PPAR γ), adipocyte fatty acid binding protein 4 (FABP4) and leptin [83, 87, 90]. In contrast, miR-143 silencing attenuates adipogenesis [87, 88].

miR-143 alteration may contribute to the development of obesity [87, 89, 91]. Several reports revealed that increased miR-143 level in adipocytes is associated with the development of obesity or an elevated body weight in mice fed with a high-fat diet [87, 89], although one study indicated that miR-143 is decreased in ob/ob mice in contrast to control mice [88].

In addition, miR-143 is also up-regulated in the liver of ob/ob mice or high-fat diet treated mice [91]. Overexpression of miR-143 in transgenic mice impairs insulin sensitivity by targeting oxysterol-binding-protein-related protein 8 (ORP8), and further inhibiting insulin-stimulated activation of AKT, a serine–threonine kinase also known as protein kinase B (PKB) [91]. Conversely, miR-143 knock-out mice attenuate the development of obesity [91], suggesting miR-143 as a potential therapeutic target for the treatment of obesity-associated diabetes.

miR-29a/b/c

As discussed previously, miR-29a/b/c negatively regulate insulin secretion and β -cell survival [27]. miR-29a/b/c are not only enriched and increased in islets of prediabetic NOD mice, but also abnormally induced in three insulin-dependent tissues (i.e. skeletal muscle, liver and fat) from diabetic GK rats [92, 93]. miR-29a/b/c expression is highly upregulated by hyperglycemia and hyperinsulinemia in adipose tissue [94], suggesting that miR-29 family may increase insulin resistance in type 2 diabetes. Indeed, overexpression of miR-29a/b/c largely represses insulin-stimulated glucose uptake through inhibiting p85 and Akt involved insulin signaling in 3T3-L1 adipocytes [92, 95].

In addition, abnormal induction of miR-29c was also observed in the kidney of *db/db* mice that are defect in leptin receptor [96]. Induced miR-29c promotes cell apoptosis by directly targeting Sprouty homolog 1 (SPRY1), an antagonist of fibroblast growth factor (FGF) signaling. Knockdown of miR-29c using antisense inhibitor reduced kidney extracellular matrix protein accumulation in the *db/db* mice *in vivo* [96]. Taken together, cytokine or glucose stimulated miR-29c plays a broader role by repressing different targets in insulin producing beta cells or insulin target tissues, indicating miR-29 family can be a potential therapeutic target in preventing multiple diabetic complications including insulin resistance, type 2 diabetes or diabetes associated nephropathy [96].

miR-320

Similarly, miR-320 expression is highly increased in insulin resistant adipocytes [97]. Blocking miR-320 using antisense oligos increases insulin-stimulated glucose uptake by increasing the p85 subunit expression of phosphatidylinositol 3-kinase (PI3-K), AKT phosphorylation and thus glucose transporter type 4 (GLUT4) protein expression [97]. Upregulation of miRNA-320 was also observed in microvascular endothelial cells from

diabetic GK rats and thus application of miR-320 inhibitor may be a therapeutic approach for treating impaired angiogenesis in diabetes [98].

MyomiRs: miR-1, miR-133, and miR-206

Due to their muscle-specific expressions, miR-1, miR-133, and miR-206 are commonly termed myomiRNAs (myomiRs), having specific roles in the development of skeletal muscle and in the regulation of insulin sensitivities. miR-1 and miR-133 have distinct roles in modulating skeletal muscle proliferation and differentiation [99]. miR-1 and miR-133 are clustered on the same chromosomal locus and transcribed together in a muscle tissue-specific manner during development. Myocyte enhancer factor 2C (MEF2C), an essential regulator of muscle development, directly activates the transcription of miR-1 and miR-133 [100].

Normally, insulin represses the transcription of miR-1 and miR-133a in human skeletal muscle by inhibiting MEF2C [101]. However, the repression of miR-133a and miR-1 in response to insulin levels is impaired in the skeletal muscle of diabetic patients, which contributes to the impairment of MEF2C and sterol regulatory element-binding protein 1c (SREBP-1c, a transcription factor that binds to the sequence motif TCACNCCAC) in diabetic patients [101]. A recent study has shown that the expression of miR-133a is strongly downregulated in the skeletal muscle of type 2 diabetic patients, and altered levels of this miRNA correlated with higher fasting glucose levels and other important clinical parameters including homeostasis model assessment 1 (HOMA1, index of insulin sensitivity) and glycosylated hemoglobin (HbA1c, estimated average glucose level over the previous 3 months) [102]. Thus these miRNAs are good therapeutic targets for improving insulin sensitivity at the early stage of type-2 diabetes.

Similarly, miR-206 is another muscle specific miRNA and induces muscle cell differentiation. A decrease of miR-206 was observed in the type 2 diabetic patients when compared with the glucose-tolerant patients and healthy subjects [103]. miR-206 is significantly upregulated by high glucose and promotes cardiomyocyte apoptosis by miR1/miR206 mediated suppression of heat shock protein 60 (Hsp60), an important protector against diabetic myocardial injury [104].

Among the three myomiRs, the most promising biomarker and therapeutic target in clinical applications might be the miR-133, a miRNA that can determine the fate of the bipotent stem cells called skeletal muscle satellite cells or myosatellite cells. These small mononuclear progenitor cells have recently been found not only giving rise to new muscle cells, but also are able to differentiate into brown adipocytes by simply switching off miR-133 [105]. miR-133 binds to the mRNA 3' UTR of the transcriptional regulator PRDM16 (PRD1-BF1-RIZ1 homologous domain containing 16), a well recognized brown fat/skeletal muscle switcher [106, 107]. Loss of miR-133 function turns satellite cells into brown adipocytes [105]. Thus, miR-133 becomes a key switcher of fat/muscle differentiation.

4. miRNAs in regulating glucose and lipid metabolism in Liver

The presence of glucagon secretion critically regulates hepatic glucose output by controlling the pathways of gluconeogenesis, glycolysis and glycogenolysis in liver [108]. Any metabolic imbalances such as increased de novo lipogenesis, reduced fatty acid oxidation or impaired triglyceride secretion from liver often leads to abnormal glucose levels within the body. miRNA deregulation in liver can alter glucose and lipid metabolism and promote the progression of diabetes [109].

miR-103/107

The sequences of mature miR-103 and miR-107 differ by one nucleotide at position 21 and both miRNAs levels are most upregulated in the livers of both *ob/ob* mice and diabetic GK rats [90, 93]. Furthermore, the expression of miR-103/miR-107 is also increased in liver biopsies from diabetes-associated human patients including alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), indicating an association of these miRNAs with insulin resistance [110]. Silencing of miR-103/107 with antagomirs decreases hepatic glucose production and increases glucose uptake in adipose tissue, leading to an improved glucose homeostasis and insulin sensitivity in both *ob/ob* and diet-induced obesity (DIO) mice. In contrast, gain of miR-103/107 function with recombinant adenovirus expressing miR-103 or miR-107 enhances hepatic glucose production and decreases insulin sensitivity [90].

One of the mechanisms by which miR-103/107 negatively regulate insulin sensitivity is by targeting caveolin-1, a major component of membrane invaginations called caveolae and is critically regulating insulin signaling and glucose uptake [90]. Silencing of miR-103/107 increases caveolin-1 level in the fat and liver of *ob/ob* mice, which is concomitant with decreased adipocyte size, enhanced insulin signaling and insulin-stimulated glucose uptake. Therefore, miR-103/107 appears as a key regulator of insulin sensitivity and identifies as novel targets for the treatment of type 2 diabetes and obesity.

In addition, miR-103/107 have been shown to accelerate adipogenesis (Fig. 2) [88]. Overexpression of miR-103 in preadipocytes increases the expression of adipogenesis and adipocyte differentiation markers such as CCAAT/enhancer binding protein (C/EBP)- β , peroxisome proliferator activated receptor- γ (PPAR γ), adipocyte fatty acid binding protein 4 (FABP4) and leptin [83, 87, 90]. TNF- α treatment markedly reduces the expression of miR-103 and miR-143 and attenuate adipogenesis [88] [87], suggesting that cytokine release triggers changes in miRNA expression, which in turn affects lipid levels and adipogenesis in obesity.

miR-122

miR-122 is the most abundant miRNA that promotes cholesterol synthesis and lipoprotein secretion in the liver [111]. Indeed, in vivo inhibition of miR-122 using antagomir-122, a cholesterol-conjugated antisense oligonucleotide, led to improved insulin sensitivity by increasing fatty acid oxidation, decreasing hepatic fatty acid synthesis rate [111]. Reduced cholesterol level is observed in both liver and serum after administration of antagomir-122 [111]. miR-122 modulates the hepatic fatty acid metabolism by targeting the key lipogenic enzymes, such as Fatty Acid Synthase (FASN), HMG-CoA reductase, SREBP-1c and SREBP-2. miR-122 is specifically down-regulated in STZ-induced type 1 diabetic mice in contrast to normal mice [112].

miR-122 is also circulating in the serum and plasma miR-122 is increased in patients with hyperlipidemia [113]. In contrast, surgery-induced weight loss in obese patients led to a marked decrease of plasma miR-122 [114]. These findings implicate therapeutic interventions may be imposed on this miRNA for regaining a normal function of diabetic livers.

miR-122 is not only critical involved in fat and cholesterol metabolism, but also has a tumor suppressor role in hepatocytes [115]. However, it is known that miR-122 stimulates hepatitis C virus (HCV) replication and LNA based anti-miR-122 therapeutics, the first miRNA targeted drug, has been initiated for treatment of HCV infection. Therefore, therapeutic applications of miR-122 may differ based on the underlying disease.

miR-181a

miR-181a negatively regulates insulin sensitivity in liver by directly targeting Sirtuin-1, a NAD(+) dependent-histone deacetylase mediated in metabolic process and energy homeostasis [116]. Overexpression of miR-181a by adenovirus decreases SIRT1 protein levels, impairs hepatic insulin signaling and attenuates insulin sensitivity in hepatic cells [116]. In contrast, blockage of miR-181a by intraperitoneal injection of locked nucleic acid (LNA) antisense oligo increases SIRT1 protein levels and reduces blood glucose level in high-fat diet induced obese mouse, indicating miR-181a might be used for a potential new strategy for treating insulin resistance and type 2 diabetes [116].

Interestingly, an upregulation of miR-181a was recently observed in the sera from children with newly diagnosed type 1 diabetes [117], suggesting miR-181a can be utilized as a potential predictive biomarker.

miR-802

The increase of miR-802 was revealed in the liver of high-fat diet mice, *db/db* mice and obese human subjects [118]. Transgenic overexpression of miR-802 in mice causes impaired glucose tolerance and attenuates insulin sensitivity, whereas reduction of miR-802 expression improves glucose tolerance and insulin action [118]. miR-802 directly targets hepatocyte nuclear factor 1 homeobox B (HNF1b), also known as transcription factor 2 (TCF2). Silencing HNF1b in liver impairs insulin signalling and causes glucose intolerance. In turn, overexpression of HNF1b improves insulin sensitivity in *db/db* mice [118]. Thus, deregulated miR-802 may contribute the development of obesity-associated impairment of glucose metabolism, which can be corrected by modulating miR-802 levels through down regulation of this miRNA by various in vitro and in vivo technologies such as injecting antagomiR or expressing recently developed small tandem target mimic (STTM) [116, 119].

5. Circulating miRNAs are promising clinical biomarker for diabetes

miRNAs have been detected in body fluids such as blood, saliva, urine, and serum [120, 121]. Circulating miRNAs are remarkably stable and circulate in the bloodstream, which make them even more attractive agents as long distant communicators and diagnostic biomarkers for predicting their abnormal functions. Although the cellular machinery responsible for the secretion of miRNA is not completely understandable, it has been demonstrated that miRNAs are packaged into exosomes, microvesicles, lipid particles and apoptotic bodies by a broad range of cell types [122–124]. Changes in the abundance and profile of miRNAs in plasma reflects the development of various chronic diseases from normal functions to cellular dysfunctions. In fact, altered circulating miRNAs have been reported in diverse diseases including cancer, heart failure and liver injury, and can be useful for early diagnosis as well as to predict the clinical outcome and treatment response [125–128].

Identification of circulating miRNA signature has been performed in blood samples from diabetic subjects and led to the identification of a group of differentially expressed plasma miRNAs [44, 66, 117]. Although at its nascent stage, the altered plasma miRNA signature offers a strong possibility to be exploited as clinical biomarkers for the development of diabetes. These miRNAs may also become important therapeutic targets since introduction of antagomiR and miRNA mimic through the blood stream avenue is a viable approach to small RNA therapeutics.

miR-375

Not only enriched in islets, miR-375 has recently been detected in the plasma of both human and mice [30, 44]. Streptozotocin (STZ) administration dramatically increases the circulating level of miR-375 prior to the onset of hyperglycemia in mice [30]. Similarly, plasma miR-375 levels are significantly increased two weeks before diabetes onset in the NOD mouse model of autoimmune diabetes [30]. Most importantly, the increase of serum miR-375 is also detected in human individuals with newly diagnosed type 2 diabetes, compared with matched non-diabetic controls and individuals with impaired glucose tolerance [44]. The elevation of plasma miR-375 level could be a reflection of higher islet miR-375 expression in individuals with type 2 diabetes or diabetic animal models [26, 31]. Taken together, plasma miR-375 can be used not only as a target for therapeutic interventions, but also may be served as a clinical predictor of diabetes.

miR-126

Zampetaki et. al explored plasma miRNA profiles in patients with diabetes and age- and sex-matched controls [66]. A group of five plasma miRNAs (miR-15a, miR-28-3p, miR-126, miR-223, and miR-320) has been identified to form a unique miRNA signature pattern to correctly distinguish between individuals with diabetes from healthy controls [66]. Importantly, the expression of miR-15a, miR-126, and miR-223 was already significantly reduced compared to matched controls years before the manifestation of diabetes, suggesting that these small RNAs might become extremely promising clinical biomarker for earlier diabetes diagnosis.

Whereas most plasma miRNAs are ubiquitously expressed, miR-126 is highly enriched in endothelial cells and plays a critical role in maintaining endothelial homeostasis and vascular integrity [129, 130]. Most importantly, the reduction of miR-126 in plasma strongly correlates with diabetes-associated coronary heart disease, suggesting that loss of plasma miR-126 can be a good diagnostic biomarker for the onset of diabetic vascular complications [66]. Interestingly, Aspirin treatment in patients with type 2 diabetes reduces the level of circulating miR-126 [131]. Aspirin is an antiplatelets medication and helps patients with diabetes prevent future heart problems. Thus, the use of platelet inhibitors should be taken into account when using plasma levels of miR-126 as a biomarker.

However, miR-126 is a tumor suppressor and loss of miR-126 level has been associated with various cancer patients [132–134]. miR-375 is also found to be elevated in the serum of patients with metastatic prostate cancer [135]. Thus, future studies are required to determine whether they are sensitive or specific enough for use as a single diagnostic test for detecting diabetes in clinical settings. The combined detection of miR-375 increase and miR-126 reduction may potentially improve the accuracy of detection. Moreover, the miRNA biomarker could be adopted with other biomarker for accurate analyses of the developmental stage of diabetes.

Other circulating miRNAs, such as miR-25 [117], miR-29a [136] and miR-181a [117] are also altered in the earlier stages of type 1, type 2 or gestational diabetes subjects using transcriptome microarray analyses. However, the analyses of miRNAs in serum is still a challenge with respect to the miRNA recovery from very limited sources of biomaterial and relatively small patient cohorts, which limit the validity and the clinical application of potential miRNA biomarkers. Therefore, the validity and specificity of a unique miRNA signature as a diagnostic biomarker remains to be confirmed by large independent studies. Taken together, identification of circulating miRNA signature in diabetes is just at the beginning but nevertheless suggest a promising aspect of their use as potential clinical biomarkers in diabetes.

6. miRNAs as pharmacological targets in diabetes

The discovery of small and conserved miRNAs has generated enormous research interest as an intriguing pharmacological target in the treatment of complex diseases like cancer, cardiovascular disease and diabetes [2, 137]. Since miRNAs are naturally endogenous regulators of cell processes that are often dysregulated in diabetes, restoration of any given miRNA functions to normal levels will be the ultimate therapeutic goal. Two main therapeutic approaches have been developed: restoring the expression of miRNAs that are downregulated in diabetes using miRNA mimics, or inhibiting the activity of miRNAs that are significantly above the normal expression using miRNA inhibitor.

miRNA inhibitors are antisense oligonucleotides with the reverse complementary sequence of the target miRNA. Because miRNAs typically act as repressors of target gene expression, a miRNA inhibitor binds to the mature miRNA, and thereby activates target gene expression. Locked nucleic acid (LNA) anti-miRs, antagomirs, and morpholinos are efficient inhibitors with different modifications and have proven effective *in vivo* [138, 139]. Among them, LNA anti-miRs, which has high affinity with miRNAs, high efficiency and low toxicity *in vivo*, shows promise for the future development of therapies [140]. LNA anti-miR-122 against miR-122 has been successfully administrated in mice and results in reduced plasma cholesterol with no indication of hepatic toxicity [141].

siRNA-like miRNA mimics are synthesized RNA duplexes and designed to “mimic” the function of endogenous miRNA. Insulin-producing cells can be regenerated from induced pluripotent stem cells (iPSCs) [142, 143] and specific miRNA clusters are reported to regulate human iPSCs reprogramming [144, 145]. miRNA mimic have been introduced to enhance the iPSCs regeneration *in vitro* [146, 147]. However this strategy is limited to *in vitro*, but not *in vivo* because miRNA mimics could easily access to certain tissues like eyes, liver, and lung, producing unexpected side effects yet to overcome [148]. It is still promising to improve the efficiency of miRNA mimics to be functioning *in vivo* since there are many designed formulations are now in testing, such as lipid encapsulated miRNA mimics, miRNA sponges, adeno-associated virus (AAV) packaged system [116, 119, 149–152].

There are big challenges remaining to overcome to promote miRNAs as a viable therapeutic target. First, miRNA mimics are relatively unstable and chemical modification alters their biological properties. Second, the approach to target these molecules selectively in β -cells as well as insulin-target tissues is not yet available. Third, nonspecific systemic delivery affects miRNA expression globally. The techniques for *in vivo* delivery of miRNA mimics or miRNA inhibitors will have to be developed. Finally, It is critical to restore the altered miRNA level to the physiological level of therapeutic miRNAs. Pharmacological over-inhibition or overexpression by administration of miRNA mimics or miRNA inhibitors may potentially have off-targets effects. In addition, the development of single miRNA targeting multiple tissues or multiple miRNA combination therapy may have more potential for the treatment of complex diabetes.

Conclusions and Perspectives

Glucose homeostasis requires coordinated metabolic regulation among multiple tissues/organs via inter-organ communication. miRNA may play important roles in this inter-organ metabolic communication (Fig. 1). Many dysregulated miRNA has been observed in β -cells and insulin-target tissues from various diabetic subjects. Restoration of dysregulated miRNA functions to normal levels has demonstrated to improve insulin sensitivity in insulin-target tissues or insulin production and secretion in β -cells. In addition, circulating miRNAs are also revealed and associated with diabetes pathogenesis. Although the origin of circulating miRNAs are less clear, these miRNAs serve as long-distance communicators and are

circulated to different organs along with the glucose in the blood vessels. Some diabetes-associated miRNAs are also abundantly expressed in the brain, but their functions in relation to glucose homeostasis remain to be determined. Taken together, miRNAs will not only be potential pharmacological targets in treating the diabetes, but also be clinical biomarkers for earlier diagnosis and intervening the development of diabetes. The potential of miRNA-based therapies will offer an exciting and powerful alternative to attenuate and hopefully cure diabetes and their complications.

Acknowledgments

This work was supported by grants from the National Institute of Health (K01DK078648 and R03DK084166) to Xiaoqing Tang. We thank Dr. Guiliang Tang for a critical reading of this manuscript.

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Fig. 1. Role of miRNAs in crosstalk between tissues in the regulation of glucose homeostasis Pancreatic α -cells and β -cells secrete glucagon and insulin, respectively, in response to the change of blood glucose level. Elevated glucose-stimulated insulin secretion increases glucose uptake in adipose and muscle, and decreases glucose production in the liver. Adipose fat cells release free fatty acids (FFAs) and adipokines (leptin, adiponectin and TNF- α) to regulate insulin sensitivity, food intake and energy expenditure. Liver cells can release factors such as betatrophin to regulate the β -cell proliferation. These inter-organ crosstalk are controlled by miRNAs through targeting the expression of key components required for glucose homeostasis. These miRNAs are tissue-specific or differentially expressed in pancreas or insulin targeting tissues. The circulating miRNAs may serve as long-distance communicators. Some diabetes-associated miRNAs are also abundantly expressed in the brain, but their functions in relation to glucose homeostasis remain to be determined.

Table 1

Major miRNAs and their functions in pancreatic beta cells

miRNA name	Expressions	Targets	Functions	Ref
<i>miR-375</i>	1 increased in <i>ob/ob</i> mice and human subjects with type 2 diabetes	Myotrophin, Pdk1	1 Inhibit insulin secretion and transcription; 2 Maintain β -cell mass, proliferation and regeneration; 3 Promote embryonic pancreas development.	[20] [21] [26] [31] [38] [39] [40] [42] [43]
<i>miR-7</i>	1 most abundant endocrine miRNA,	Pax6, p70S6K, eIF4E, Mknk1, Mknk2, and Mapkap1	1 Promote β -cell and α -cell differentiation; 2 Fine-tune β -cell development and regeneration; 3 Inhibit adult β -cell proliferation.	[43] [45] [46] [47] [48] [52]
miR-29a/b/c	1 increased in NOD mice	Mcl1, Onecut-2, Mct1	1 Promote cytokine-mediated β -cell apoptosis; 2 Maintain glucose-stimulated insulin secretion.	[27] [53] [54] [55]
<i>miR-21</i>	1 Increased in NOD and <i>db/db</i> mice	Piccolo, Pdcd4	1) Impairs insulin secretion; 2) Prevents cytokine-mediated β -cell death 2) Controls immune rejection in transplanted islets.	[28] [57] [58] [60]
<i>miR-9</i>	1 preferentially expressed in brain and β -cells	Onecut-2, Sirt1	1 Regulates glucose-challenged insulin secretion	[67] [68]
<i>miR-124a</i>	1 preferentially expressed in brain and embryonic pancreas, 2 undetectable in mature mouse islets	Foxa2, Rab27a	1 Regulates pancreas development; 2 Inhibits insulin secretion	[22] [53] [71] [72]
<i>miR-30d</i>	1 decreased in <i>db/db</i> mice	Map4k4	1 Stimulates insulin secretion and production; 2 Activates MafA expression; 3 Promotes pancreatic islet-derived mesenchymal cell differentiation	[29] [75] [80] [81]
<i>miR-338-3p</i>	1 decreased during rat pregnancy and young <i>db/db</i> mice	Gpr30; Glp1.	1 A reduction is required for β -cell mass expansion during pregnancy; 2 inhibits β -cell proliferation and survival.	[82]