
# Therapeutic potential of microRNAs in diabetes mellitus

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**Summary**

MicroRNAs are major regulators of gene expression that are emerging as central players in the development of many human diseases, including diabetes mellitus. In fact, diabetes manifestation is associated with alterations in the microRNA profile in insulin-secreting cells, insulin target tissues and, in case of long-term diabetes complications, in many additional organs. Diabetes results also in changes in the profile of microRNAs detectable in blood and other body fluids. This has boosted an ever increasing interest in the use of circulating microRNAs as potential biomarkers to predict the development of diabetes and its devastating complications. Moreover, promising approaches to correct the level of selected microRNAs are emerging, permitting to envisage new therapeutic strategies to treat diabetes and its complications.

**Key words:** Diabetes mellitus; Insulin; pancreatic islet; microRNA; Gene expression; biomarker; diabetic complication; Adeno-associated virus

## Expert commentary

### Introduction

Diabetes mellitus is a very common metabolic disorder characterized by chronically elevated blood glucose levels. Recent estimates indicate that more than 350 million people are affected by this disease and, due to population ageing and increasing sedentary life style, the situation is expected to dramatically worsen in the coming years [1]. Insulin released by pancreatic β-cells plays a pivotal role in the control of blood glucose homeostasis. Diabetes mellitus develops if the amount of insulin secreted by β-cells is insufficient to cover the organism needs. This occurs if the β-cells are destroyed by the immune system (Type 1 diabetes, T1D) or when β-cells are unable to compensate for the diminished sensitivity of insulin target tissues typically occurring in relationship to obesity and ageing (Type 2 diabetes, T2D) or to pregnancy (gestational diabetes, GD). All forms of diabetes are associated with major changes in gene expression in the endocrine pancreas, in insulin target tissues (liver, skeletal muscles and fat) and in blood vessels (in case of vascular complications). Indeed, under pre-diabetic and diabetic conditions the cells composing these tissues are chronically exposed to elevated concentrations of glucose, fatty acids, pro-inflammatory mediators etc., all conditions impacting on gene expression and that can lead to organ dysfunction and failure. So far most of the studies that attempted to identify the causes of diabetes and its long-term complications focused on protein-coding genes. However, protein-coding genes account for less than 2% of the human genome and we now know that human cells express thousands of RNA transcripts with little or no protein-coding potential but exerting essential regulatory activities. These non-coding RNA molecules include the microRNAs (miRNAs) that will be the focus of this review.

### Contribution of microRNAs to the development of diabetes and its long-term complications

MiRNAs are small (typically 20-23 nucleotides) non-coding RNAs that act as translational repressors and play major roles in the control of gene expression [2]. Today the miRNA family includes about 2500 members in humans and almost 2000 in mice. MiRNAs are transcribed by RNA polymerase II yielding pri-miRNA transcripts that are recognized by the microprocessor complex component DGCR8

(DiGeorge syndrome critical region 8) (Fig.1). They are then cleaved by Drosha to form precursor-miRNA hairpin structures of about 70 nucleotides. These molecules are exported from the nucleus by Exportin 5 and are successfully cleaved by Dicer to generate double-stranded RNA molecules of 20-23 nucleotides. After being cleaved by Dicer the mature miRNA strands associate to members of the Argonaute protein family and are loaded in to the RNA-Induced Silencing Complex (RISC). This enables them to bind to MicroRNA Recognition Elements (MRE) located in the 3'untranslated region of target mRNAs that are initially recognized through base pairing to a conserved "seed" sequence corresponding to nucleotides 2-8 of the miRNAs, leading to inhibition of mRNA translational and/or to a decrease in messenger stability [2, 3]. A single miRNA typically controls hundreds of targets and each mRNA can be targeted by different miRNAs, conferring to this class of non-coding RNA molecules a huge regulatory potential [4]. In the past decade, a large body of evidence has been accumulated pointing to a role for miRNAs in the etiology and pathogenesis of diabetes and its complications. Indeed, alterations in the level of these non-coding RNAs has been observed both in insulin-secreting cells and in insulin-target tissues isolated from diabetes animal models or diabetic patients [5]. Moreover, changes in miRNA expression have been associated with long-term diabetes complications including neuropathy, retinopathy, renal failure and macrovascular diseases.

The first demonstration of the involvement of miRNAs in the control of specialized β-cell functions has been provided ten years ago by Poy et al. who showed that inappropriate levels of miR-375, one of the most abundant miRNAs present in β-cells, can affect insulin secretion [6]. Later on, this and several other miRNAs including miR-15a/b, miR-16, miR-195, miR-503, miR-451, miR-214, miR-9, miR-124a, miR-7 and miR-376 were demonstrated to play important roles in the differentiation of pancreatic islet cells [7-12]. Moreover, changes in the levels of many miRNAs, including miR-9, miR-124a, miR-24, miR-26, miR-148 and miR-182 were found to exert a deleterious influence on β-cell activities by targeting key genes involved in insulin biosynthesis, insulin secretion and cell survival [13-16].

Several studies have attempted to establish a link between the changes in islet miRNA expression and the development of both T1D and T2D. MiR-34a, miR-21, miR-146a and miR-29 family members were found to be up-regulated in the islets of pre-diabetic NOD mice, a well-characterized model of T1D [17, 18]. The up-regulation of these miRNAs is deleterious for the secretory activity and for the survival of β-cells in the presence of pro-inflammatory cytokines released by leucocytes infiltrating the islets of Langerhans, suggesting that they may contribute to the progression of the disease. T1D was also linked to altered miRNA expression in immune cells. Indeed, Herzova et al. observed up-regulation of miR-510 and down-regulation of miR-191 and miR-342 in regulatory T-cells ($T_{reg}$ cells) isolated from T1D patients [19]. Since these cells are known to be critical controllers of the immune reaction, alterations in their miRNA profile may favor the development of an autoimmune attack directed toward the β-cells and hence the manifestation of T1D diabetes.

Several independent studies have reported modifications in miRNA expression occurring in the islets of T2D animal models, including *ob/ob* and *db/db* mice, lacking leptin or its receptor, mice fed a high fat diet and Goto-Kakizaki rats, a spontaneous model of T2D that in contrast to the other is not associated with obesity [20]. Systematic analysis of the differentially expressed miRNAs highlighted changes in the level of numerous miRNAs with a deleterious impact on insulin secretion and β-cell survival under pro-apoptotic conditions including among others miR-34a, miR-124, miR-146a, miR-199a-3p, miR-203, miR-210, miR-335 and miR-383 [17, 21-23]. However, not all the modifications in miRNA expression occurring in the islets of T2D animals were found to have a negative effect on β-cells. Indeed, the down-regulation of miR-184 and miR-338-3p and the up-regulation of miR-132 observed in different animal models were demonstrated to trigger β-cell mass expansion and to improve insulin secretion [22, 24, 25]. Thus, the changes in the level of these miRNAs are likely to be part of the compensatory mechanisms attempting to counterbalance the diminished sensitivity of insulin target tissues that typically develops in obese individuals and during pregnancy. Significant differences in islet miRNA expression were also detected in the islets of human T2D patients. Indeed, the islets of T2D donors were found to display an up-regulation of miR-187 [26] and the down-

regulation of several members of a large miRNA cluster generated from the imprinted DLK1-MEG locus [27]. It is not yet clear whether the differential levels of these miRNAs are inherited, predisposing the affected individuals to develop T2D, or whether they are acquired later in life, promoting the manifestation of the disease.

Obesity and insulin resistance are also leading to a strong dysregulation of the miRNA profile in adipose tissue, liver and in skeletal muscles. The most affected miRNAs, including among others miR-29, miR-103/107, miR-143, miR-802 and Let-7, were shown to target key components of the insulin signaling pathway and to contribute to the loss of insulin sensitivity observed in obese individuals [28-30]. Moreover, miR-133 and miR-1 dysregulation has been shown to contribute to impaired muscle function in T2D [31-33]. Indeed, repression of miR-133 and miR-1 in response to insulin was shown to be impaired in T2D patients [31, 34].

MiRNAs are also suspected to be central players in the development of long-term microvascular (neuropathy, nephropathy and retinopathy) and macrovascular (cardiovascular and peripheral vascular diseases) diabetes complications. Indeed, several studies point to a role for miRNAs including miR-29, miR-192, miR-200, miR-216 and miR-217 in TGFβ signaling and glomerular fibrosis associated with diabetic nephropathy [35]. Moreover, miRNAs have been reported to regulate the expression of VEGF (vascular endothelial growth factor) in the retina and to participate to the development of diabetic retinopathy [36]. Kovacs et al. identified a set of up-regulated NF-κB-responsive miRNAs in the retina of diabetic rats, including miR-146a/b, miR-155, miR-132 and miR-21 [37]. MiR-155 is known to be involved in immuno-modulatory signaling, whereas miR-21 and mir-146a/b participate to fibrotic responses and miR-132 to angiogenesis.

MiR-1 and miR-133 were shown to play controversial roles also in diabetic cardiomyopathy. Xiao et al. observed exaggerated expression of miR-133 in the heart of diabetic rabbits causing the prolongation of QT interval, a typical event observed in T2D [38]. However miR-133 as well as miR-1 are down-regulated in cardiac hypertrophy and in the hearts of STZ-induced diabetic mice suggesting complex relationship between these miRNAs and cardiac dysfunction [39].

**Circulating microRNAs as biomarkers for diabetes**

The majority of the miRNAs reside inside the cells where they accomplish most of their regulatory activity. However, these non-coding RNAs can also be detected in virtually all body fluids including blood, bile, saliva, urine, breast milk, vaginal secretions, semen, tears, amniotic fluid, cerebrospinal fluid, bronchial lavage, pleural fluid, seminal fluid and peritoneal fluid [40-43]. Beyond any expectation, extracellular miRNAs are remarkably stable, suggesting that they are resistant to degradation by RNases present in the circulation. In contrast to other RNAs, even harsh conditions such as extreme pH variation, multiple freeze and thaw cycles as well as storage at room temperature do not significantly affect the stability of circulating miRNAs [44]. This unexpected stability is due to their inclusion in vesicles which provide a protected environment [45] or to the formation of stable complexes with proteins or lipoprotein particles [41, 46]. The exact mechanism by which miRNAs are released into the circulation are not-yet fully understood. Most miRNAs are secreted as single-stranded RNAs, but precursor hairpins have also been detected in extracellular fluids [47-50]. Vesicle-borne miRNAs are actively secreted inside exosomes [51, 52] or are released from damaged cells in apoptotic bodies [47, 53, 54]. MiRNAs transported in vesicle-free form are associated with proteins such as Nucleophosmin 1 or Argonaute 2 [55] or with lipoproteins (HDL, LDL) [47-49]. In pathological conditions such as tissue damage or inflammation, part of the miRNAs can also be passively shed upon cell lyses or necrosis [53, 56].

There is growing evidence for distinct miRNA signatures in the exosomal, lipoprotein-bound and protein-bound fractions [48, 49]. The transport mechanism of individual miRNAs seems to be specific and may be altered depending on the type of disease or injury. In a study performed using different liver disease models, plasma and serum miR-122 and miR-155 were found to be associated with exosomes upon inflammatory liver injury and alcoholic liver disease, whereas upon drug-induced liver injuries (acetaminophen, APAP) these miRNAs were mainly recovered in a protein-bound form [57]. This suggests different roles for each mode of transport. *In vitro* experiments have shown that extracellular miRNAs can be actively taken up by target cells by receptor-mediated capture,

endocytosis or fusion of exosomes with the cell membrane [51, 58-61] and to further alter the gene expression profile of recipient cells [62, 63]. Additionally, plant miRNAs were observed to be transported to the liver, proving their capability to cross the intestinal barrier and enter the blood stream [48, 64]. Therefore, circulating miRNAs may potentially act as signaling molecules traveling over long distances to elicit a response in cells located at distant organs. However, the precise mechanisms permitting the targeting of the secreted miRNAs to specific target cells remains unclear.

MiRNAs can be easily isolated from body fluids in a non-invasive manner, which makes them perfect candidates as biomarkers. In principle, they can be measured both in plasma and in serum samples. However, if serum is used, care should be taken to avoid lysis of blood cells during the coagulation process [65]. Beside their elevated stability, these molecules can be rapidly and accurately detected using highly specific and sensitive methods. There is a hope that the use of miRNAs as biomarkers may facilitate early detection of diseases and allow timely intervention to delay complications. Furthermore, changes in the level of some of these small non-coding RNAs may be useful for the prognosis of disease progression and to monitor the efficacy of the treatments. Indeed, several miRNAs were shown to be altered in the circulation in a growing number of diseases including diabetes, several types of cancer and Alzheimer (see Supplementary Table 1 for an exhaustive list). Interestingly, in some cases the modifications in the circulating miRNA profile were already detectable years before the manifestation of the disease suggesting that they may be exploited to identify the individuals at risk to develop the disorder.

Along this line, a growing number of studies have attempted to predict the occurrence of diabetes and its long-term complications by detecting early changes in plasma miRNAs. Zampetaki and coworkers were the first to identify a plasma miRNA profile characteristic of T2D. Five miRNAs (miR-15a, miR-28-3p, miR-29b, miR-126 and miR-223) were found to be deregulated several years before the onset of the disease [46] and permitted to predict the occurrence of vascular complications. Furthermore, a recent study by Yang *et al.* detected significantly lower levels of miR-23a in the serum of T2D patients compared to healthy controls that were already detectable in pre-diabetic individuals

[66]. A similar type of analysis was also performed in animal models to identify potential biomarkers predicting the development of T1D. For this purpose, circulating miRNAs were measured in the blood of non-obese diabetic (NOD) mouse, a well-characterized T1D model and in B57BL/6 mice treated with streptozotocin, a pharmacological agent inducing a rapid and specific loss of pancreatic β-cells. In both models the level of miR-375, a miRNA highly enriched in β-cells was found to be increased in plasma, prior to T1D onset [67]. In humans the loss of β-cells and the phases preceding the manifestation of T1D is likely to span over a much longer period compared to NOD mice or mice treated with streptozotocin. Therefore, it is not yet clear whether a slow and progressive loss of β-cells will lead to detectable modifications in circulating miR-375 levels. Changes in circulating miRNAs were also used to predict the development of GD. Indeed, miR-29a, miR-132 and miR-222 were already decreased after 16-19 weeks of gestation in the serum of pregnant women that were later diagnosed with GD at weeks 25-28 [68].

Interestingly, nine miRNAs (miR-27a, miR-29a, miR-29b, miR-126, miR-142, miR-144, miR-199, miR-342 and miR-1307) were identified in peripheral blood mononuclear cells (PBMCs) as potential biomarkers in all three diabetes types which might imply their association with the diabetes condition per se. This may be due to the fact that several of these common miRNAs play important regulatory functions in pathways related to diabetes, such as metabolic or immunological processes [69].

Although miRNAs are now viewed as potentially interesting biomarkers for an increasing number of diseases there are still concerns about their generalized use in the clinics. In fact, the majority of the circulating miRNAs identified as potential biomarkers for diabetes mellitus are also altered in many other diseases, which somehow may reflect their biological functions (Supplementary Table 1). A good example is miR-155, which is associated to epithelial-mesenchymal transition [70], as well as, autoimmunity [71] and has been shown to be deregulated in several cancers and cardiovascular diseases. Lung cancer and diabetes also share a large number of modified miRNAs, possibly reflecting the presence of a general inflammatory response [72].

The miRNA profiles are believed to be stable over long time [73] and

serum miRNAs are similar between individuals of different gender or ages [44]. Furthermore, feeding state, smoking and time of blood draw do not seem to have major influences on the miRNA profile [74]. However, the impact of factors such as female hormone cycle, pregnancy and life style remain to be elucidated. Hemolysis is another problem with which investigators have to deal during blood samplings. It has been linked to a direct release of large numbers of miRNAs significantly altering the blood miRNA profile [74]. For this reason, biomarkers for hemolysis might be useful to prevent faulty quantifications.

Studies performed by different groups often show discrepant results in their miRNA analysis. This may be caused by technical challenges such as different RNA processing, sample handling, storage, measurement technologies, as well as, different sources for miRNA analysis. A problem investigators are faced with is the missing standardization and the lack of a "housekeeping" miRNA for normalization. The latter may be solved by using additional spike-in standards which can be introduced prior to cDNA synthesis.

Zhang and coworkers analyzed the Zampetaki miRNA signature in plasma of healthy individuals, T2D-susceptible individuals and T2D patients [75]. In their study, miR-29b and miR-28-3p were not detectable and miR-15a and miR-223 showed comparable expression levels in all three groups. Only miR-126 showed an altered expression profile in both studies. These discrepancies may be explained by the differences in size and ethnicity of the cohorts. Zampetaki *et al.* analyzed plasma samples of around 800 Italians, while Zhang *et al.* worked with plasma samples of 90 Chinese individuals. This emphasizes the need to confirm the results in larger prospective populations.

So far, the question remains open whether the deregulation in the circulating miRNA profile is the cause or the consequence of the diseases. Furthermore, the cellular origin of circulating miRNAs remains also unclear. The miRNA profile in the circulation does not seem to merely mirror the one present in the cells, as e.g. the miR-29 family members are down-regulated in serum of diabetic patients, but are up-regulated in pancreas [76].

Although, research of miRNAs as potential biomarkers is still in its infancy, several studies have identified miRNAs which may help to predict and diagnose diabetes. However, as a single miRNA shows often alterations in more than one disease, measurement of a group of miRNAs will probably better achieve the required specificity and sensitivity needed for the clinics.

**Therapeutic strategies to modulate the level of microRNAs in diabetes**

As described above, diabetes and its long-term complications are characterized by major alterations in the miRNA profile in a large number of cells. The biochemical and biophysical properties of miRNAs are well defined and different strategies to deliver them in active form or to specifically block their activity *in vitro* and in animal models are already available. MiRNA-based therapeutics offer unprecedented possibilities to target multiple genes belonging to the same pathological pathway, in particular when the target miRNA is tissue or cell-type specific, or plays a central role in the pathological process. However, future use of miRNA-based therapeutics will require careful evaluation of potentially severe adverse effects caused by the elevated number of targets controlled by each single miRNA. This will be particularly important for chronic diseases such as diabetes that may require life-long treatments and for which alternative cures are already available.

**Reparative miRNA therapies**

The pivotal position of miRNAs in metabolism and associated disorders combined with the existence of approaches permitting to modulate their expression *in vivo* opens drug design opportunities for the development of new classes of anti-diabetic agents. Whether diabetes or its complications are a direct cause of altered miRNA expression or this altered expression occurs as a consequence of the pathological state is still unknown. Nonetheless, restoration of miRNA expression to normal levels appears as a potentially attractive therapeutic strategy. Several trials aiming at modulating the expression of specific miRNAs and restore their physiological levels are already on the way for other pathological conditions [77, 78]. Depending on the expression levels of the candidate miRNA in diseased tissues and of the function of the non-coding RNA, two main strategies can be envisaged: replacement therapy or silencing therapy. These two strategies will involve the generation of miRNA

mimics or miRNA inhibitors permitting to restore or inhibit the level of expression of the miRNAs that drive disease initiation and/or progression.

**Replacement therapy**

The restoration of miRNA levels can be achieved by delivering hairpin-containing RNA molecules analogous to the miRNA precursors or oligonucleotides mimicking the mature form of the miRNA of interest (Fig.1). The small size of the latter molecules permits their delivery as double-stranded chemically-modified oligonucleotides analogous to small interfering RNAs. Since this requires an efficient miRNA processing machinery, an alternative approach is the use of single-stranded molecules that are immediately effective. The purpose of the chemical modifications is multiple. They help protecting the oligonucleotide from nuclease cleavage, they ameliorate target specificity and increase the binding affinity and they permit to improve *in vivo* delivery [78-80] (Fig.2). Once entered the cells, these molecules are processed by the cellular machinery enabling them to work as the endogenous miRNA [81, 82]. A major drawback of this approach is that *in vivo* delivery of these miRNA mimics is not organ specific, potentially leading to severe side effects. To circumvent this problem, overexpression of miRNAs can be obtained using viral vectors that integrate into the host genome and that are engineered to express a miRNA precursor or an artificial shRNA (short hairpin RNA) under the control of a specific promoter. Due to the ability to provide long-lasting gene silencing, this approach is of great interest for gene therapy applications [83]. Adeno-associated viruses (AAVs) engineered to drive the expression of miRNA precursors are among the most attractive vectors to restore appropriate miRNA levels. Numerous AAV serotypes are available with a natural tropism towards specific organs. For example AAV6, AVV8 and AVV9 have been shown to efficiently target the pancreatic islets *in vivo* [84]. In this context, the level of miR-26a which is down-regulated in liver cancer cells has been corrected by administration of an AVV8 construct through the animal tail vein, resulting in a significant protection from hepatic cancer progression with no sign of toxicity [85].

**Silencing therapy**

As described above, many miRNAs are up-regulated in diabetic conditions. The aim of the silencing **therapy is to reduce the excess of miRNA and restore proper target gene expression**. This is usually achieved using antisense oligonucleotides (anti-miRs) that bind to the miRNA causing the blockade of its activity and its degradation. Anti-miRs are widely used for the modulation of miRNA expression in experimental models and, if conveniently modified, they have proved effective also *in vivo* [86, 87]. Different types of anti-miRs are currently available involving specific chemical modifications of the nucleotides [79, 80] (Fig.2). AntagomiRs are O-methyl-modified oligonucleotides coupled to cholesterol and were the first molecules capable of blocking miRNA expression *in vivo* [88]. Another common modification involves the inclusion of Locked nucleic acids (LNAs) that strongly increase the binding affinity of the anti-miRs (Fig.2). In view of the superior properties of LNAs, it is possible to design not only anti-miR specifically directed to a single miRNA but also anti-miRs capable of blocking an entire family of closely-related miRNAs. This is achieved using shorter "8-mer" LNA oligonucleotides (Tiny LNAs) complementary to the seed sequence shared by all members of the same miRNA family [89].

Recently, Wang et al. showed that miR-7a, a miRNA highly expressed in adult β-cells, targets five components of the mTOR signaling pathway. Inhibition of miR-7a was found to activate the mTOR signaling and to promote adult β-cell replication. These findings suggest that miR-7a acts as a brake on adult β-cell proliferation and represents a potential therapeutic target for diabetes [90].

Interestingly, a circular RNA has been shown to possess numerous miR-7 binding sites and to regulate the availability of this miRNA [91]. Thus, in theory strategies raising the level of this circular RNA may potentially be exploited to trigger adult β-cell proliferation.

MiR-122 is a liver specific miRNA that regulates both lipid metabolism [92] and HCV replication [93, 94]. MiR-122 inhibition in a diet-induced obesity mouse model resulted in decreased plasma cholesterol levels and a significant improvement in liver steatosis, accompanied by reductions in several lipogenic genes [92]. On the other hand, HCV replication was shown to be inhibited by a 2'-O-methyl-modified anti-miR-122 oligonucleotide opening the doors for the use of miR-122 inhibitors as

a treatment for HCV infection in humans [93]. “Miravirsen” is a 15-mer LNA containing a phosphorothioate-modification providing an extremely high affinity for miR-122. A study carried out in a non-human primate showed that intravenous administration of Miravirsen leads to inhibition of miR-122 and a considerable lowering of plasma cholesterol levels [95, 96]. Miravirsen was also efficient in the treatment of chronic HCV infection Chimpanzees [97]. Studies on healthy primates and humans did not reveal any significant toxic effect [95, 96]. In a phase II clinical study, Miravirsen has been successfully administered subcutaneously resulting in a decrease in the HCV RNA level in patient’s serum and was well tolerated after 18 weeks treatment [98, 99]. However, potential long-term adverse effects of miRNA inhibition still need to be assessed.

In view of the observed modifications in their expression level occurring in association with T1D, T2D and GD, in principle several miRNAs would elect as attractive targets for diabetes miRNA-based therapies. Because of the difficulty in specifically targeting a very small cell population such as the one represented by pancreatic β-cells, so far most of the studies focused on miRNAs altered in liver and in other insulin target cells.

Let-7 family members have been discovered to play central and unexpected roles in glucose metabolism in many organs [100]. Several members of this family are up-regulated in the liver of *ob/ob* and diet-induced obesity mice. Interestingly, impaired glucose tolerance in diet-induced obesity mice could be prevented and treated upon systemic injection of anti-Let-7 [100], suggesting that blockade of this miRNA family may constitute a potential strategy to treat T2D. The members of the Let-7 family promote cell differentiation and suppress tumor initiation because of their multiple targets that are involved in cell cycle and mitotic signaling. Furthermore, let-7 is down-regulated in a variety of malignancies [101]. Thus, the potential use of anti-let-7 therapy to counteract the diabetic state should take into consideration the long-term risk of developing cancer [102]. Another miRNA family that is being scrutinized as a candidate for clinical use is miR-103/miR-107. These two miRNAs differ only by one nucleotide in their 3’ region. The expression of miR-103/107 has been shown to be elevated in *ob/ob* mice and to target Caveolin-1, which is a critical regulator of insulin receptor

signaling. In *ob/ob* mice antagomiR-based silencing of miR-103/107 led to improved glucose homeostasis and insulin sensitivity in peripheral tissues [29], making these two miRNAs very attractive new targets for the treatment of T2D and obesity. Currently, an anti-miR is being developed by Regulus Therapeutics in partnership with AstraZeneca, and is at the preclinical stage [78].

MiR-184 and miR-338-3p are two other miRNAs with an interesting potential for the treatment of T2D and/or GD. The level of these two miRNAs is reduced under conditions of insulin resistance associated with obesity and pregnancy [24, 25]. Treatments leading to diminished expression of miR-184 and miR-338-3p resulted in β-cell mass expansion compensating for the insulin resistant state. Thus, molecules blocking the activity of these miRNAs specifically delivered to β-cells would be anticipated to boost the physiological response of the organism to insulin resistance and may permit to prevent or treat T2D or GD.

As for miRNA mimics, one of the major obstacles inherent to the use of anti-miRs *in vivo* is represented by the difficulty in targeting a specific cell type. An alternative strategy to inhibit miRNA activity involves the use of so-called “miRNA sponges”. MiRNA sponges are expression vectors engineered to generate RNA molecules containing multiple artificial miRNA binding sites. These constructs, which can be driven by cell-specific promoters, permit to sequester the endogenous miRNA and to relieve its inhibitory activity on the target mRNAs [103]. Sponges have been widely used *in vitro* to investigate miRNA functions and the efficacy of this approach has also been proven *in vivo* [104].

**MicroRNA target protection strategy**

Each miRNA can target and modulate the expression of multiple genes. Under certain circumstances, it may not be appropriate to affect the level of all potential targets of the selected miRNA and it will be preferable to focus on a single target. The target protection strategy centralizes the effect of the treatment on specific target genes by introducing a single-stranded oligonucleotide that is complementary to the mRNA region recognized by the miRNA (Target Site Blocker, TSB). The target

site blocker is longer than the binding site of the miRNA conferring an improved specificity. This approach can reduce off-target effects, however it won't be suitable when there is a need to target multiple pathways.

The proof of concept of the efficacy of this technology has been obtained *in vitro* by preventing the repressive action of miR-199a-5p on its target Caveolin-1. This permitted to restore the expression of Caveolin-1 and to avoid myofibroblast differentiation in response to TGF-β stimulation [105]. An analogous approach has also been used in β-cells to prevent the deleterious effect of miR-29 by introducing oligonucleotides specifically masking the binding site of this miRNA present in the 3'UTR of the mRNA coding for Mcl1, a member of the Bcl2 family [18]. This permitted to protect insulin-secreting cells from cytokine-mediated death.

The first study showing the effectiveness of this technique *in vivo* was conducted by Tao Sun team [106]. This group used a vector expressing oligonucleotide sequences of different sizes (20, 40 and 60 nucleotides) that were fully complementary to the binding site of miR-19a on the 3'UTR of the PTEN mRNA. They found that oligonucleotides of 60nt are the most effective in preventing the suppression of PTEN during the development of the mice cortex [106]. This technique is still at its infancy and more studies are required to validate the potential of this therapy *in vivo*.

**Reparative microRNA therapies in diabetes complications**

Reasonable progression in the development of miRNAs-based therapeutics has been accomplished in diabetes complications. For example, subcutaneous delivery of LNA-anti-miR-192 in streptozotocin-induced diabetic mice resulted in decreased expression of the target miRNA. This was paralleled by an increase in the levels of the transcription factors ZEB1/2, resulting in diminished collagen, fibronectin and TGF-β expression and improved renal function [107, 108].

Regarding vascular complications, modulation of miR-23 and miR-27 resulted in an important decrease in neovascularization within the eye in response to laser-mediated injury to the choroid layer [109]. Inhibition of miR-208a has also been reported to reduce cardiac remodeling and to increase survival after hypertension-induced heart failure [110]. Moreover, the inhibition of this

miRNA also improved glucose metabolism and reduced plasma lipid content [111]. Anti-miR-208 is in preclinical development by miRagen Therapeutics for chronic heart failure [102].

MiR-21 is another promising target for the treatment of diabetic nephropathy. MiR-21 has been successfully repressed using LNA inhibitors in different mice models including renal diseases induced by UUO (unilateral uretal obstruction). In this system, inhibition of miR-21 resulted in reduced expression of ECM proteins and prevented TGF-β-induced renal fibrosis [112].

**Five-year view**

MiRNAs are emerging as key players in the development of different forms of diabetes mellitus. Several strategies are already available to efficiently modulate the level of selected miRNAs. Taking advantage of these techniques, restoration of deregulated miRNA expression to normal levels *in vitro* and in animal models has been demonstrated to improve insulin sensitivity in target tissues or ameliorate insulin production and secretion from β-cells. Although the efficiency of the majority of these molecules remains to be confirmed in preclinical and clinical trials, the results obtained so far are encouraging and open new real perspectives for the treatment of T2D and insulin resistance.

A major advantage provided by miRNA-based therapeutics is the possibility to target a complex disease affecting multiple organs with small molecules that can be easily synthesized and manipulated. However, a number of key issues concerning these new pharmacological principles remain to be addressed and will need to be solved before the use of miRNA-based therapeutics can become reality. One of the biggest challenges will be the development of delivery strategies to target the miRNA mimics or the anti-miRs to the appropriate cells or tissues. This is a critical point to avoid potentially severe side-effects. In fact, the vast majority of the miRNAs are expressed in a wide variety of cells and many of them are major players in the development of devastating diseases such as cancer. Even very low probabilities to favor cancer development are certainly not acceptable for an anti-diabetic drug. The design of efficient approaches to specifically deliver oligonucleotides to selected cells would also open new possibilities for miRNA-based treatments targeting the β-cells. Although dysfunction of these cells is known to be one of the major causes of diabetes manifestation, the difficulty in specifically delivering miRNA mimics or anti-miRs to β-cells has so far hampered the modulation of β-cell miRNAs *in vivo*. Today it is arduous to anticipate which strategy will be chosen by the investigators to achieve cell-specific delivery of miRNA mimics or anti-miRs but the use of exosome-like vesicles carrying ligands recognized by the targeted cells may represent an attractive option.

Beside their therapeutic potential, the presence of characteristic miRNA signatures in easily accessible body fluids opens new perspectives for the diagnosis and prevention of diabetes and its long-term complications. The two major issues about the use of circulating miRNAs as effective biomarkers for diabetes concern the specificity of the miRNA signature and its reproducibility in different populations and laboratories around the world. It can be anticipated that the accumulation of studies analyzing the profile of circulating miRNAs under many different physiological and pathological conditions will help defining the predictive value of the changes in the extracellular level of specific miRNAs. We presently don't know which cells are contributing to the pool of circulating miRNAs and, if any, what is the precise role of these extracellular RNAs. A better definition of the origin of circulating miRNAs and of their potential regulatory function will guide the selection of the most relevant miRNA changes to be used as biomarkers for diabetes and its complications.

The discovery of miRNAs has revealed entirely new and unpredicted possibilities for the diagnosis and treatment of diabetes mellitus. The number of studies focusing on therapeutic approaches aiming at correcting miRNA expression in relevant tissues or scanning the pool of circulating miRNAs in search for diabetes biomarkers is increasing exponentially. In the next five years we will know whether these studies can hold all their promises. Should this the case, the way physicians manage diabetes mellitus and its devastating complications will probably be revolutionized.

**KEY ISSUES**

- MicroRNA are small non-coding RNAs that play important role in the regulation of gene expression.

- Diabetes mellitus is associated with major alterations in the microRNA expression profile in insulin-secreting cells, in insulin target tissues and in several other organs in case of long-term micro- and macrovascular complications.

- MicroRNAs are also detectable in most biological fluids and their level is modified under pre-diabetic and diabetic conditions, suggesting that they may be useful to predict the manifestation of the disease and its complications.

- The level of specific microRNAs can be modulated *in vivo* using short chemically-modified oligonucleotides or viral vectors, permitting to restore appropriate levels of the non-coding RNA and to prevent or treat diabetes.

- The recent advances in the analysis of circulating microRNAs and in the strategies permitting the modulation miRNA expression in relevant organs provide hope for better prevention and treatment of diabetes and its complications.

**FIGURES LEGENDS**

**Figure 1: miRNA biogenesis and strategies for reparative miRNA-based therapeutics**

The figure illustrates the key steps of miRNAs biogenesis and the methods available to either increase or diminish miRNA function. **MiRNA biogenesis** (blue boxes)**:** miRNA genes are transcribed into long primary miRNA transcripts (pri-miRNAs) that are processed in the nucleus to ~70 nt pre-miRNAs by the nuclear Microprocessor complex, consisting of DGCR8 and the RNase III enzyme Drosha. Pre-miRNAs are exported by Exportin-5 and further processed in the cytoplasm by Dicer to yield ~22 nt double-stranded miRNA duplexes. The mature miRNA is loaded into a RISC (miRISC) containing Argonaute 2. This complex binds to the 3' UTRs of target mRNAs to promote translational repression or deadenylation and degradation of the messenger. **Strategies for miRNA-based therapeutics** (black boxes): miRNA expression is increased by introduction of miRNA mimics or by transduction of miRNA-encoding viruses (replacement therapy). Inhibition of miRNAs function is achieved using anti-miRs (antisense oligonucleotides directly targeting miRNAs), miRNA Target Site Blockers or AAVs that express long mRNAs 'sponges' which contain multiple miRNA 'seed' sequences (silencing therapy). **miRISC**, miRNA loaded into the RISC ; **AVV**, Adeno Associated Virus

**Figure 2: Chemical modifications used for miRNA-based therapeutics**

**A)** Chemical modifications can be incorporated into anti-miR oligonucleotides. Most affect the 2' position of the sugar ring, Locked Nucleic Acid (LNA), 2'-O-methyl (2'-OMe), 2-O-methoxyethyl (2'-MOE) and 2'-fluoro (2'-F) modifications. Oligonucleotide modifications permit to improve the pharmacological and pharmacokinetic properties. Ubiquitous nucleases cleave the phosphodiester linkage which makes unmodified nucleic acids unstable in biological systems. The morpholino oligomer is the result of the replacement of the sugar moiety by a six-membered morpholine ring. 2'-OMe RNA contains a methyl group at the 2'-OH position that enhances nuclease resistance. A sulfur substitution of a non-bridging oxygen generates a phosphorothioate linkage between nucleotides. The substitution of the oxygen by sulfur enhances resistance to nucleases and promotes plasma

protein binding, which prevents renal clearance and increases tissue delivery. Binding affinity to the specific target can be improved by the drug design. 2'-MOE RNA contains a methoxy group which confers a higher affinity and specificity to RNA than their OMe-analogs. The 2'-F modification involves the introduction of a fluorine atom at the ribose 2' position and locks the sugar ring into a high 3'-endo conformation resulting in a very high affinity for target RNAs. LNA modification introduces a 2', 4' methylene bridge in the ribose to form a rigid bicyclic nucleotide locked into a C3'-endo (RNA) sugar conformation, which confers an extremely high affinity. **B)** Schematic representation of miRNA inhibition possibilities. The exceptional binding affinity of LNA oligonucleotides allows the use of shorter sequences. The tiny-LNAs allow specific targeting of a whole family of miRNAs sharing the same seed sequence.

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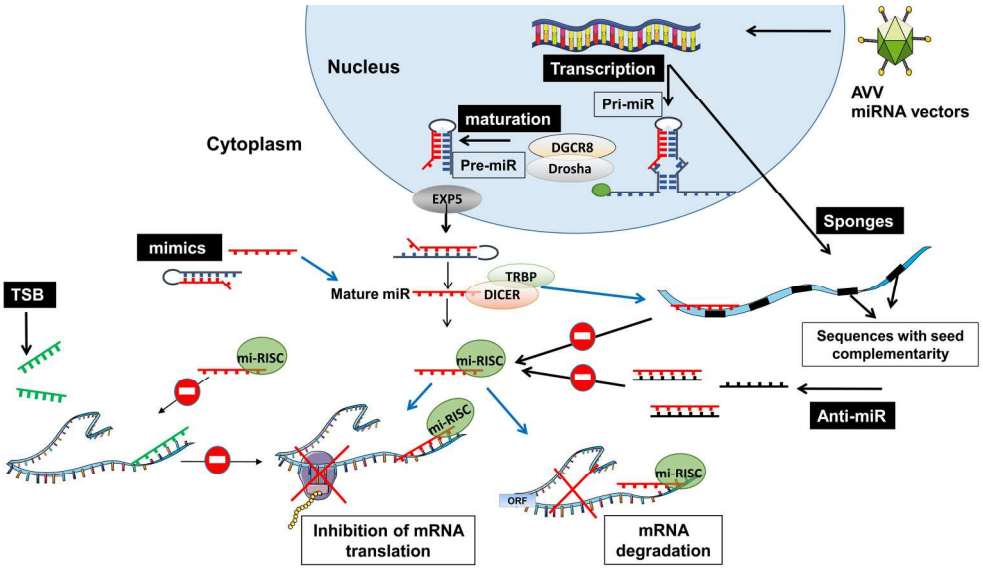


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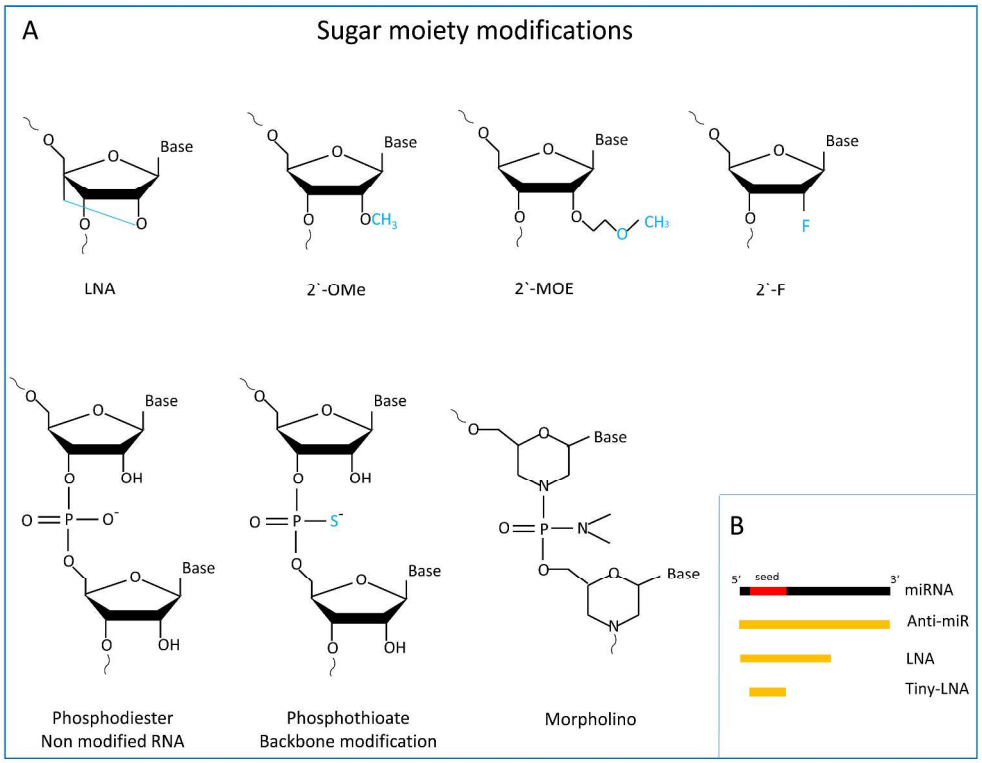


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**Supplemental Table 1 |** Circulating miRNAs associated to Diabetes

| miRNA | Diabetes Type | Source | other associated diseases |
|---|---|---|---|
| ebv-miR-BART12 | T1D | PBMC [1] | |
| let-7f | T1D | PBMC [1, 2] | Breast Cancer [3], AD (Blood, PBMC) [4, 5], Lung cancer (Plasma) [6] |
| let-7g | T1D | PBMC [1, 2] | Breast Cancer [3], AD (Plasma) [4, 5], MetS (Serum) [7] |
| let-7i | T2D | Serum [8] | Breast Cancer, Lung Cancer [3], AD (CSF) [5] |
| miR-7 | T1D | PBMC [1] | Colorectal Cancer (Plasma) [9], Dermatomyositis (Serum) [10], Acute Pancreatitis (Serum) [11] |
| miR-9 | T1D<br>T2D | Serum [12, 13]<br>Serum [4, 7, 12, 16-18] | HD (Blood MVs) [14], Biliary Tract Cancer (Bile) [3], AD (CSF, Serum) [4, 5, 15] |
| miR-10a | T1D | PBMC [1] | Esophageal Cancer (Serum) [9], Acute Pancreatitis (Serum) [11] |
| miR-15a | T2D | Plasma [4, 7, 12, 16, 18-20] | Diabetic NP (Urine) [21], Obesity (Plasma) [22], AD (Plasma, CSF) [4, 5], HF (Plasma) [23] |
| miR-15b* | T1D | PBMC [1] | |
| miR-16 | T1D | PBMC [1] | Childhood Obesity (Plasma), NAFLD (Serum) [7]<br>Prostate Cancer, HCC [3], PD (Leukocytes) [5, 24] |
| miR-18b | T1D | PBMC [1] | Breast Cancer (Plasma) [25] |
| miR-19a | T1D | PBMC [1] | CAD (Blood, Plasma MPs) [4, 16, 23], Lung Cancer (Plasma) [6, 26], ACS (Plasma MPs) [7] |
| miR-20a* | T1D | PBMC [1] | |
| miR-20b | T1D<br>T2D | PBMC [1, 2]<br>Plasma [4, 7, 18, 19] | Fetal Hypoxia [27] |
| miR-21a | T1D<br><br>T2D | PBMC [1, 2, 12, 18, 28], Plasma [29], Urine [29]<br><br>Plasma [4, 7, 18, 19] | CAD (Plasma MPs), Arteriosclerosis (Serum) [16, 23], Obesity (Blood) [7], HCC (Plasma) [9]<br>Lung Cancer, Ovarian Cancer (Plasma ExoS) [30], HF (Blood MVs) [14]<br>Diabetic NP (Urine), NAFLD (Serum) [31], AMI, CVD (Plasma), AD (CSF) [15] |
| miR-23 | T2D | Serum [8] | Dyslipidemia (Blood) [32], Brain Tumors (Serum) [9] |
| miR-24a | T1D<br>T2D | Serum [4, 12, 18, 33]<br>Plasma [4, 18, 19] | Lung Cancer (Serum) [9, 26], Rheumatic Arthritis (Plasma) [10] |
| miR-25 | T1D | Serum [4, 12, 18, 33] | CAD (Plasma, Plasma MPs), AMI (PBMC, Platelets, Plasma) [23]<br>NSCLC (Serum) [30], Lung Cancer (Serum) [9] |
| miR-26a | T1D<br>GD | Serum [4, 12, 18, 33]<br>PBMC [2] | Pancreatic Tumors, Gastric Cancer, Sarcoidosis, AMI, Pancreatitis (Blood) [34]<br>PD (PBMC) [35], AMI (Serum) [36], HCC (Plasma) [9], AD (Blood) [5] |
| miR-26b | T1D | PBMC [1] | Pancreatic Tumors, Gastric Cancer, MS, Sarcoidosis, AMI (Blood) [34], AD (Blood) [5] |

| | | | |
|---|---|---|---|
| miR-27a | T1D | Serum [4, 12, 18, 33], PBMC [2] | MetS (Blood) [32], Gastric Cancer, HCC (Plasma) [9], AD (CSF) [5] |
| | T2D | Blood [12, 32], PBMC [2] | Stroke (Plasma) [4] |
| | GD | PBMC [2] | |
| miR-27b | T1D | Serum [4, 12, 18, 33], PBMC [1] | Arteriosclerosis (Serum) [4, 7, 16], Obesity (Blood) [7] |
| miR-28 | T2D | Plasma [4, 7, 12, 14, 16, 18-20] | PD (PBMC) [35], Prepubertal Obesity (Plasma) [7, 37] |
| miR-29a | T1D | Serum [4, 12, 18, 33] | CAD (Blood) [4, 16], IgA NP (Urine) [21], AD (Serum), PD (Blood) [4, 5] |
| | T2D | Serum [4, 7, 12, 16-18], Blood [7, 16, 38], Urine [4, 39] | Liver Fibrosis (Serum) [40] |
| | GD | Serum [4, 7, 18, 41], PBMC [2] | |
| miR-29b | T1D | PBMC [2] | PD (PBMC) [35], AD (Serum) [4, 5], Aortic Aneurysm (Plasma) [4] |
| | T2D | Plasma [4, 7, 12, 16, 19, 42, 43], PBMC [2] | Cardiomyopathy (PBMC) [23] |
| | GD | PBMC [2] | |
| miR-29c | GD | PBMC [2] | Lung Cancer [3] |
| miR-30a | T1D | Serum [4, 12, 18, 33] | PD (Blood) [4, 18], AMI (Plasma), HF (Serum) [23] |
| miR-30b | GD | PBMC [2] | PD (PBMC) [35], Lung Cancer (Plasma) [26], HF (Plasma) [23] |
| miR-30d | T2D | Serum [4, 7, 12, 16-18], Blood [7, 16, 38] | FSGS (Urine) [21], Lung Cancer (Serum) [26], AMI (Plasma) [23] |
| miR-30e | T2D | PBMC [2] | CAD (Blood) [4, 16, 23], Crohn's Disease (Serum) [8], NSCLC (Plasma) [6] |
| miR-30e* | T1D | PBMC [1] | |
| miR-31 | T1D | Serum [12, 13] | Oral Cancer [3] |
| miR-32 | T1D | PBMC [1] | |
| miR-33a | T1D | PBMC [1] | Hyperlipidemia (Serum) [44] |
| miR-34a | T1D | Serum [12, 13] | NAFLC (Serum) [31], Gastric Cancer (Serum) [9], AD (PBMC) [4, 18], Ageing (Blood) [45] |
| | T2D | Serum [4, 7, 12, 16-18] | |
| miR-93 | T1D | PBMC [12, 18, 28] | IgA NP (Urine) [21], Colorectal Cancer (Plasma) [9], Crohn's Disease (Serum) [10] |
| miR-96 | T2D | Serum [8] | |
| miR-98 | T1D | PBMC [1] | HCC (Serum) [46] |
| miR-101 | T1D | PBMC [1] | Autism (Serum) [47] |
| | GD | PBMC [2] | |
| miR-103 | T1D | PBMC [2] | AD (Blood) [5], HF (Plasma) [23], Obesity (Blood) [7] |
| miR-122 | T2D | Serum [8] | NAFLD (Serum) [31], HCC (Plasma) [9], CAD (Plasma) [7] |
| | | | ATI (Serum) [30], MI, HF (Plasma) [16, 20], NP (Urine) [48] |
| miR-124a | T2D | Serum [4, 7, 12, 17, 18] | ATI (Serum) [30], Aortic Aneurysm (Plasma) [4] |

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| miR-126 | T1D | Urine [29], PBMC [1, 2] | Bladder Cancer (Urine) [30], PD (PBMC) [35], HF, CAD (Plasma) [20], Lung Cancer (Plasma) [26] |
| | T2D | Plasma [4, 7, 12, 15, 18-20, 49], PBMC [2] | AMI (Plasma, Serum) [4, 36], Congestive HF (Plasma) [4], SLE (Plasma) [10] |
| | GD | PBMC [2] | |
| miR-126* | T1D | PBMC [2] | PD (PBMC) [35], CAD (Plasma, Plasma MPs) [23] |
| miR-130a | T1D | PBMC [2] | HF (Plasma) [23], Autism (Serum) [47] |
| | | | Arteriosclerosis (Serum) [4, 16], Hypertension (Blood) [32] |
| miR-132 | GD | Serum [4, 18, 41] | AD (Plasma) [5], Rheumatic Arthritis (Plasma) [10], Obesity (Blood) [7] |
| miR-140 | T1D | PBMC [1] | CAD (Blood) [16, 20, 23], Obesity (Plasma) [22], Crohn's Disease (Serum) [10] |
| | T2D | PBMC [2] | Childhood Obesity, Morbid Obesity (Plasma) [47] |
| miR-142 | T1D | PBMC [2] | Cardiomyopathy (PBMC) [16, 23], Obesity (Plasma) [22], Prepubertal Obesity (Plasma) [37] |
| | T2D | PBMC [2] | HF (Plasma) [23], SLE (Plasma) [10], AD (Plasma) [4, 5] |
| | GD | PBMC [2] | |
| miR-144 | T1D | PBMC [2] | Diabetic NP (Urine) [31] |
| | T2D | Blood [3, 7, 16, 31, 38], PBMC [2] | |
| | GD | PBMC [2] | |
| miR-146a | T1D | Serum [12, 13] | Sepsis (Serum, Blood MVs) [14, 30], Rheumatoid Arthritis (Blood MVs) [14] |
| | T2D | Plasma [4, 7, 15, 50, 51], PBMC [7, 50] | AMI, CVD (Plasma) [15], SLE (Serum) [10] |
| | | Serum [4, 7, 8, 12, 16-18] Blood [7, 16, 38] | AD (CSF) [4, 5, 15], Lung Cancer (Serum) [26], CAD (PBMC, Plasma MPs) [16, 23] |
| miR-148a | T1D | Serum [4, 12, 18, 33], PBMC [1] | Lung Cancer, Pancreatic Tumors, MS, Pancreatitis, Sarcoidosis, Prostate Cancer (Blood) [34] |
| miR-148b | T1D | PBMC [1] | Esophageal Cancer (Serum) [9] |
| miR-150 | T1D | PBMC [2] | CAD (Blood) [4, 16, 23], CLL [3], PD (Leukocytes) [24], AMI (Serum) [36] |
| | T2D | Blood [7, 12, 16, 32, 38], Plasma [4, 7, 19, 43] | |
| miR-152 | T1D | Serum [4, 12, 18, 33] | Lung Cancer (Serum) [9], Preeclampsia [3] |
| miR-155 | T1D | Serum [12, 13] | Pancreatic Cancer (PJ) [3], AMI (Plasma) [15], SLE (Blood MVs) [14] |
| | | | CAD (Plasma, Serum, Blood, Plasma MPs) [16], AD (CSF) [5]], Obesity (Blood) [7] |
| miR-181a | T1D | Serum [4, 12, 18, 33] | Azoospermia (Semen) [52], AMI (Blood) [4], SLE (Plasma), Psoriasis (Serum) [10] |
| | GD | PBMC [2] | |
| miR-181d | GD | PBMC [2] | CAD (Blood) [7] |
| miR-182 | T2D | Blood [7, 16, 38] | CAD (Blood) [7, 16, 20, 23], Asthenozoospermia (Semen) [52], Lung Cancer (Serum) [26] |
| miR-186 | T1D | PBMC [1] | Dyslipidemia (HDL) [53], AMI (Serum, Platelets) [4, 23] |
| | T2D | Serum [8] | |

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|---|---|---|---|
| miR-191 | T2D | Plasma [4, 7, 18, 19], Serum [8] | AD (Plasma) [4, 5], AMI (Serum) [36]<br>Dyslipidemia (HDL) [53], Lung Adenocarcinoma (Blood) [6] |
| miR-192 | T2D | Blood [7, 12, 16, 32, 38], Serum [8] | Diabetic NP (Urine), IgA NP (Urine) [31], Crohn's Disease (Serum) [10]<br>ATI (Serum) [30], SLE (Serum, Urine) [3], FSGS (Serum) [54], HCC (Plasma) [9] |
| miR-195 | T1D | PBMC [1] | HF, AMI (Plasma) [23], Crohn's Disease (Serum) [10], Autism (Serum) [47]<br>Hypertension (Blood) [32], Breast Cancer (Blood MVs) [14] |
| miR-197 | T2D | Plasma [4, 7, 18, 19] | Dyslipidemia (Blood) [32], Brain Tumors (Serum) [9], AMI (Plasma) [4]<br>Pancreatic Tumors, Gastric Cancer, MS, Pancreatitis (Blood) [34] |
| miR-199 | T1D<br>T2D<br>GD | Serum [12, 13], PBMC [1, 2]<br>PBMC [2], Serum [8]<br>PBMC [2] | PD (PBMC) [35], Lung Cancer (Serum) [9], CAD (Plasma, Serum) [7] |
| miR-200a | T1D | Serum [4, 12, 18, 33] | SLE (Serum, Urine) [3], AD (PBMC) [4], Breast Cancer (Serum) [6]<br>Ovarian Cancer (Plasma ExoS), Oral Cancer (Saliva) [30] |
| miR-210 | T1D | Serum [4, 12, 18, 33], Plasma [29], Urine [29] | Lung Cancer (Blood MVs, Plasma) [14, 26], Congestive HF (PBMC) [4], CLBCL (Serum) [30]<br>Arteriosclerosis (Serum) [16], HF (Plasma, Serum, MC) [23] |
| miR-222 | T2D<br>GD | PBMC [2]<br>Serum [4, 7, 18, 41] | Obesity (Plasma) [22], PD (Plasma) [4]<br>Dyslipidemia (HDL) [53], CAD (Blood, EPC) [16], Lung Cancer, Thyroid Cancer (Serum) [9]<br>Arteriosclerosis (Blood) [45], Morbid Obesity, Childhood Obesity (Plasma) [7] |
| miR-223 | T2D | Plasma [4, 7, 12, 16, 18-20] | AMI (Plasma, Serum), Aortic Aneurysm (Plasma) [4], MS (Serum) [10]<br>Sepsis (Serum) [30], Dyslipidemia (HDL) [53], CAD (Plasma MPs) [16], HCC (Serum) [40] |
| miR-301a | T1D | PBMC [1] | PD (PBMC) [35], AD (Plasma) [4, 5] |
| miR-320a | T2D | Plasma [4, 7, 18, 19], Blood [7, 12, 16, 32] | HF (Serum) [16], MetS (Blood) [32], Lung Cancer (Serum) [9]<br>Pancreatic Tumors, Gastric Cancer, Sarcoidosis, AMI, Pancreatitis (Blood) [34] |
| miR-324 | T1D | PBMC [1] | Pancreatic Tumors, Gastric Cancers, MS, Pancreatitis, AMI, Sarcoidosis (Blood) [34] |
| miR-326 | T1D | Lymphocytes [12, 18, 55] | Colorectal Cancer (Plasma) [56] |
| miR-335 | T1D | PBMC [1] | PD (PBMC) [35] |
| miR-338 | T1D | PBMC [1] | Colorectal Cancer (Blood) [57] |
| miR-340* | T1D | PBMC [1] | CAD (Platelets) [16] |
| miR-342 | T1D<br>T2D<br>GD | PBMC [1, 2]<br>PBMC [2]<br>PBMC [2] | Dyslipidemia (HDL) [53], CAD (Blood) [4, 16, 23], AMI (Platelets), HF (Plasma) [23] |

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|---|---|---|---|
| miR-375 | T2D | Serum [4, 7, 12, 16-18], Blood [12, 32] | MI (Plasma) [16], HCC (Serum) [18], Diabetic NP (Urine) [31], Prostate Cancer (Serum) [9] |
| miR-423 | T1D | PBMC [1] | Obesity, Prepubertal Obesity (Plasma) [7, 22, 37], HF (Serum, Plasma) [20, 30] Pancreatic Tumors, Gastric Cancer, AMI, Pancreatitis (Blood) [34] |
| miR-424 | T1D | PBMC [1] | Fetal Hypoxia [27] |
| miR-450a | T1D | PBMC [1] | |
| miR-451 | T1D | PBMC [2] | Lung Cancer (Plasma) [26], CAD, HF (Plasma) [23] CLL [3], NAFLD (Serum) [31], ALS (Leukocytes) [5] |
| miR-454 | T1D | PBMC [1] | MS (Plasma) [10] |
| miR-486 | T2D | Plasma [4, 7, 18, 19], Serum [8] | Prepubertal Obesity (Plasma) [7, 37], Lung Cancer (Plasma) [26], AMI (Serum) [36] |
| miR-503 | T2D | Plasma [7, 16, 58], Serum [27][7] | Obesity (Serum) [7, 59] |
| miR-542 | T1D | PBMC [1] | |
| miR-548 | T1D | PBMC [1] | |
| miR-595 | GD | PBMC [2] | Pancreatic Tumors, Gastric Cancer, MS, Sarcoidosis, Prostate Cancer (Blood) [34] |
| miR-720 | T1D | PBMC [1, 2] | CAD (Plasma, EPC) [60], Myeloma (Serum) [61] |
| miR-766 | T1D | PBMC [1] | Prostate Cancer [3] |
| miR-940 | T1D | PBMC [1] | |
| miR-1180 | GD | PBMC [2] | |
| miR-1260 | T1D | PBMC [2] | Influenza A Virus Infection (Blood) [62] |
| miR-1268 | GD | PBMC [2] | Lung Cancer (Serum) [18] |
| miR-1274a | T1D | PBMC [2] | |
| miR-1274b | T1D | PBMC [2] | PD (Leukocytes) [24] |
| miR-1275 | T1D | PBMC [1] | ALS (Leukocytes) [5] |
| miR-1307 | T1D | PBMC [2] | |
| | T2D | PBMC [2] | |
| | GD | PBMC [2] | |

ACS, Acute Coronary Syndrome; AD, Alzheimer's Disease; ALS, Amyotrophic Lateral Sclerosis; AMI, Acute Myocardial Infarction; ATI, Acute Tissue Injuries; CAD, Coronary Artery Disease; CLBCL, Cutaneous Large B-Cell Lymphoma; CSF, Cerebrospinal Fluid; CVD, Cardiovascular Disease; EPC, Endothelial Progenitor Cells; ExoS, Exosomes; FSGS, Focal Segmental Glomerulosclerosis; GD, Gestational Diabetes; HCC, Hepatocellular Carcinoma; HD, Huntington's Disease; HDL, High-Density Lipoprotein; HF, Heart Failure; MC, Mononuclear Cells; MetS, Metabolic Syndrome; MI, Myocardial Infarction; MPs, Microparticles; MS, Multiple Sclerosis; MVs, Microvesicles; NAFLD, Non-Alcoholic Fatty Liver Disease; NP, Nephropathy; NSCLC, Non-Small-Cell Lung Carcinoma; PBMC, Peripheral Blood Mononuclear Cells; PD, Parkinson's Disease; PJ, Pancreatic Juice; SLE, Systemic Lupus Erythematosus; T1D, Type 1 Diabetes; T2D, Type 2 Diabetes

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