



## 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  $\beta$ -cells plays a pivotal role in the control of blood glucose homeostasis. Diabetes mellitus develops if the amount of insulin secreted by  $\beta$ -cells is insufficient to cover the organism needs. This occurs if the  $\beta$ -cells are destroyed by the immune system (Type 1 diabetes, T1D) or when  $\beta$ -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

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3 (DiGeorge syndrome critical region 8) (Fig.1). They are then cleaved by Drosha to form precursor-  
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5 miRNA hairpin structures of about 70 nucleotides. These molecules are exported from the nucleus by  
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7 Exportin 5 and are successfully cleaved by Dicer to generate double-stranded RNA molecules of 20-  
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9 23 nucleotides. After being cleaved by Dicer the mature miRNA strands associate to members of the  
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11 Argonaute protein family and are loaded in to the RNA-Induced Silencing Complex (RISC). This  
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13 enables them to bind to MicroRNA Recognition Elements (MRE) located in the 3'untranslated region  
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15 of target mRNAs that are initially recognized through base pairing to a conserved "seed" sequence  
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17 corresponding to nucleotides 2-8 of the miRNAs, leading to inhibition of mRNA translational and/or  
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19 to a decrease in messenger stability [2, 3]. A single miRNA typically controls hundreds of targets and  
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21 each mRNA can be targeted by different miRNAs, conferring to this class of non-coding RNA  
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23 molecules a huge regulatory potential [4]. In the past decade, a large body of evidence has been  
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25 accumulated pointing to a role for miRNAs in the etiology and pathogenesis of diabetes and its  
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27 complications. Indeed, alterations in the level of these non-coding RNAs has been observed both in  
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29 insulin-secreting cells and in insulin-target tissues isolated from diabetes animal models or diabetic  
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31 patients [5]. Moreover, changes in miRNA expression have been associated with long-term diabetes  
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33 complications including neuropathy, retinopathy, renal failure and macrovascular diseases.  
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38 The first demonstration of the involvement of miRNAs in the control of specialized  $\beta$ -cell functions  
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40 has been provided ten years ago by Poy et al. who showed that inappropriate levels of miR-375, one  
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42 of the most abundant miRNAs present in  $\beta$ -cells, can affect insulin secretion [6]. Later on, this and  
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44 several other miRNAs including miR-15a/b, miR-16, miR-195, miR-503, miR-451, miR-214, miR-9,  
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46 miR-124a, miR-7 and miR-376 were demonstrated to play important roles in the differentiation of  
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48 pancreatic islet cells [7-12]. Moreover, changes in the levels of many miRNAs, including miR-9, miR-  
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50 124a, miR-24, miR-26, miR-148 and miR-182 were found to exert a deleterious influence on  $\beta$ -cell  
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52 activities by targeting key genes involved in insulin biosynthesis, insulin secretion and cell survival  
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54 [13-16].  
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3 Several studies have attempted to establish a link between the changes in islet miRNA expression  
4 and the development of both T1D and T2D. MiR-34a, miR-21, miR-146a and miR-29 family members  
5 were found to be up-regulated in the islets of pre-diabetic NOD mice, a well-characterized model of  
6 T1D [17, 18]. The up-regulation of these miRNAs is deleterious for the secretory activity and for the  
7 survival of  $\beta$ -cells in the presence of pro-inflammatory cytokines released by leucocytes infiltrating  
8 the islets of Langerhans, suggesting that they may contribute to the progression of the disease. T1D  
9 was also linked to altered miRNA expression in immune cells. Indeed, Herzova et al. observed up-  
10 regulation of miR-510 and down-regulation of miR-191 and miR-342 in regulatory T-cells ( $T_{reg}$  cells)  
11 isolated from T1D patients [19]. Since these cells are known to be critical controllers of the immune  
12 reaction, alterations in their miRNA profile may favor the development of an autoimmune attack  
13 directed toward the  $\beta$ -cells and hence the manifestation of T1D diabetes.  
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27 Several independent studies have reported modifications in miRNA expression occurring in the islets  
28 of T2D animal models, including *ob/ob* and *db/db* mice, lacking leptin or its receptor, mice fed a high  
29 fat diet and Goto-Kakizaki rats, a spontaneous model of T2D that in contrast to the other is not  
30 associated with obesity [20]. Systematic analysis of the differentially expressed miRNAs highlighted  
31 changes in the level of numerous miRNAs with a deleterious impact on insulin secretion and  $\beta$ -cell  
32 survival under pro-apoptotic conditions including among others miR-34a, miR-124, miR-146a, miR-  
33 199a-3p, miR-203, miR-210, miR-335 and miR-383 [17, 21-23]. However, not all the modifications in  
34 miRNA expression occurring in the islets of T2D animals were found to have a negative effect on  $\beta$ -  
35 cells. Indeed, the down-regulation of miR-184 and miR-338-3p and the up-regulation of miR-132  
36 observed in different animal models were demonstrated to trigger  $\beta$ -cell mass expansion and to  
37 improve insulin secretion [22, 24, 25]. Thus, the changes in the level of these miRNAs are likely to be  
38 part of the compensatory mechanisms attempting to counterbalance the diminished sensitivity of  
39 insulin target tissues that typically develops in obese individuals and during pregnancy. Significant  
40 differences in islet miRNA expression were also detected in the islets of human T2D patients. Indeed,  
41 the islets of T2D donors were found to display an up-regulation of miR-187 [26] and the down-  
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3 regulation of several members of a large miRNA cluster generated from the imprinted DLK1-MEG  
4 locus [27]. It is not yet clear whether the differential levels of these miRNAs are inherited,  
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6 predisposing the affected individuals to develop T2D, or whether they are acquired later in life,  
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8 promoting the manifestation of the disease.  
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11 Obesity and insulin resistance are also leading to a strong dysregulation of the miRNA profile in  
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13 adipose tissue, liver and in skeletal muscles. The most affected miRNAs, including among others miR-  
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15 29, miR-103/107, miR-143, miR-802 and Let-7, were shown to target key components of the insulin  
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17 signaling pathway and to contribute to the loss of insulin sensitivity observed in obese individuals  
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19 [28-30]. Moreover, miR-133 and miR-1 dysregulation has been shown to contribute to impaired  
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21 muscle function in T2D [31-33]. Indeed, repression of miR-133 and miR-1 in response to insulin was  
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23 shown to be impaired in T2D patients [31, 34].  
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27 MiRNAs are also suspected to be central players in the development of long-term microvascular  
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29 (neuropathy, nephropathy and retinopathy) and macrovascular (cardiovascular and peripheral  
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31 vascular diseases) diabetes complications. Indeed, several studies point to a role for miRNAs  
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33 including miR-29, miR-192, miR-200, miR-216 and miR-217 in TGF $\beta$  signaling and glomerular fibrosis  
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35 associated with diabetic nephropathy [35]. Moreover, miRNAs have been reported to regulate the  
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37 expression of VEGF (vascular endothelial growth factor) in the retina and to participate to the  
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39 development of diabetic retinopathy [36]. Kovacs et al. identified a set of up-regulated NF- $\kappa$ B-  
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41 responsive miRNAs in the retina of diabetic rats, including miR-146a/b, miR-155, miR-132 and miR-21  
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43 [37]. MiR-155 is known to be involved in immuno-modulatory signaling, whereas miR-21 and miR-  
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45 146a/b participate to fibrotic responses and miR-132 to angiogenesis.  
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49 MiR-1 and miR-133 were shown to play controversial roles also in diabetic cardiomyopathy. Xiao et  
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51 al. observed exaggerated expression of miR-133 in the heart of diabetic rabbits causing the  
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53 prolongation of QT interval, a typical event observed in T2D [38]. However miR-133 as well as miR-1  
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55 are down-regulated in cardiac hypertrophy and in the hearts of STZ-induced diabetic mice suggesting  
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57 complex relationship between these miRNAs and cardiac dysfunction [39].  
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### 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,

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3 endocytosis or fusion of exosomes with the cell membrane [51, 58-61] and to further alter the gene  
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5 expression profile of recipient cells [62, 63]. Additionally, plant miRNAs were observed to be  
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7 transported to the liver, proving their capability to cross the intestinal barrier and enter the blood  
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9 stream [48, 64]. Therefore, circulating miRNAs may potentially act as signaling molecules traveling  
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11 over long distances to elicit a response in cells located at distant organs. However, the precise  
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13 mechanisms permitting the targeting of the secreted miRNAs to specific target cells remains unclear.  
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15 MiRNAs can be easily isolated from body fluids in a non-invasive manner, which makes them perfect  
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17 candidates as biomarkers. In principle, they can be measured both in plasma and in serum samples.  
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19 However, if serum is used, care should be taken to avoid lysis of blood cells during the coagulation  
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21 process [65]. Beside their elevated stability, these molecules can be rapidly and accurately detected  
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23 using highly specific and sensitive methods. There is a hope that the use of miRNAs as biomarkers  
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25 may facilitate early detection of diseases and allow timely intervention to delay complications.  
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27 Furthermore, changes in the level of some of these small non-coding RNAs may be useful for the  
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29 prognosis of disease progression and to monitor the efficacy of the treatments. Indeed, several  
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31 miRNAs were shown to be altered in the circulation in a growing number of diseases including  
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33 diabetes, several types of cancer and Alzheimer (see Supplementary Table 1 for an exhaustive list).  
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35 Interestingly, in some cases the modifications in the circulating miRNA profile were already  
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37 detectable years before the manifestation of the disease suggesting that they may be exploited to  
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39 identify the individuals at risk to develop the disorder.  
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44 Along this line, a growing number of studies have attempted to predict the occurrence of diabetes  
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46 and its long-term complications by detecting early changes in plasma miRNAs. Zampetaki and  
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48 coworkers were the first to identify a plasma miRNA profile characteristic of T2D. Five miRNAs (miR-  
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50 15a, miR-28-3p, miR-29b, miR-126 and miR-223) were found to be deregulated several years before  
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52 the onset of the disease [46] and permitted to predict the occurrence of vascular complications.  
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54 Furthermore, a recent study by Yang *et al.* detected significantly lower levels of miR-23a in the serum  
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56 of T2D patients compared to healthy controls that were already detectable in pre-diabetic individuals  
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3 [66]. A similar type of analysis was also performed in animal models to identify potential biomarkers  
4 predicting the development of T1D. For this purpose, circulating miRNAs were measured in the blood  
5 of non-obese diabetic (NOD) mouse, a well-characterized T1D model and in B57BL/6 mice treated  
6 with streptozotocin, a pharmacological agent inducing a rapid and specific loss of pancreatic  $\beta$ -cells.  
7 In both models the level of miR-375, a miRNA highly enriched in  $\beta$ -cells was found to be increased in  
8 plasma, prior to T1D onset [67]. In humans the loss of  $\beta$ -cells and the phases preceding the  
9 manifestation of T1D is likely to span over a much longer period compared to NOD mice or mice  
10 treated with streptozotocin. Therefore, it is not yet clear whether a slow and progressive loss of  $\beta$ -  
11 cells will lead to detectable modifications in circulating miR-375 levels. Changes in circulating miRNAs  
12 were also used to predict the development of GD. Indeed, miR-29a, miR-132 and miR-222 were  
13 already decreased after 16-19 weeks of gestation in the serum of pregnant women that were later  
14 diagnosed with GD at weeks 25-28 [68].

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16 Interestingly, nine miRNAs (miR-27a, miR-29a, miR-29b, miR-126, miR-142, miR-144, miR-199, miR-  
17 342 and miR-1307) were identified in peripheral blood mononuclear cells (PBMCs) as potential  
18 biomarkers in all three diabetes types which might imply their association with the diabetes  
19 condition per se. This may be due to the fact that several of these common miRNAs play important  
20 regulatory functions in pathways related to diabetes, such as metabolic or immunological processes  
21 [69].

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

1 The miRNA profiles are believed to be stable over long time [73] and  
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5 serum miRNAs are similar between individuals of different gender or ages [44]. Furthermore, feeding  
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7 state, smoking and time of blood draw do not seem to have major influences on the miRNA profile  
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9 [74]. However, the impact of factors such as female hormone cycle, pregnancy and life style remain  
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11 to be elucidated. Hemolysis is another problem with which investigators have to deal during blood  
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13 samplings. It has been linked to a direct release of large numbers of miRNAs significantly altering the  
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15 blood miRNA profile [74]. For this reason, biomarkers for hemolysis might be useful to prevent faulty  
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17 quantifications.  
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20 Studies performed by different groups often show discrepant results in their miRNA analysis. This  
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22 may be caused by technical challenges such as different RNA processing, sample handling, storage,  
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24 measurement technologies, as well as, different sources for miRNA analysis. A problem investigators  
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26 are faced with is the missing standardization and the lack of a “housekeeping” miRNA for  
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28 normalization. The latter may be solved by using additional spike-in standards which can be  
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30 introduced prior to cDNA synthesis.  
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33 Zhang and coworkers analyzed the Zampetaki miRNA signature in plasma of healthy individuals, T2D-  
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35 susceptible individuals and T2D patients [75]. In their study, miR-29b and miR-28-3p were not  
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37 detectable and miR-15a and miR-223 showed comparable expression levels in all three groups. Only  
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39 miR-126 showed an altered expression profile in both studies. These discrepancies may be explained  
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41 by the differences in size and ethnicity of the cohorts. Zampetaki *et al.* analyzed plasma samples of  
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43 around 800 Italians, while Zhang *et al.* worked with plasma samples of 90 Chinese individuals. This  
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45 emphasizes the need to confirm the results in larger prospective populations.  
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48 So far, the question remains open whether the deregulation in the circulating miRNA profile is the  
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50 cause or the consequence of the diseases. Furthermore, the cellular origin of circulating miRNAs  
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52 remains also unclear. The miRNA profile in the circulation does not seem to merely mirror the one  
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54 present in the cells, as e.g. the miR-29 family members are down-regulated in serum of diabetic  
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56 patients, but are up-regulated in pancreas [76].  
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3 Although, research of miRNAs as potential biomarkers is still in its infancy, several studies have  
4 identified miRNAs which may help to predict and diagnose diabetes. However, as a single miRNA  
5 shows often alterations in more than one disease, measurement of a group of miRNAs will probably  
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7 better achieve the required specificity and sensitivity needed for the clinics.  
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### 10 11 **Therapeutic strategies to modulate the level of microRNAs in diabetes**

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

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

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

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3 As described above, many miRNAs are up-regulated in diabetic conditions. The aim of the silencing  
4 **therapy is to reduce the excess of miRNA and restore proper target gene expression.** This is usually  
5 achieved using antisense oligonucleotides (anti-miRs) that bind to the miRNA causing the blockade of  
6 its activity and its degradation. Anti-miRs are widely used for the modulation of miRNA expression in  
7 experimental models and, if conveniently modified, they have proved effective also *in vivo* [86, 87].  
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9 Different types of anti-miRs are currently available involving specific chemical modifications of the  
10 nucleotides [79, 80] (Fig.2). AntagomiRs are O-methyl-modified oligonucleotides coupled to  
11 cholesterol and were the first molecules capable of blocking miRNA expression *in vivo* [88]. Another  
12 common modification involves the inclusion of Locked nucleic acids (LNAs) that strongly increase the  
13 binding affinity of the anti-miRs (Fig.2). In view of the superior properties of LNAs, it is possible to  
14 design not only anti-miR specifically directed to a single miRNA but also anti-miRs capable of blocking  
15 an entire family of closely-related miRNAs. This is achieved using shorter “8-mer” LNA  
16 oligonucleotides (Tiny LNAs) complementary to the seed sequence shared by all members of the  
17 same miRNA family [89].  
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33 Recently, Wang et al. showed that miR-7a, a miRNA highly expressed in adult  $\beta$ -cells, targets five  
34 components of the mTOR signaling pathway. Inhibition of miR-7a was found to activate the mTOR  
35 signaling and to promote adult  $\beta$ -cell replication. These findings suggest that miR-7a acts as a brake  
36 on adult  $\beta$ -cell proliferation and represents a potential therapeutic target for diabetes [90].  
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40 Interestingly, a circular RNA has been shown to possess numerous miR-7 binding sites and to  
41 regulate the availability of this miRNA [91]. Thus, in theory strategies raising the level of this  
42 circular RNA may potentially be exploited to trigger adult  $\beta$ -cell proliferation.  
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48 MiR-122 is a liver specific miRNA that regulates both lipid metabolism [92] and HCV replication [93,  
49 94]. MiR-122 inhibition in a diet-induced obesity mouse model resulted in decreased plasma  
50 cholesterol levels and a significant improvement in liver steatosis, accompanied by reductions in  
51 several lipogenic genes [92]. On the other hand, HCV replication was shown to be inhibited by a 2'-O-  
52 methyl-modified anti-miR-122 oligonucleotide opening the doors for the use of miR-122 inhibitors as  
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3 a treatment for HCV infection in humans [93]. “Miravirsen” is a 15-mer LNA containing a  
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5 phosphorothioate-modification providing an extremely high affinity for miR-122. A study carried out  
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7 in a non-human primate showed that intravenous administration of Miravirsen leads to inhibition of  
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9 miR-122 and a considerable lowering of plasma cholesterol levels [95, 96]. Miravirsen was also  
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11 efficient in the treatment of chronic HCV infection Chimpanzees [97]. Studies on healthy primates  
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13 and humans did not reveal any significant toxic effect [95, 96]. In a phase II clinical study, Miravirsen  
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15 has been successfully administered subcutaneously resulting in a decrease in the HCV RNA level in  
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17 patient’s serum and was well tolerated after 18 weeks treatment [98, 99]. However, potential long-  
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19 term adverse effects of miRNA inhibition still need to be assessed.  
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22 In view of the observed modifications in their expression level occurring in association with T1D, T2D  
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24 and GD, in principle several miRNAs would elect as attractive targets for diabetes miRNA-based  
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26 therapies. Because of the difficulty in specifically targeting a very small cell population such as the  
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28 one represented by pancreatic  $\beta$ -cells, so far most of the studies focused on miRNAs altered in liver  
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30 and in other insulin target cells.  
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33 Let-7 family members have been discovered to play central and unexpected roles in glucose  
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35 metabolism in many organs [100]. Several members of this family are up-regulated in the liver of  
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37 *ob/ob* and diet-induced obesity mice. Interestingly, impaired glucose tolerance in diet-induced  
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39 obesity mice could be prevented and treated upon systemic injection of anti-Let-7 [100], suggesting  
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41 that blockade of this miRNA family may constitute a potential strategy to treat T2D. The members of  
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43 the Let-7 family promote cell differentiation and suppress tumor initiation because of their multiple  
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45 targets that are involved in cell cycle and mitotic signaling. Furthermore, let-7 is down-regulated in a  
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47 variety of malignancies [101]. Thus, the potential use of anti-let-7 therapy to counteract the diabetic  
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49 state should take into consideration the long-term risk of developing cancer [102]. Another miRNA  
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51 family that is being scrutinized as a candidate for clinical use is miR-103/miR-107. These two miRNAs  
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53 differ only by one nucleotide in their 3’ region. The expression of miR-103/107 has been shown to be  
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55 elevated in *ob/ob* mice and to target Caveolin-1, which is a critical regulator of insulin receptor  
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3 signaling. In *ob/ob* mice antagomiR-based silencing of miR-103/107 led to improved glucose  
4 homeostasis and insulin sensitivity in peripheral tissues [29], making these two miRNAs very  
5 attractive new targets for the treatment of T2D and obesity. Currently, an anti-miR is being  
6 developed by Regulus Therapeutics in partnership with AstraZeneca, and is at the preclinical stage  
7 [78].  
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13 MiR-184 and miR-338-3p are two other miRNAs with an interesting potential for the treatment of  
14 T2D and/or GD. The level of these two miRNAs is reduced under conditions of insulin resistance  
15 associated with obesity and pregnancy [24, 25]. Treatments leading to diminished expression of miR-  
16 184 and miR-338-3p resulted in  $\beta$ -cell mass expansion compensating for the insulin resistant state.  
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18 Thus, molecules blocking the activity of these miRNAs specifically delivered to  $\beta$ -cells would be  
19 anticipated to boost the physiological response of the organism to insulin resistance and may permit  
20 to prevent or treat T2D or GD.  
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28 As for miRNA mimics, one of the major obstacles inherent to the use of anti-miRs *in vivo* is  
29 represented by the difficulty in targeting a specific cell type. An alternative strategy to inhibit miRNA  
30 activity involves the use of so-called “miRNA sponges”. MiRNA sponges are expression vectors  
31 engineered to generate RNA molecules containing multiple artificial miRNA binding sites. These  
32 constructs, which can be driven by cell-specific promoters, permit to sequester the endogenous  
33 miRNA and to relieve its inhibitory activity on the target mRNAs [103]. Sponges have been widely  
34 used *in vitro* to investigate miRNA functions and the efficacy of this approach has also been proven *in*  
35 *vivo* [104].  
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#### 46 **MicroRNA target protection strategy**

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48 Each miRNA can target and modulate the expression of multiple genes. Under certain circumstances,  
49 it may not be appropriate to affect the level of all potential targets of the selected miRNA and it will  
50 be preferable to focus on a single target. The target protection strategy centralizes the effect of the  
51 treatment on specific target genes by introducing a single-stranded oligonucleotide that is  
52 complementary to the mRNA region recognized by the miRNA (Target Site Blocker, TSB). The target  
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3 site blocker is longer than the binding site of the miRNA conferring an improved specificity. This  
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5 approach can reduce off-target effects, however it won't be suitable when there is a need to target  
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7 multiple pathways.

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9 The proof of concept of the efficacy of this technology has been obtained *in vitro* by preventing the  
10  
11 repressive action of miR-199a-5p on its target Caveolin-1. This permitted to restore the expression of  
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13 Caveolin-1 and to avoid myofibroblast differentiation in response to TGF- $\beta$  stimulation [105]. An  
14  
15 analogous approach has also been used in  $\beta$ -cells to prevent the deleterious effect of miR-29 by  
16  
17 introducing oligonucleotides specifically masking the binding site of this miRNA present in the 3'UTR  
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19 of the mRNA coding for Mcl1, a member of the Bcl2 family [18]. This permitted to protect insulin-  
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21 secreting cells from cytokine-mediated death.

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24 The first study showing the effectiveness of this technique *in vivo* was conducted by Tao Sun team  
25  
26 [106]. This group used a vector expressing oligonucleotide sequences of different sizes (20, 40 and 60  
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28 nucleotides) that were fully complementary to the binding site of miR-19a on the 3'UTR of the PTEN  
29  
30 mRNA. They found that oligonucleotides of 60nt are the most effective in preventing the suppression  
31  
32 of PTEN during the development of the mice cortex [106]. This technique is still at its infancy and  
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34 more studies are required to validate the potential of this therapy *in vivo*.

### 35 36 37 **Reparative microRNA therapies in diabetes complications**

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

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49 Regarding vascular complications, modulation of miR-23 and miR-27 resulted in an important  
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51 decrease in neovascularization within the eye in response to laser-mediated injury to the choroid  
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53 layer [109]. Inhibition of miR-208a has also been reported to reduce cardiac remodeling and to  
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55 increase survival after hypertension-induced heart failure [110]. Moreover, the inhibition of this  
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3 miRNA also improved glucose metabolism and reduced plasma lipid content [111]. Anti-miR-208 is in  
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5 preclinical development by miRagen Therapeutics for chronic heart failure [102].  
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7 MiR-21 is another promising target for the treatment of diabetic nephropathy. MiR-21 has been  
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9 successfully repressed using LNA inhibitors in different mice models including renal diseases induced  
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11 by UUO (unilateral ureteral obstruction). In this system, inhibition of miR-21 resulted in reduced  
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13 expression of ECM proteins and prevented TGF- $\beta$ -induced renal fibrosis [112].  
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**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  $\beta$ -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  $\beta$ -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  $\beta$ -cells has so far hampered the modulation of  $\beta$ -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.

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3 Beside their therapeutic potential, the presence of characteristic miRNA signatures in easily  
4 accessible body fluids opens new perspectives for the diagnosis and prevention of diabetes and its  
5 long-term complications. The two major issues about the use of circulating miRNAs as effective  
6 biomarkers for diabetes concern the specificity of the miRNA signature and its reproducibility in  
7 different populations and laboratories around the world. It can be anticipated that the accumulation  
8 of studies analyzing the profile of circulating miRNAs under many different physiological and  
9 pathological conditions will help defining the predictive value of the changes in the extracellular level  
10 of specific miRNAs. We presently don't know which cells are contributing to the pool of circulating  
11 miRNAs and, if any, what is the precise role of these extracellular RNAs. A better definition of the  
12 origin of circulating miRNAs and of their potential regulatory function will guide the selection of the  
13 most relevant miRNA changes to be used as biomarkers for diabetes and its complications.  
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26 The discovery of miRNAs has revealed entirely new and unpredicted possibilities for the diagnosis  
27 and treatment of diabetes mellitus. The number of studies focusing on therapeutic approaches  
28 aiming at correcting miRNA expression in relevant tissues or scanning the pool of circulating miRNAs  
29 in search for diabetes biomarkers is increasing exponentially. In the next five years we will know  
30 whether these studies can hold all their promises. Should this the case, the way physicians manage  
31 diabetes mellitus and its devastating complications will probably be revolutionized.  
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**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 ; **AAV**, 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

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

1. Danaei, G., et al., *National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants*. *Lancet*, 2011. **378**(9785): p. 31-40.
2. Bartel, D.P., *MicroRNAs: genomics, biogenesis, mechanism, and function*. *Cell*, 2004. **116**(2): p. 281-97.
3. Wilczynska, A. and M. Bushell, *The complexity of miRNA-mediated repression*. *Cell Death Differ*, 2014.
4. Doench, J.G. and P.A. Sharp, *Specificity of microRNA target selection in translational repression*. *Genes Dev*, 2004. **18**(5): p. 504-11.
5. Guay, C., et al., *Diabetes mellitus, a microRNA-related disease?* *Transl Res*, 2011. **157**(4): p. 253-64.
6. Poy, M.N., et al., *A pancreatic islet-specific microRNA regulates insulin secretion*. *Nature*, 2004. **432**(7014): p. 226-30.
7. Joglekar, M.V., V.M. Joglekar, and A.A. Hardikar, *Expression of islet-specific microRNAs during human pancreatic development*. *Gene Expr Patterns*, 2009. **9**(2): p. 109-13.
8. Lynn, F.C., et al., *MicroRNA expression is required for pancreatic islet cell genesis in the mouse*. *Diabetes*, 2007. **56**(12): p. 2938-45.
9. Joglekar, M.V., et al., *MicroRNA profiling of developing and regenerating pancreas reveal post-transcriptional regulation of neurogenin3*. *Dev Biol*, 2007. **311**(2): p. 603-12.
10. Baroukh, N., et al., *MicroRNA-124a regulates Foxa2 expression and intracellular signaling in pancreatic beta-cell lines*. *J Biol Chem*, 2007. **282**(27): p. 19575-88.
11. Correa-Medina, M., et al., *MicroRNA miR-7 is preferentially expressed in endocrine cells of the developing and adult human pancreas*. *Gene Expr Patterns*, 2009. **9**(4): p. 193-9.
12. Kloosterman, W.P., et al., *Targeted inhibition of miRNA maturation with morpholinos reveals a role for miR-375 in pancreatic islet development*. *PLoS Biol*, 2007. **5**(8): p. e203.
13. Plaisance, V., et al., *MicroRNA-9 controls the expression of Granuphilin/Slp4 and the secretory response of insulin-producing cells*. *J Biol Chem*, 2006. **281**(37): p. 26932-42.
14. Cheng, L.C., et al., *miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche*. *Nat Neurosci*, 2009. **12**(4): p. 399-408.
15. Melkman-Zehavi, T., et al., *miRNAs control insulin content in pancreatic beta-cells via downregulation of transcriptional repressors*. *EMBO J*, 2011. **30**(5): p. 835-45.
16. Latreille, M., et al., *MicroRNA-7a regulates pancreatic beta cell function*. *J Clin Invest*, 2014. **124**(6): p. 2722-35.
17. Roggli, E., et al., *Involvement of microRNAs in the cytotoxic effects exerted by proinflammatory cytokines on pancreatic beta-cells*. *Diabetes*, 2010. **59**(4): p. 978-86.
18. Roggli, E., et al., *Changes in microRNA expression contribute to pancreatic beta-cell dysfunction in prediabetic NOD mice*. *Diabetes*, 2012. **61**(7): p. 1742-51.
19. Hezova, R., et al., *microRNA-342, microRNA-191 and microRNA-510 are differentially expressed in T regulatory cells of type 1 diabetic patients*. *Cell Immunol*, 2010. **260**(2): p. 70-4.
20. Rees, D.A. and J.C. Alcolado, *Animal models of diabetes mellitus*. *Diabet Med*, 2005. **22**(4): p. 359-70.
21. Lovis, P., et al., *Alterations in microRNA expression contribute to fatty acid-induced pancreatic beta-cell dysfunction*. *Diabetes*, 2008. **57**(10): p. 2728-36.
22. Nesca, V., et al., *Identification of particular groups of microRNAs that positively or negatively impact on beta cell function in obese models of type 2 diabetes*. *Diabetologia*, 2013. **56**(10): p. 2203-12.
23. Esguerra, J.L., et al., *Differential glucose-regulation of microRNAs in pancreatic islets of non-obese type 2 diabetes model Goto-Kakizaki rat*. *PLoS One*, 2011. **6**(4): p. e18613.

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24. Jacovetti, C., et al., *MicroRNAs contribute to compensatory beta cell expansion during pregnancy and obesity*. J Clin Invest, 2012. **122**(10): p. 3541-51.
25. Tattikota, S.G., et al., *Argonaute2 mediates compensatory expansion of the pancreatic beta cell*. Cell Metab, 2014. **19**(1): p. 122-34.
26. Locke, J.M., et al., *Increased expression of miR-187 in human islets from individuals with type 2 diabetes is associated with reduced glucose-stimulated insulin secretion*. Diabetologia, 2014. **57**(1): p. 122-8.
27. Kameswaran, V., et al., *Epigenetic regulation of the DLK1-MEG3 microRNA cluster in human type 2 diabetic islets*. Cell Metab, 2014. **19**(1): p. 135-45.
28. Kornfeld, J.W., et al., *Obesity-induced overexpression of miR-802 impairs glucose metabolism through silencing of Hnf1b*. Nature, 2013. **494**(7435): p. 111-5.
29. Trajkovski, M., et al., *MicroRNAs 103 and 107 regulate insulin sensitivity*. Nature, 2011. **474**(7353): p. 649-53.
30. Herrera, B.M., et al., *Global microRNA expression profiles in insulin target tissues in a spontaneous rat model of type 2 diabetes*. Diabetologia, 2010. **53**(6): p. 1099-109.
31. Granjon, A., et al., *The microRNA signature in response to insulin reveals its implication in the transcriptional action of insulin in human skeletal muscle and the role of a sterol regulatory element-binding protein-1c/myocyte enhancer factor 2C pathway*. Diabetes, 2009. **58**(11): p. 2555-64.
32. Sayer, A.A., et al., *Type 2 diabetes, muscle strength, and impaired physical function: the tip of the iceberg?* Diabetes Care, 2005. **28**(10): p. 2541-2.
33. Gallagher, I.J., et al., *Integration of microRNA changes in vivo identifies novel molecular features of muscle insulin resistance in type 2 diabetes*. Genome Med, 2010. **2**(2): p. 9.
34. Liu, N., et al., *An intragenic MEF2-dependent enhancer directs muscle-specific expression of microRNAs 1 and 133*. Proc Natl Acad Sci U S A, 2007. **104**(52): p. 20844-9.
35. Kato, M., et al., *TGF-beta activates Akt kinase through a microRNA-dependent amplifying circuit targeting PTEN*. Nat Cell Biol, 2009. **11**(7): p. 881-9.
36. Long, J., et al., *Identification of microRNA-93 as a novel regulator of vascular endothelial growth factor in hyperglycemic conditions*. J Biol Chem, 2010. **285**(30): p. 23457-65.
37. Kovacs, B., et al., *MicroRNAs in early diabetic retinopathy in streptozotocin-induced diabetic rats*. Invest Ophthalmol Vis Sci, 2011. **52**(7): p. 4402-9.
38. Xiao, J., et al., *MicroRNA miR-133 represses HERG K+ channel expression contributing to QT prolongation in diabetic hearts*. J Biol Chem, 2007. **282**(17): p. 12363-7.
39. Chen, J.F., T.E. Callis, and D.Z. Wang, *microRNAs and muscle disorders*. J Cell Sci, 2009. **122**(Pt 1): p. 13-20.
40. Khazaie, Y. and M.H. Nasr Esfahani, *MicroRNA and Male Infertility: A Potential for Diagnosis*. Int J Fertil Steril, 2014. **8**(2): p. 113-8.
41. Batkai, S. and T. Thum, *Analytical approaches in microRNA therapeutics*. J Chromatogr B Analyt Technol Biomed Life Sci, 2014. **964**: p. 146-52.
42. Zeng, L., et al., *The emerging role of circulating microRNAs as biomarkers in autoimmune diseases*. Autoimmunity, 2014: p. 1-11.
43. Farid, W.R., et al., *The Ins and Outs of MicroRNAs as Biomarkers in Liver Disease and Transplantation*. Transpl Int, 2014.
44. Allegra, A., et al., *Circulating microRNAs: new biomarkers in diagnosis, prognosis and treatment of cancer (review)*. Int J Oncol, 2012. **41**(6): p. 1897-912.
45. Olivieri, F., et al., *Circulating inflamma-miRs in aging and age-related diseases*. Front Genet, 2013. **4**: p. 121.
46. Zampetaki, A., et al., *Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes*. Circ Res, 2010. **107**(6): p. 810-7.
47. Creemers, E.E., A.J. Tijssen, and Y.M. Pinto, *Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease?* Circ Res, 2012. **110**(3): p. 483-95.

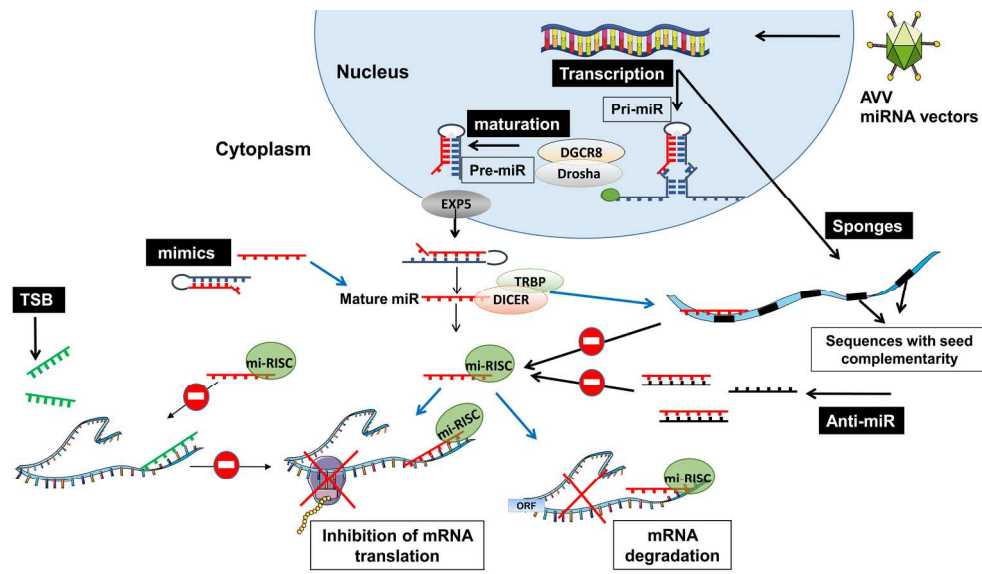


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48. Boon, R.A. and K.C. Vickers, *Intercellular transport of microRNAs*. *Arterioscler Thromb Vasc Biol*, 2013. **33**(2): p. 186-92.
49. Vickers, K.C., et al., *MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins*. *Nat Cell Biol*, 2011. **13**(4): p. 423-33.
50. Chen, T.S., et al., *Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs*. *Nucleic Acids Res*, 2010. **38**(1): p. 215-24.
51. Valadi, H., et al., *Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells*. *Nat Cell Biol*, 2007. **9**(6): p. 654-9.
52. Hunter, M.P., et al., *Detection of microRNA expression in human peripheral blood microvesicles*. *PLoS One*, 2008. **3**(11): p. e3694.
53. Grasso, M., et al., *Circulating miRNAs as biomarkers for neurodegenerative disorders*. *Molecules*, 2014. **19**(5): p. 6891-910.
54. Zernecke, A., et al., *Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection*. *Sci Signal*, 2009. **2**(100): p. ra81.
55. Wang, K., et al., *Export of microRNAs and microRNA-protective protein by mammalian cells*. *Nucleic Acids Res*, 2010. **38**(20): p. 7248-59.
56. Zen, K. and C.Y. Zhang, *Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers*. *Med Res Rev*, 2012. **32**(2): p. 326-48.
57. Bala, S., et al., *Circulating microRNAs in exosomes indicate hepatocyte injury and inflammation in alcoholic, drug-induced, and inflammatory liver diseases*. *Hepatology*, 2012. **56**(5): p. 1946-57.
58. Guay, C. and R. Regazzi, *Circulating microRNAs as novel biomarkers for diabetes mellitus*. *Nat Rev Endocrinol*, 2013. **9**(9): p. 513-21.
59. Chevillet, J.R., et al., *Issues and prospects of microRNA-based biomarkers in blood and other body fluids*. *Molecules*, 2014. **19**(5): p. 6080-105.
60. Morello, M., et al., *Large oncosomes mediate intercellular transfer of functional microRNA*. *Cell Cycle*, 2013. **12**(22): p. 3526-36.
61. Stoorvogel, W., *Functional transfer of microRNA by exosomes*. *Blood*, 2012. **119**(3): p. 646-8.
62. Rottiers, V. and A.M. Naar, *MicroRNAs in metabolism and metabolic disorders*. *Nat Rev Mol Cell Biol*, 2012. **13**(4): p. 239-50.
63. Kosaka, N., et al., *Secretory mechanisms and intercellular transfer of microRNAs in living cells*. *J Biol Chem*, 2010. **285**(23): p. 17442-52.
64. Zhang, L., et al., *Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA*. *Cell Res*, 2012. **22**(1): p. 107-26.
65. Wang, K., et al., *Comparing the MicroRNA spectrum between serum and plasma*. *PLoS One*, 2012. **7**(7): p. e41561.
66. Yang, Z., et al., *Serum miR-23a, a potential biomarker for diagnosis of pre-diabetes and type 2 diabetes*. *Acta Diabetol*, 2014.
67. Erenner, S., et al., *Circulating miR-375 as a biomarker of beta-cell death and diabetes in mice*. *Endocrinology*, 2013. **154**(2): p. 603-8.
68. Zhao, C., et al., *Early second-trimester serum miRNA profiling predicts gestational diabetes mellitus*. *PLoS One*, 2011. **6**(8): p. e23925.
69. Collares, C.V., et al., *Identifying common and specific microRNAs expressed in peripheral blood mononuclear cell of type 1, type 2, and gestational diabetes mellitus patients*. *BMC Res Notes*, 2013. **6**: p. 491.
70. Gwak, J.M., et al., *MicroRNA-9 is associated with epithelial-mesenchymal transition, breast cancer stem cell phenotype, and tumor progression in breast cancer*. *Breast Cancer Res Treat*, 2014. **147**(1): p. 39-49.
71. Lee, H.M., D.T. Nguyen, and L.F. Lu, *Progress and challenge of microRNA research in immunity*. *Front Genet*, 2014. **5**: p. 178.
72. Chen, X., et al., *Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases*. *Cell Res*, 2008. **18**(10): p. 997-1006.

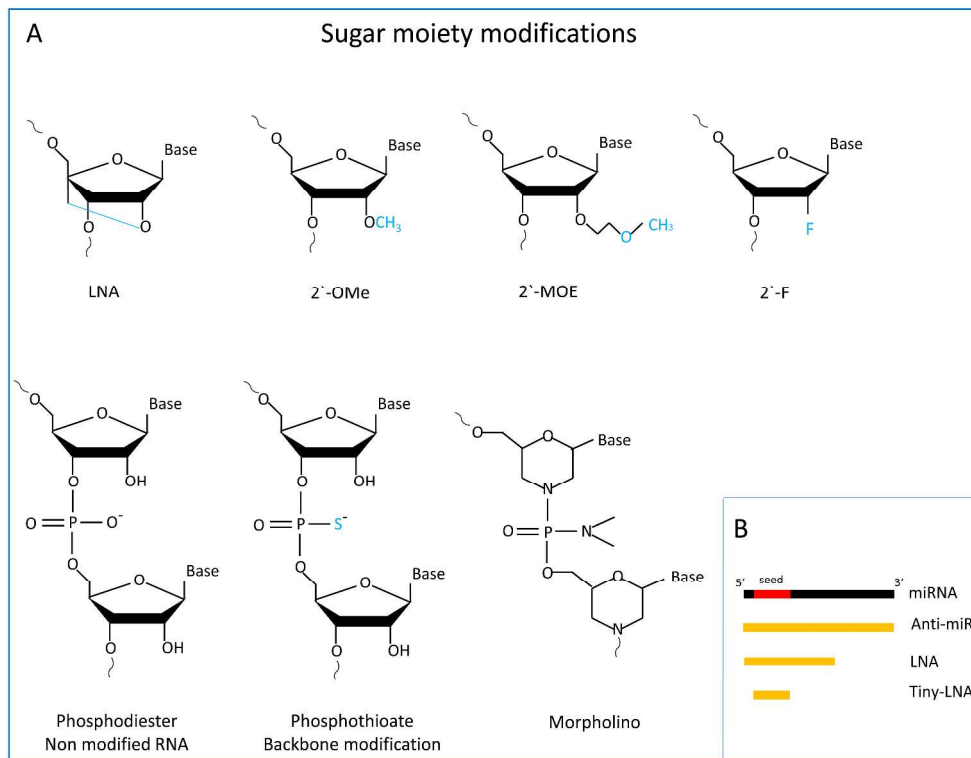
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- 3 73. Leidinger, P., et al., *The human miRNA repertoire of different blood compounds*. BMC
- 4 Genomics, 2014. **15**: p. 474.
- 5 74. MacLellan, S.A., et al., *Pre-profiling factors influencing serum microRNA levels*. BMC Clin
- 6 Pathol, 2014. **14**: p. 27.
- 7 75. Zhang, T., et al., *Plasma miR-126 is a potential biomarker for early prediction of type 2*
- 8 *diabetes mellitus in susceptible individuals*. Biomed Res Int, 2013. **2013**: p. 761617.
- 9 76. McClelland, A., S. Hagiwara, and P. Kantharidis, *Where are we in diabetic nephropathy:*
- 10 *microRNAs and biomarkers?* Curr Opin Nephrol Hypertens, 2014. **23**(1): p. 80-6.
- 11 77. Mao, Y., et al., *MicroRNAs as pharmacological targets in diabetes*. Pharmacol Res, 2013. **75**:
- 12 p. 37-47.
- 13 78. Li, Z. and T.M. Rana, *Therapeutic targeting of microRNAs: current status and future*
- 14 *challenges*. Nat Rev Drug Discov, 2014. **13**(8): p. 622-38.
- 15 79. Bennett, C.F. and E.E. Swayze, *RNA targeting therapeutics: molecular mechanisms of*
- 16 *antisense oligonucleotides as a therapeutic platform*. Annu Rev Pharmacol Toxicol, 2010. **50**:
- 17 p. 259-93.
- 18 80. Bumcrot, D., et al., *RNAi therapeutics: a potential new class of pharmaceutical drugs*. Nat
- 19 Chem Biol, 2006. **2**(12): p. 711-9.
- 20 81. Thomson, D.W., C.P. Bracken, and G.J. Goodall, *Experimental strategies for microRNA target*
- 21 *identification*. Nucleic Acids Res, 2011. **39**(16): p. 6845-53.
- 22 82. Broderick, J.A. and P.D. Zamore, *MicroRNA therapeutics*. Gene Ther, 2011. **18**(12): p. 1104-
- 23 10.
- 24 83. Wang, Z., et al., *RNA interference and cancer therapy*. Pharm Res, 2011. **28**(12): p. 2983-95.
- 25 84. Jimenez, V., et al., *In vivo genetic engineering of murine pancreatic beta cells mediated by*
- 26 *single-stranded adeno-associated viral vectors of serotypes 6, 8 and 9*. Diabetologia, 2011.
- 27 **54**(5): p. 1075-86.
- 28 85. Kota, J., et al., *Therapeutic microRNA Delivery Suppresses Tumorigenesis in a Murine Liver*
- 29 *Cancer Model*. Cell, 2009. **137**(6): p. 1005-1017.
- 30 86. Vester, B. and J. Wengel, *LNA (locked nucleic acid): high-affinity targeting of complementary*
- 31 *RNA and DNA*. Biochemistry, 2004. **43**(42): p. 13233-41.
- 32 87. Orom, U.A., S. Kauppinen, and A.H. Lund, *LNA-modified oligonucleotides mediate specific*
- 33 *inhibition of microRNA function*. Gene, 2006. **372**: p. 137-41.
- 34 88. Krutzfeldt, J., et al., *Silencing of microRNAs in vivo with 'antagomirs'*. Nature, 2005.
- 35 **438**(7068): p. 685-9.
- 36 89. Obad, S., et al., *Silencing of microRNA families by seed-targeting tiny LNAs*. Nat Genet, 2011.
- 37 **43**(4): p. 371-8.
- 38 90. Wang, Y., et al., *MicroRNA-7 regulates the mTOR pathway and proliferation in adult*
- 39 *pancreatic beta-cells*. Diabetes, 2013. **62**(3): p. 887-95.
- 40 91. Hansen, T.B., et al., *Natural RNA circles function as efficient microRNA sponges*. Nature, 2013.
- 41 **495**(7441): p. 384-8.
- 42 92. Esau, C., et al., *miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting*.
- 43 *Cell Metab*, 2006. **3**(2): p. 87-98.
- 44 93. Jopling, C.L., et al., *Modulation of hepatitis C virus RNA abundance by a liver-specific*
- 45 *MicroRNA*. Science, 2005. **309**(5740): p. 1577-81.
- 46 94. Jopling, C.L., K.L. Norman, and P. Sarnow, *Positive and negative modulation of viral and*
- 47 *cellular mRNAs by liver-specific microRNA miR-122*. Cold Spring Harb Symp Quant Biol, 2006.
- 48 **71**: p. 369-76.
- 49 95. Elmen, J., et al., *LNA-mediated microRNA silencing in non-human primates*. Nature, 2008.
- 50 **452**(7189): p. 896-9.
- 51 96. Hildebrandt-Eriksen, E.S., et al., *A locked nucleic acid oligonucleotide targeting microRNA 122*
- 52 *is well-tolerated in cynomolgus monkeys*. Nucleic Acid Ther, 2012. **22**(3): p. 152-61.
- 53 97. Lanford, R.E., et al., *Therapeutic silencing of microRNA-122 in primates with chronic hepatitis*
- 54 *C virus infection*. Science, 2010. **327**(5962): p. 198-201.
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98. Lindow, M. and S. Kauppinen, *Discovering the first microRNA-targeted drug*. J Cell Biol, 2012. **199**(3): p. 407-12.
99. Janssen, H.L., et al., *Treatment of HCV infection by targeting microRNA*. N Engl J Med, 2013. **368**(18): p. 1685-94.
100. Frost, R.J. and E.N. Olson, *Control of glucose homeostasis and insulin sensitivity by the Let-7 family of microRNAs*. Proc Natl Acad Sci U S A, 2011. **108**(52): p. 21075-80.
101. Kent, O.A. and J.T. Mendell, *A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes*. Oncogene, 2006. **25**(46): p. 6188-96.
102. van Rooij, E., A.L. Purcell, and A.A. Levin, *Developing microRNA therapeutics*. Circ Res, 2012. **110**(3): p. 496-507.
103. Ebert, M.S., J.R. Neilson, and P.A. Sharp, *MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells*. Nat Methods, 2007. **4**(9): p. 721-6.
104. Xie, J., et al., *Long-term, efficient inhibition of microRNA function in mice using rAAV vectors*. Nat Methods, 2012. **9**(4): p. 403-9.
105. Lino Cardenas, C.L., et al., *miR-199a-5p Is upregulated during fibrogenic response to tissue injury and mediates TGFbeta-induced lung fibroblast activation by targeting caveolin-1*. PLoS Genet, 2013. **9**(2): p. e1003291.
106. Bian, S., et al., *MicroRNA cluster miR-17-92 regulates neural stem cell expansion and transition to intermediate progenitors in the developing mouse neocortex*. Cell Rep, 2013. **3**(5): p. 1398-406.
107. Kato, M., et al., *A microRNA circuit mediates transforming growth factor-beta1 autoregulation in renal glomerular mesangial cells*. Kidney Int, 2011. **80**(4): p. 358-68.
108. Putta, S., et al., *Inhibiting microRNA-192 ameliorates renal fibrosis in diabetic nephropathy*. J Am Soc Nephrol, 2012. **23**(3): p. 458-69.
109. Zhou, Q., et al., *Regulation of angiogenesis and choroidal neovascularization by members of microRNA-23~27~24 clusters*. Proc Natl Acad Sci U S A, 2011. **108**(20): p. 8287-92.
110. Montgomery, R.L., et al., *Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure*. Circulation, 2011. **124**(14): p. 1537-47.
111. Grueter, C.E., et al., *A cardiac microRNA governs systemic energy homeostasis by regulation of MED13*. Cell, 2012. **149**(3): p. 671-83.
112. Chau, B.N., et al., *MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways*. Sci Transl Med, 2012. **4**(121): p. 121ra18.

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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	Serum [12, 13]	HD (Blood MVs) [14], Biliary Tract Cancer (Bile) [3], AD (CSF, Serum) [4, 5, 15]
	T2D	Serum [4, 7, 12, 16-18]	
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]
			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	PBMC [1, 2]	Fetal Hypoxia [27]
	T2D	Plasma [4, 7, 18, 19]	
miR-21a	T1D	PBMC [1, 2, 12, 18, 28], Plasma [29], Urine [29]	CAD (Plasma MPs), Arteriosclerosis (Serum) [16, 23], Obesity (Blood) [7], HCC (Plasma) [9] Lung Cancer, Ovarian Cancer (Plasma ExoS) [30], HF (Blood MVs) [14]
	T2D	Plasma [4, 7, 18, 19]	
miR-23	T2D	Serum [8]	Dyslipidemia (Blood) [32], Brain Tumors (Serum) [9]
miR-24a	T1D	Serum [4, 12, 18, 33]	Lung Cancer (Serum) [9, 26], Rheumatic Arthritis (Plasma) [10]
	T2D	Plasma [4, 18, 19]	
miR-25	T1D	Serum [4, 12, 18, 33]	CAD (Plasma, Plasma MPs), AMI (PBMC, Platelets, Plasma) [23]
			NSCLC (Serum) [30], Lung Cancer (Serum) [9]
miR-26a	T1D	Serum [4, 12, 18, 33]	Pancreatic Tumors, Gastric Cancer, Sarcoidosis, AMI, Pancreatitis (Blood) [34] PD (PBMC) [35], AMI (Serum) [36], HCC (Plasma) [9], AD (Blood) [5]
	GD	PBMC [2]	
miR-26b	T1D	PBMC [1]	Pancreatic Tumors, Gastric Cancer, MS, Sarcoidosis, AMI (Blood) [34], AD (Blood) [5]

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4	miR-27a	T1D T2D GD	Serum [4, 12, 18, 33], PBMC [2] Blood [12, 32], PBMC [2] PBMC [2]
5			MetS (Blood) [32], Gastric Cancer, HCC (Plasma) [9], AD (CSF) [5] Stroke (Plasma) [4]
6			
7	miR-27b	T1D	Serum [4, 12, 18, 33], PBMC [1]
8	miR-28	T2D	Plasma [4, 7, 12, 14, 16, 18-20]
9			Arteriosclerosis (Serum) [4, 7, 16], Obesity (Blood) [7]
10			PD (PBMC) [35], Prepubertal Obesity (Plasma) [7, 37]
11	miR-29a	T1D T2D GD	Serum [4, 12, 18, 33] Serum [4, 7, 12, 16-18], Blood [7, 16, 38], Urine [4, 39] Serum [4, 7, 18, 41], PBMC [2]
12			CAD (Blood) [4, 16], IgA NP (Urine) [21], AD (Serum), PD (Blood) [4, 5] Liver Fibrosis (Serum) [40]
13			
14	miR-29b	T1D T2D GD	PBMC [2] Plasma [4, 7, 12, 16, 19, 42, 43], PBMC [2] PBMC [2]
15			PD (PBMC) [35], AD (Serum) [4, 5], Aortic Aneurysm (Plasma) [4] Cardiomyopathy (PBMC) [23]
16			
17	miR-29c	GD	PBMC [2]
18	miR-30a	T1D	Serum [4, 12, 18, 33]
19	miR-30b	GD	PBMC [2]
20	miR-30d	T2D	Serum [4, 7, 12, 16-18], Blood [7, 16, 38]
21	miR-30e	T2D	PBMC [2]
22	miR-30e*	T1D	PBMC [1]
23			Lung Cancer [3]
24	miR-31	T1D	Serum [12, 13]
25	miR-32	T1D	PBMC [1]
26	miR-33a	T1D	PBMC [1]
27			PD (Blood) [4, 18], AMI (Plasma), HF (Serum) [23] PD (PBMC) [35], Lung Cancer (Plasma) [26], HF (Plasma) [23]
28	miR-34a	T1D T2D	Serum [12, 13] Serum [4, 7, 12, 16-18]
29			Hyperlipidemia (Serum) [44]
30	miR-93	T1D	PBMC [12, 18, 28]
31	miR-96	T2D	Serum [8]
32	miR-98	T1D	PBMC [1]
33	miR-101	T1D GD	PBMC [1] PBMC [2]
34			NAFLC (Serum) [31], Gastric Cancer (Serum) [9], AD (PBMC) [4, 18], Ageing (Blood) [45] IgA NP (Urine) [21], Colorectal Cancer (Plasma) [9], Crohn's Disease (Serum) [10]
35	miR-103	T1D	PBMC [2]
36			HCC (Serum) [46] Autism (Serum) [47]
37	miR-122	T2D	Serum [8]
38			AD (Blood) [5], HF (Plasma) [23], Obesity (Blood) [7] NAFLD (Serum) [31], HCC (Plasma) [9], CAD (Plasma) [7] ATI (Serum) [30], MI, HF (Plasma) [16, 20], NP (Urine) [48]
39			
40	miR-124a	T2D	Serum [4, 7, 12, 17, 18]
41			ATI (Serum) [30], Aortic Aneurysm (Plasma) [4]
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4	miR-126	T1D	Urine [29], PBMC [1, 2]
5		T2D	Plasma [4, 7, 12, 15, 18-20, 49], PBMC [2]
6		GD	PBMC [2]
7	miR-126*	T1D	PBMC [2]
8			Bladder Cancer (Urine) [30], PD (PBMC) [35], HF, CAD (Plasma) [20], Lung Cancer (Plasma) [26]
9	miR-130a	T1D	PBMC [2]
10			AMI (Plasma, Serum) [4, 36], Congestive HF (Plasma) [4], SLE (Plasma) [10]
11	miR-132	GD	Serum [4, 18, 41]
12			HF (Plasma) [23], Autism (Serum) [47]
13	miR-140	T1D	PBMC [1]
14		T2D	PBMC [2]
15			Arteriosclerosis (Serum) [4, 16], Hypertension (Blood) [32]
16	miR-142	T1D	PBMC [2]
17		T2D	PBMC [2]
18		GD	PBMC [2]
19	miR-144	T1D	PBMC [2]
20		T2D	Blood [3, 7, 16, 31, 38], PBMC [2]
21		GD	PBMC [2]
22			Diabetic NP (Urine) [31]
23	miR-146a	T1D	Serum [12, 13]
24		T2D	Plasma [4, 7, 15, 50, 51], PBMC [7, 50]
25			Serum [4, 7, 8, 12, 16-18] Blood [7, 16, 38]
26	miR-148a	T1D	Serum [4, 12, 18, 33], PBMC [1]
27	miR-148b	T1D	PBMC [1]
28			Sepsis (Serum, Blood MVs) [14, 30], Rheumatoid Arthritis (Blood MVs) [14]
29	miR-150	T1D	PBMC [2]
30		T2D	Blood [7, 12, 16, 32, 38], Plasma [4, 7, 19, 43]
31	miR-152	T1D	Serum [4, 12, 18, 33]
32			AMI, CVD (Plasma) [15], SLE (Serum) [10]
33	miR-155	T1D	Serum [12, 13]
34			AD (CSF) [4, 5, 15], Lung Cancer (Serum) [26], CAD (PBMC, Plasma MPs) [16, 23]
35	miR-181a	T1D	Serum [4, 12, 18, 33]
36		GD	PBMC [2]
37	miR-181d	GD	PBMC [2]
38	miR-182	T2D	Blood [7, 16, 38]
39			Lung Cancer, Pancreatic Tumors, MS, Pancreatitis, Sarcoidosis, Prostate Cancer (Blood) [34]
40	miR-186	T1D	PBMC [1]
41		T2D	Serum [8]
42			Esophageal Cancer (Serum) [9]
43			CAD (Blood) [4, 16, 23], CLL [3], PD (Leukocytes) [24], AMI (Serum) [36]
44			Lung Cancer (Serum) [9], Preeclampsia [3]
45			Pancreatic Cancer (PJ) [3], AMI (Plasma) [15], SLE (Blood MVs) [14]
46			CAD (Plasma, Serum, Blood, Plasma MPs) [16], AD (CSF) [5], Obesity (Blood) [7]
47			Azoospermia (Semen) [52], AMI (Blood) [4], SLE (Plasma), Psoriasis (Serum) [10]
			CAD (Blood) [7]
			CAD (Blood) [7, 16, 20, 23], Asthenozoospermia (Semen) [52], Lung Cancer (Serum) [26]
			Dyslipidemia (HDL) [53], AMI (Serum, Platelets) [4, 23]



miR-191	T2D	Plasma [4, 7, 18, 19], Serum [8]	AD (Plasma) [4, 5], AMI (Serum) [36] 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] 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] 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] Pancreatic Tumors, Gastric Cancer, MS, Pancreatitis (Blood) [34]
miR-199	T1D	Serum [12, 13], PBMC [1, 2]	PD (PBMC) [35], Lung Cancer (Serum) [9], CAD (Plasma, Serum) [7]
	T2D	PBMC [2], Serum [8]	
	GD	PBMC [2]	
miR-200a	T1D	Serum [4, 12, 18, 33]	SLE (Serum, Urine) [3], AD (PBMC) [4], Breast Cancer (Serum) [6] 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] Arteriosclerosis (Serum) [16], HF (Plasma, Serum, MC) [23]
miR-222	T2D	PBMC [2]	Obesity (Plasma) [22], PD (Plasma) [4] Dyslipidemia (HDL) [53], CAD (Blood, EPC) [16], Lung Cancer, Thyroid Cancer (Serum) [9]
	GD	Serum [4, 7, 18, 41]	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] 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] 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	PBMC [1, 2]	Dyslipidemia (HDL) [53], CAD (Blood) [4, 16, 23], AMI (Platelets), HF (Plasma) [23]
	T2D	PBMC [2]	
	GD	PBMC [2]	

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

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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1. Takahashi, P., et al., *MicroRNA expression profiling and functional annotation analysis of their targets in patients with type 1 diabetes mellitus*. *Gene*, 2014. **539**(2): p. 213-23.
2. Collares, C.V., et al., *Identifying common and specific microRNAs expressed in peripheral blood mononuclear cell of type 1, type 2, and gestational diabetes mellitus patients*. *BMC Res Notes*, 2013. **6**: p. 491.
3. Allegra, A., et al., *Circulating microRNAs: new biomarkers in diagnosis, prognosis and treatment of cancer (review)*. *Int J Oncol*, 2012. **41**(6): p. 1897-912.
4. Chevillet, J.R., et al., *Issues and prospects of microRNA-based biomarkers in blood and other body fluids*. *Molecules*, 2014. **19**(5): p. 6080-105.
5. Grasso, M., et al., *Circulating miRNAs as biomarkers for neurodegenerative disorders*. *Molecules*, 2014. **19**(5): p. 6891-910.
6. Salido-Guadarrama, I., et al., *MicroRNAs transported by exosomes in body fluids as mediators of intercellular communication in cancer*. *Onco Targets Ther*, 2014. **7**: p. 1327-38.
7. Price, N.L., C.M. Ramirez, and C. Fernandez-Hernando, *Relevance of microRNA in metabolic diseases*. *Crit Rev Clin Lab Sci*, 2014: p. 1-16.
8. Yang, Z., et al., *Serum miR-23a, a potential biomarker for diagnosis of pre-diabetes and type 2 diabetes*. *Acta Diabetol*, 2014.
9. Schwarzenbach, H., et al., *Clinical relevance of circulating cell-free microRNAs in cancer*. *Nat Rev Clin Oncol*, 2014. **11**(3): p. 145-56.
10. Zeng, L., et al., *The emerging role of circulating microRNAs as biomarkers in autoimmune diseases*. *Autoimmunity*, 2014: p. 1-11.
11. Liu, P., et al., *Identification of serum microRNAs as diagnostic and prognostic biomarkers for acute pancreatitis*. *Pancreatology*, 2014. **14**(3): p. 159-66.
12. Guay, C. and R. Regazzi, *Circulating microRNAs as novel biomarkers for diabetes mellitus*. *Nat Rev Endocrinol*, 2013. **9**(9): p. 513-21.
13. Sebastiani, G., *MicroRNA expression fingerprint in serum of type 1 diabetic patients*. *Diabetologia*, 2012. **55**: p. S48.
14. Ciesla, M., et al., *MicroRNAs as biomarkers of disease onset*. *Anal Bioanal Chem*, 2011. **401**(7): p. 2051-61.
15. Olivieri, F., et al., *Circulating inflamma-miRs in aging and age-related diseases*. *Front Genet*, 2013. **4**: p. 121.
16. Tijssen, A.J., Y.M. Pinto, and E.E. Creemers, *Circulating microRNAs as diagnostic biomarkers for cardiovascular diseases*. *Am J Physiol Heart Circ Physiol*, 2012. **303**(9): p. H1085-95.
17. Kong, L., et al., *Significance of serum microRNAs in pre-diabetes and newly diagnosed type 2 diabetes: a clinical study*. *Acta Diabetol*, 2011. **48**(1): p. 61-9.
18. Chen, H., et al., *Application of microRNAs in diabetes mellitus*. *J Endocrinol*, 2014. **222**(1): p. R1-R10.
19. Zampetaki, A., et al., *Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes*. *Circ Res*, 2010. **107**(6): p. 810-7.
20. Creemers, E.E., A.J. Tijssen, and Y.M. Pinto, *Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease?* *Circ Res*, 2012. **110**(3): p. 483-95.
21. McClelland, A., S. Hagiwara, and P. Kantharidis, *Where are we in diabetic nephropathy: microRNAs and biomarkers?* *Curr Opin Nephrol Hypertens*, 2014. **23**(1): p. 80-6.
22. Ortega, F.J., et al., *Targeting the circulating microRNA signature of obesity*. *Clin Chem*, 2013. **59**(5): p. 781-92.
23. Bronze-da-Rocha, E., *MicroRNAs expression profiles in cardiovascular diseases*. *Biomed Res Int*, 2014. **2014**: p. 985408.
24. Soreq, L., et al., *Small RNA sequencing-microarray analyses in Parkinson leukocytes reveal deep brain stimulation-induced splicing changes that classify brain region transcriptomes*. *Front Mol Neurosci*, 2013. **6**: p. 10.
25. Cookson, V.J., et al., *Circulating microRNA profiles reflect the presence of breast tumours but not the profiles of microRNAs within the tumours*. *Cell Oncol (Dordr)*, 2012. **35**(4): p. 301-8.

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26. Nair, V.S., et al., *Design and Analysis for Studying microRNAs in Human Disease: A Primer on - Omic Technologies*. Am J Epidemiol, 2014. **180**(2): p. 140-52.
27. Whitehead, C.L., et al., *Circulating MicroRNAs in maternal blood as potential biomarkers for fetal hypoxia in-utero*. PLoS One, 2013. **8**(11): p. e78487.
28. Salas-Perez, F., et al., *MicroRNAs miR-21a and miR-93 are down regulated in peripheral blood mononuclear cells (PBMCs) from patients with type 1 diabetes*. Immunobiology, 2013. **218**(5): p. 733-7.
29. Osipova, J., et al., *Diabetes-associated microRNAs in paediatric patients with type 1 diabetes mellitus: a cross-sectional cohort study*. J Clin Endocrinol Metab, 2014: p. jc20133868.
30. Zen, K. and C.Y. Zhang, *Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers*. Med Res Rev, 2012. **32**(2): p. 326-48.
31. Yang, Y., et al., *Urine miRNAs: potential biomarkers for monitoring progression of early stages of diabetic nephropathy*. Med Hypotheses, 2013. **81**(2): p. 274-8.
32. Karolina, D.S., et al., *Circulating miRNA profiles in patients with metabolic syndrome*. J Clin Endocrinol Metab, 2012. **97**(12): p. E2271-6.
33. Nielsen, L.B., et al., *Circulating levels of microRNA from children with newly diagnosed type 1 diabetes and healthy controls: evidence that miR-25 associates to residual beta-cell function and glycaemic control during disease progression*. Exp Diabetes Res, 2012. **2012**: p. 896362.
34. Keller, A., et al., *Toward the blood-borne miRNome of human diseases*. Nat Methods, 2011. **8**(10): p. 841-3.
35. Martins, M., et al., *Convergence of miRNA expression profiling, alpha-synuclein interacton and GWAS in Parkinson's disease*. PLoS One, 2011. **6**(10): p. e25443.
36. Hsu, A., et al., *Systemic approach to identify serum microRNAs as potential biomarkers for acute myocardial infarction*. Biomed Res Int, 2014. **2014**: p. 418628.
37. Prats-Puig, A., et al., *Changes in circulating microRNAs are associated with childhood obesity*. J Clin Endocrinol Metab, 2013. **98**(10): p. E1655-60.
38. Karolina, D.S., et al., *MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus*. PLoS One, 2011. **6**(8): p. e22839.
39. Peng, H., et al., *Urinary miR-29 correlates with albuminuria and carotid intima-media thickness in type 2 diabetes patients*. PLoS One, 2013. **8**(12): p. e82607.
40. Farid, W.R., et al., *The Ins and Outs of MicroRNAs as Biomarkers in Liver Disease and Transplantation*. Transpl Int, 2014.
41. Zhao, C., et al., *Early second-trimester serum miRNA profiling predicts gestational diabetes mellitus*. PLoS One, 2011. **6**(8): p. e23925.
42. Wang, X., et al., *Determination of 14 circulating microRNAs in Swedes and Iraqis with and without diabetes mellitus type 2*. PLoS One, 2014. **9**(1): p. e86792.
43. Zampetaki, A., et al., *Profiling of circulating microRNAs: from single biomarkers to re-wired networks*. Cardiovasc Res, 2012. **93**(4): p. 555-62.
44. Simionescu, N., et al., *Analysis of circulating microRNAs that are specifically increased in hyperlipidemic and/or hyperglycemic sera*. Mol Biol Rep, 2014. **41**(9): p. 5765-73.
45. Jiang, Y., et al., *Peripheral blood miRNAs as a biomarker for chronic cardiovascular diseases*. Sci Rep, 2014. **4**: p. 5026.
46. Sukata, T., et al., *Circulating microRNAs, possible indicators of progress of rat hepatocarcinogenesis from early stages*. Toxicol Lett, 2011. **200**(1-2): p. 46-52.
47. Mundalil Vasu, M., et al., *Serum microRNA profiles in children with autism*. Mol Autism, 2014. **5**: p. 40.
48. Argyropoulos, C., et al., *Urinary microRNA profiling in the nephropathy of type 1 diabetes*. PLoS One, 2013. **8**(1): p. e54662.
49. Zhang, T., et al., *Plasma miR-126 is a potential biomarker for early prediction of type 2 diabetes mellitus in susceptible individuals*. Biomed Res Int, 2013. **2013**: p. 761617.
50. Balasubramanyam, M., et al., *Impaired miR-146a expression links subclinical inflammation and insulin resistance in Type 2 diabetes*. Mol Cell Biochem, 2011. **351**(1-2): p. 197-205.

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51. Rong, Y., et al., *Increased microRNA-146a levels in plasma of patients with newly diagnosed type 2 diabetes mellitus*. PLoS One, 2013. **8**(9): p. e73272.
52. Khazaie, Y. and M.H. Nasr Esfahani, *MicroRNA and Male Infertility: A Potential for Diagnosis*. Int J Fertil Steril, 2014. **8**(2): p. 113-8.
53. Vickers, K.C., et al., *MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins*. Nat Cell Biol, 2011. **13**(4): p. 423-33.
54. Kato, M., N.E. Castro, and R. Natarajan, *MicroRNAs: potential mediators and biomarkers of diabetic complications*. Free Radic Biol Med, 2013. **64**: p. 85-94.
55. Sebastiani, G., et al., *Increased expression of microRNA miR-326 in type 1 diabetic patients with ongoing islet autoimmunity*. Diabetes Metab Res Rev, 2011. **27**(8): p. 862-6.
56. Kjersem, J.B., et al., *Plasma microRNAs predicting clinical outcome in metastatic colorectal cancer patients receiving first-line oxaliplatin-based treatment*. Mol Oncol, 2014. **8**(1): p. 59-67.
57. Yong, F.L., C.W. Law, and C.W. Wang, *Potentiality of a triple microRNA classifier: miR-193a-3p, miR-23a and miR-338-5p for early detection of colorectal cancer*. BMC Cancer, 2013. **13**: p. 280.
58. Caporali, A., et al., *Deregulation of microRNA-503 contributes to diabetes mellitus-induced impairment of endothelial function and reparative angiogenesis after limb ischemia*. Circulation, 2011. **123**(3): p. 282-91.
59. Pescador, N., et al., *Serum circulating microRNA profiling for identification of potential type 2 diabetes and obesity biomarkers*. PLoS One, 2013. **8**(10): p. e77251.
60. Wang, H.W., et al., *Deficiency of the microRNA-31-microRNA-720 pathway in the plasma and endothelial progenitor cells from patients with coronary artery disease*. Arterioscler Thromb Vasc Biol, 2014. **34**(4): p. 857-69.
61. Jones, C.I., et al., *Identification of circulating microRNAs as diagnostic biomarkers for use in multiple myeloma*. Br J Cancer, 2012. **107**(12): p. 1987-96.
62. Tambyah, P.A., et al., *microRNAs in circulation are altered in response to influenza A virus infection in humans*. PLoS One, 2013. **8**(10): p. e76811.