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MicroRNAs as therapeutic targets for the treatment of diabetes mellitus and its complications

Abstract

Introduction: Diabetes mellitus is a very common metabolic disorder affecting more than 400 million people worldwide. Currently available treatments permit to manage the disease but, in the long term, many patients develop severe micro- and macrovascular complications that decrease life quality and expectancy. Better therapeutic tools to prevent and treat diabetes are therefore urgently needed.

Areas covered: MicroRNAs are key regulators of gene expression and central players in a variety of physiological and pathological processes. This review summarizes the role of microRNAs in insulin-secreting cells and in insulin target tissues as well as their involvement in the development of diabetes and its long term complications.

Expert opinion: Because of their physicochemical properties and their capacity to regulate a wide range of physiopathological events, microRNAs are attractive therapeutic targets. There is accumulating evidence that approaches permitting to correct the level of specific microRNAs can successfully prevent or treat diabetes and its complications. Pharmacological tools that efficiently modulate the level of microRNAs are already available. However, before these tools can be allowed to integrate the arsenal for the treatment of diabetic patients, new innovative strategies will be needed to achieve selective delivery of these pharmacological principles to the appropriate target cells.

Keywords: Aptamer, diabetes mellitus, diabetic complications, exosome, microRNA

1. Introduction

Diabetes mellitus is a very common metabolic disorder affecting more than 400 million people worldwide (https://www.idf.org/). Because of population aging and lifestyle changes, the number of individuals affected by this disease is expected to further rise in the next decades and is forecasted to exceed 600 million by year 2040. Diabetes mellitus is a chronic disease and, even if appropriately treated, people suffering from this metabolic disorder are at increased risk for developing serious micro- and macrovascular complications potentially leading to heart and kidney failure, stroke, blindness or lower limb amputations[1, 2]. Thus, the management of this chronic disease and of its associated long-term complications constitutes a major public health challenge and a very heavy socioeconomic burden. Despite improvements in recent years, the therapeutic arsenal currently available for the treatment of diabetic patients remains insufficient. For this reason, new therapeutic strategies to prevent and treat this very common metabolic disorder are urgently needed.

Diabetes mellitus is characterized by chronically elevated blood glucose levels and occurs when pancreatic β-cells, located within the islets of Langerhans, become unable to secrete enough insulin to cover the organism needs. The disease can have different etiologies but is most often associated with loss and/or dysfunction of insulin-secreting cells. Type 1 diabetes develops when all (or near all) the β-cells are eliminated by an autoimmune reaction directed against the insulin-secreting cells[1]. This form of the disease usually manifests during childhood or in young adults and represents about 10% of all diabetes cases. Multiple daily injections of insulin are necessary for the treatment of these patients. Type 2 diabetes is the most frequent form of the disease and is often associated with obesity and with a diminished insulin sensitivity of peripheral tissues[2]. It is usually diagnosed in older individuals compared to type 1 diabetes and its incidence increases with aging. In type 2 diabetic patients, β-cells are still present but are unable to expand and raise sufficiently their secretory activity to

compensate for the insulin resistant state. This form of diabetes is initially treated with drugs that increase the sensitivity of insulin target tissues and/or stimulate the secretory activities of β-cells. However, in the long term the patients become often refractory to oral pharmacological treatments and may also require insulin injections. Gestational diabetes is a metabolic disorder occurring in about 5% of the pregnancies that is associated with complications to both mother and newborn[3]. It usually resolves after delivery, but women suffering from gestational diabetes as well as their offspring have a higher propensity to develop type 2 diabetes later in life.

Predisposition to diabetes mellitus is usually determined by multiple genes, but in some cases the disease can also result from mutations in single genes that are essential for the differentiation and/or function of the insulin-secreting cells. According to the functional impact of the mutation, the disease manifests at birth (neonatal diabetes)[4] or may become apparent only later in life (Maturity Onset Diabetes of the Young)[5].

2. Alterations in gene expression associated with diabetes mellitus

Under pre-diabetic and diabetic conditions, many tissues in the body are chronically exposed to elevated concentrations of nutrients (glucose, fatty acids), inflammatory mediators (cytokines, adipokines) and hormones (insulin). This leads to the activation of signaling cascades culminating in major changes in gene expression in a variety of cells, including β-cells, hepatocytes, adipocytes, skeletal muscle cells and endothelial cells. In the last decades, substantial efforts have been made to determine the contribution of these alterations in gene expression in the development of diabetes and its long-term complications[6]. Most of these studies focused on genes coding for proteins. However, protein-coding genes account for less than 2% of the 3.2 billion base pairs constituting the human genome and recent technological advances revealed that most genome sequences can be transcribed to RNA. In fact, human

cells contain a very large number of transcripts that are not coding for proteins but play nonetheless essential regulatory roles in most physiological and pathological processes[7, 8]. These non-coding RNAs have been subdivided in different categories according to their length, physico-chemical properties and functions.

Long non-coding RNAs (lncRNAs) form a heterogeneous class of transcripts that contain more than 200 nucleotides[9]. They can modulate gene expression by affecting a variety of processes, including chromatin remodeling, gene transcription as well as mRNA splicing and translation. LncRNAs accomplish these tasks by binding to other RNAs, to DNA or to proteins. This allows them to act as signals for the initiation of the transcriptional activity, as decoy molecules sequestering RNAs or proteins, as guides for the localization of ribonucleoprotein complexes or as scaffolds for the assembly of proteins or RNAs[10]. Although the study of the role of lncRNAs has just started, there is already evidence indicating that lncRNAs play an important role in a variety of physiological and pathological processes, including diabetes mellitus[11-13].

Small non-coding RNAs are shorter than 200 nucleotides and include, among others, microRNAs (miRNAs), piRNAs and tRNA-derived fragments[14-16]. MiRNAs are central regulators of gene expression[14] and important players in the development of different forms of diabetes mellitus. Indeed, as outlined below, miRNAs control the expression of several key genes in β-cells and in insulin target tissues. Moreover, changes in the level of these small non-coding RNAs are important determinants of long-term diabetes complications such as renal fibrosis, visual loss and lower limb ischemia[17-19].

3. General properties of miRNAs

The properties of miRNAs make them attractive targets for the treatment of several human diseases, including diabetes mellitus. In fact, these molecules are small (typically 21 to 24

nucleotides) and can be easily synthesized and modified to improve their stability, their efficacy or their delivery to the cells. Moreover, different approaches permitting to specifically block the activity of the miRNAs are already available.

Today, more than 2500 different miRNAs have been identified in humans (http://www.mirbase.org/). These small RNAs are usually produced from intergenic or intronic sequences. In the latter case, they are often co-regulated with their hosting genes but their expression may also be controlled independently[20]. Many miRNAs are ubiquitously expressed but some of them are restricted to a subset of cells. They are usually generated in the nucleus from long precursor molecules (pri-miRNAs) transcribed by RNA polymerase II[21]. Once produced, pri-miRNAs are processed by an enzymatic complex including the RNase III enzyme Drosha, releasing a ~70 nucleotide hairpin-shaped precursor called pre-miRNA. Pre-miRNA hairpins are translocated by the Exportin-5/Ran GTPase complex from the nucleus to the cytoplasm where they are further cleaved by Dicer, another RNase III-type endonuclease. This generates a short RNA duplex (21-24bp), including the mature miRNA (guide strand) and a partially complementary sequence called the passenger strand (or miRNA*) which is usually rapidly degraded. The biogenesis of some miRNAs does not occur via the canonical pathway described above but are produced via alternative routes that bypass Drosha or Dicer cleavage[22].

After completing the maturation process, miRNAs are delivered to an Argonaute protein and are included in a RNA/protein complex (RISC) capable of recognizing specific sequences (seed sequences) in the 3' untranslated regions of target mRNAs. This results in translational repression and/or in a decrease in target mRNA stability. The interaction between the miRNAs and their targets follows complex rules but does not require a perfect sequence complementarity, enabling each miRNA to simultaneously regulate the expression of hundreds of transcripts, often encoding multiple components of the same signaling network[23].

4. Role of miRNAs in pancreatic β-cells

Pancreatic β-cells are central players in the control of blood glucose homeostasis. MiRNAs are involved both in the differentiation of insulin-secreting cells and in the control of the activities of fully mature β-cells. At birth, β-cells display an elevated proliferation rate and are still functionally immature[24]. Indeed, the capacity to secrete insulin in response to glucose, a unique feature of fully functional β-cells, is only acquired after a major gene reprogramming occurring in newborns or in young children[24, 25]. There is evidence indicating that changes in miRNA expression driven by the nutritional shift occurring at weaning may be instrumental in this maturation process[24]. MiRNAs play central roles also in fully mature β-cells. Indeed, numerous miRNAs contribute to the regulation of insulin biosynthesis and modulate the level of protein components guiding the transport and the fusion of secretory granules to the plasma membrane[26, 27].

Modifications in the miRNA expression profile appear to contribute to compensatory β-cell mass expansion occurring under insulin resistance conditions. Indeed, adaptations in the level of a set miRNAs, including miR-184, miR-338-3p and miR-375, favor β-cell mass expansion in obese and pregnant mice[28-30]. Conversely, progressive changes in the miRNA expression profile may contribute to the decline in β-cell proliferation in response to mitogenic signals that is observed during aging[31].

The miRNA expression profile is altered in the islets of different diabetes animal models and in the islets of diabetic patients, promoting β-cell dysfunction and failure. Indeed, inappropriate levels of several miRNAs have been observed in the islets of mice fed a high fat diet or in mice lacking leptin or its receptor[28, 29, 32-34]. Some of these miRNAs and many others were also differentially expressed in the islets of type 2 diabetic patients compared to the islets of healthy donors[29, 33-36]. Moreover, the expression of a number of miRNAs was found

to be affected by chronic exposure to pro-inflammatory cytokines and to be modified in the islets of pre-diabetic NOD mice, a well characterized model of type 1 diabetes[37].

5. Role of miRNAs in insulin target tissues

Besides β-cells, obesity and type 2 diabetes are also associated with changes in miRNA levels in insulin target tissues, contributing to insulin resistance and impaired glucose homeostasis. Different laboratories reported an up-regulation of miR-143 in the liver of obese and diabetic mice[38, 39]. Transgenic mice overexpressing this miRNA, display defective insulin-stimulated AKT activation and impaired glucose homeostasis. These effects were attributed to the down-regulation of oxysterol-binding-protein-related protein ORP8, a direct target of miR-143[39]. Consistent with these observations, mice deficient for miR-143 were found to be protected from obesity-associated insulin resistance[39].

The liver of obese mice contains also elevated levels of miR-103 and miR-107, two closely related miRNAs differing by just one nucleotide[40, 41]. The expression of these miRNAs in liver biopsies was found to be positively correlated with insulin resistance also in human subjects[40]. Up-regulation of these miRNAs in either hepatocytes or adipocytes caused a reduction in the level of caveolin-1, a critical regulator of insulin receptor activation, leading to impaired glucose homeostasis[40]. Another miRNA which is induced under insulin resistant conditions is miR-802[42]. Transgenic mice overexpressing this miRNA display glucose intolerance and diminished insulin sensitivity resulting from silencing of *Hnf1b* expression, a key transcription factor which is direct target of miR-802[42]. In contrast to the previous miRNAs, miR-26a was found to be down-regulated in the liver of obese mice and in overweight subjects and to be negatively correlated to insulin resistance[43]. This miRNA regulates the expression of several key genes involved in insulin signaling and in the metabolism of glucose and lipids. Indeed, transgenic mice overexpressing miR-26a in the

liver displayed increased insulin sensitivity and decreased hepatic glucose production and fatty acid synthesis[43].

Detailed analysis of the miRNA profile of skeletal muscle biopsies of type 2 diabetic patients revealed changes in more than sixty different miRNAs[44]. The differentially expressed miRNAs included a rise of miR-143 and a reduction of two muscle-specific miRNAs, miR-206 and miR-133a[44]. Many of these changes in miRNA expression were found to occur prior to the onset of clinical diabetes, suggesting an involvement in the early phases of the disease.

A large number of miRNAs have been found to positively or negatively affect the differentiation of white and brown adipocytes by controlling the expression of key transcription factors (for review see[45-47]). A subset of these miRNAs, including miR-196a[48], has also been shown to prevent or ameliorate obesity by promoting adipocyte browning. However, therapeutic approaches specifically designed to diminish the fat mass are not yet available.

Let-7 isoforms constitute a large family of miRNAs with complex effects on glucose metabolism. Indeed, Let-7 overexpressing mice display impaired glucose tolerance, reduced glucose-induced insulin secretion and a decrease in fat mass[49]. In agreement with these findings, overexpression of Lin28a/b, two RNA-binding proteins that inhibit Let-7 biogenesis, led to improvement in insulin sensitivity[50]. These metabolic effects may be linked to the capacity of Let-7 to control the level of key components of the insulin/PI3K/mTOR pathway, including Insulin and IGF1 receptors and the insulin-receptor substrate IRS2[50].

6. Contribution of miRNAs to diabetic complications

Exposure to chronically elevated blood glucose levels causes vascular damage promoting dysfunction and failure of heart, kidney, retina, peripheral nerves as well as impaired wound healing. A growing number of studies points to an important contribution of miRNAs in the

development of these diabetic complications[17, 19, 51]. Several miRNAs, including miR-21, miR-29, miR-192, miR-200b/c, miR-216/217, miR-377, were shown to be part of a signaling network participating in the development of diabetic nephropathy[52-56]. This degenerative process involves a progressive decline in glomerular filtration and is triggered by the activation of the TGFβ1 signaling pathway and the accumulation of extracellular matrix proteins[57]. Moreover, exposure of glomeruli to high glucose or to TGFβ1 was found to induce an endoplasmic reticulum stress response resulting in increased expression of a megacluster of nearly 40 miRNAs hosted in a long non-coding RNA transcript[58].

Sustained hyperglycemia can also lead to metabolic alterations in retinal endothelial cells resulting in capillary leakage, macular edema and blurred vision. The lack of oxygen, promotes the formation of new fragile capillaries that can bleed perturbing the vision and destroying the retina. Streptozotocin-induced diabetes in rats was found to be associated with major alterations in the miRNA profile in retinal endothelial cells. These expression changes included the down-regulation of miR-200b, a miRNA targeting the mRNA of VEGF and mimicking the increase in endothelial permeability and angiogenesis elicited by chronic hyperglycemia[59] and the up-regulation of miRNAs that are controlled by VEGF, p53 and NFκB[18].

Diabetic skin ulcers caused by defective wound healing can often lead to lower limb amputations and constitute a major clinical problem. A number of miRNAs have been proposed to contribute to impaired angiogenesis and revascularization under diabetic conditions. These include an up-regulation of miR-503 triggered by the activation of the NFκB pathway under prolonged hyperglycemia and ischemia conditions[60]. Moreover, reduced activity of the Inositol-Requiring Enzyme 1 (IRE1α), a key transducer of the unfolded protein response, under diabetic conditions was recently reported to improve the stability of the

precursors of several members of the miR-466 and miR-200 family, causing a rise in the level of the mature forms of these miRNAs[61].

7. miRNAs as potential therapeutic targets

As presented above, the development of different forms of diabetes mellitus is associated with alterations in the level of specific miRNAs. There is mounting evidence indicating that strategies permitting to correct the level of these non-coding RNAs can restore insulin secretion and/or insulin action and can prevent or treat the disease. In a recent study, the expression of several members of the miR-141/miR-200 family, a group of abundant miRNAs recognizing the same target sequences, was found to be increased in the islets of mice lacking the leptin receptor and to correlate with diabetes development[33]. Changes in the level of these miRNAs have a direct impact on β-cell survival. In fact, transgenic mice overexpressing two of these miRNAs display a progressive rise in blood glucose levels and become overtly diabetic after few weeks of life[33]. The authors generated mice in which the members of the miR-200/miR-141 family are not expressed in β-cells and assessed whether they were resistant to diabetes. Indeed, when treated streptozotocin, a toxic compound that specifically kills the β-cells and promotes the appearance of diabetes, these mice were less prone to develop the disease[33]. Akita mice bear a point mutation in the sequence coding for insulin. This triggers an endoplasmic reticulum stress causing severe β-cell dysfunction and loss. The knockout of all miR-200/miR-141 family members was also able to prevent diabetes manifestation in Akita mice[33].

Transgenic mice in which the level of selected miRNAs was manipulated in insulin target tissues were also reported to be less prone to develop insulin resistance and type 2 diabetes. For instance, the absence of miR-143 and miR-802, two miRNAs which are induced

under obesity conditions, was found to protect high-fat-diet fed mice against insulin resistance and to improve glucose tolerance[39, 42].

8. Conclusion

Taken together, these studies provide strong evidence for a direct involvement of miRNAs in β-cell dysfunction and insulin resistance and point at these small non-coding RNAs as interesting potential targets for the treatment of obesity associated diabetes.

9. Expert opinion

Genetic gain- or loss-of-function of miRNAs in animal models have evidenced a strong potential for therapeutics based on the modulation of the level of these small regulatory RNAs. As mentioned above, each miRNA controls the expression of multiple targets, often involved in the same signaling pathways or participating in the same functional processes. Thus, in contrast to siRNA-based strategies that target a single gene, therapeutic approaches modulating the level of selected miRNAs would allow a global control of gene networks. This represents a significant advantage for therapeutic interventions aiming at the treatment of complex diseases such as diabetes mellitus.

We already dispose of approaches permitting to modulate the level of miRNAs *in vivo*. MiRNAs are short RNA sequences with well-defined physico-chemical properties that can be easily synthesized to overexpress the miRNAs (miRNA mimics). These synthetic oligonucleotides can be used to raise the level of cellular miRNAs exerting a beneficial effect in disease settings. MiRNAs can also be specifically and very efficiently inactivated using short antisense oligonucleotides (anti-miRs) that block their activity and promote their degradation[62-64]. The delivery of anti-miRs permits to attenuate the impact of miRNAs contributing to diabetes development or progression. RNAs are usually very sensitive to

endogenous RNases present in the blood or in the cells. However, miRNA mimics and anti-miRs can be chemically modified to increase their stability and avoid premature nuclease degradation[62]. Moreover, the inclusion of chemically modified nucleotides permits to enhance target affinity, to reduce glomerular filtration and to facilitate cellular uptake. MiRNA mimics and anti-miRs usually contain phosphorothioate backbone linkages to escape nuclease degradation and to favor binding to plasma proteins[62, 63]. Anti-miRs most often contain also 2' sugar modifications that contribute to nuclease resistance and strongly improve the affinity for complementary RNAs[62, 63]. In some cases, anti-miRs have also been conjugated to cholesterol via a 2'-*O*-methyl linkage to enhance hepatic uptake and to lower delivery to other tissues[64].

An increasing number of studies have demonstrated the efficacy of these oligonucleotide derivatives in modulating the level of miRNAs in different diabetes models (Table 1). Several groups have succeeded in ameliorating insulin sensitivity under diabetic conditions by blocking the activity of specific miRNAs. Indeed, silencing of miR-103/107 using antisense oligonucleotides in either liver or adipocytes led to enhanced insulin sensitivity and improved glucose homeostasis[40]. Moreover, blockade of Let-7 family members with anti-miRNAs ameliorated insulin sensitivity in liver and muscle and prevented impaired glucose tolerance in mice with diet-induced obesity[49]. Similar results were obtained by down-regulating miR-181a and consequently up-regulating the expression of its target sirtuin-1[65].

Intraperitoneal injection of antisense oligonucleotides reducing the level of miR-21 were recently found to ameliorate diabetic nephropathy[66]. Indeed, blockade of this miRNA in diabetic mice attenuated a number of pathological hallmarks of diabetic kidney disease, including mesangial cell hypertrophy, interstitial fibrosis, podocyte loss and inflammation. Similar effects were observed upon silencing of miR-192, a miRNA induced by TGFβ signaling[67].

The use of miRNA mimics or of anti-miRs was also successful in modulating the activity of specific miRNAs in the eye. Indeed, introduction of miR-200b mimics in the vitreous cavity of the eye of diabetic rats decreased VEGF expression and prevented the diabetes-induced increase in retinal vascular permeability[59]. Moreover, intravitreal anti-miR injection permitted to decrease the level of miR-195 in retinal cells and to reduce tissue damage and retinopathy in diabetic rats[68].

Anti-miRs have also been used to stimulate wound healing in diabetic mice. In fact, local administration of anti-miR-26 or of a miR-27b mimic were found to promote angiogenesis and to accelerate wound healing in diabetic *db/db* mice[69, 70].

Recently, oligonucleotides (including anti-miRs) conjugated to triantennary *N*-acetylgalactosamine have been shown to be exceptionally stable and to be very efficiently targeted to the liver[71-74]. In fact, these oligonucleotide conjugates bind to the asialoglycoprotein receptor which is very abundant in hepatocytes and enter the cells by endocytosis. Clinical trials involving the use of *N*-acetylgalactosamine conjugates for the delivery of anti-miRs are already ongoing[71, 73, 74] and this approach will most probably become the dominant strategy for RNA-based therapeutics targeting the hepatocytes. Conjugate-mediated delivery of therapeutic oligonucleotides to other metabolically relevant tissues such as fat, skeletal muscle or pancreatic islets will require further investigations to identify mechanisms allowing cell-specific uptake.

While oligonucleotide derivatives have proven successful in modulating the level of selected miRNAs and in treating diabetes or its associated long-term complications, the use of these compounds presents some limitations that have so far prevented their general usage for therapeutic purposes.

Long-term treatments with these compounds may potentially lead to unacceptable side effects for the patients. In fact, most if not all the miRNAs that have been targeted so far for

the treatment of diabetes or its associated complications are not restricted to specific cells but are ubiquitously expressed. While having a positive impact in the target cells, changes in the level of these miRNAs may trigger deleterious effects in other organs. Thus, systemic and chronic delivery of miRNA mimics or anti-miRs could potentially lead to malignancies or to other severe disorders. Moreover, as mentioned above, these nucleotide-based molecules contain phosphorothioate backbone modifications to protect them from plasma and intracellular nucleases. The presence of phosphorothioate backbone modifications was recently found to trigger the activation of platelets and to promote thrombus formation[75]. If confirmed, this observation may have relevant implications for future therapeutic utilization of these molecules in humans.

Besides potentially leading to off-target effects, intraperitoneal injections are not efficient in delivering miRNA mimics or anti-miRs in some relevant tissues such as for instance the pancreatic islets. To circumvent these problems, several studies have taken advantage of viral constructs driven by cell-specific promoters (Fig.1A). These constructs can be engineered to generate either miRNA precursors or miRNA decoy molecules capable of sequestering the small non-coding RNAs and of blocking their interaction with the endogenous targets[76]. Adeno-associated viruses (AAV) are popular vectors for the delivery of miRNA precursors or miRNA decoys[77]. Several AAV serotypes differing for their cellular tropism are available, facilitating the preferential delivery to specific organs. An AAV-based approach has been used to block the activity of miR-503 in ischemic limb muscles of diabetic mice and to promote reparative angiogenesis[60]. Another AAV construct driven by the insulin promoter was employed to express a miR-338-3p decoy molecule in pancreatic β-cells and to promote their proliferation[78]. Other viral vectors have also been used to modulate the level of miRNAs relevant for diabetes treatment. For instance, the level of miR-146a in the retina was

increased by intravitreal injection of a lentiviral construct expressing the precursor of this miRNA, resulting in a decrease in microvascular leakage and retinal functional defects[79].

Several alternative strategies enabling cell-specific delivery of molecules capable of regulating the level of selected miRNAs are currently scrutinized. An attractive approach would be to couple them to aptamers (Fig.1B). Aptamers are DNA or RNA oligonucleotides that are selected for their capacity to bind a target molecule thanks to their stable three-dimensional structure[80, 81]. These short chemically synthesized molecules are obtained by a selection process named SELEX (Systematic Evolution of Ligands by Experimental enrichment) that involves iterative cycles of binding and amplification of a large pool of random sequences[82, 83]. Aptamers can be internalized by receptor-mediated endocytosis[84, 85], allowing intracellular delivery of their cargo. Selection protocols to identify synthetic oligonucleotides binding to whole living cells (cell-SELEX) are already available[86] and could permit the design of aptamers for the targeting of miRNA mimics and anti-miRs to specific tissues.

Another interesting approach would be to encapsulate the molecules regulating the level of the miRNAs inside liposomes (Fig.1C). These artificial vesicles would protect miRNA mimics and anti-miRs from degradation. Moreover, they could be engineered to bear at their surface, antibodies, ligands or receptors directing them to the appropriate target cells. Alternatively, the oligonucleotides could be introduced in exosomes (Fig.2C), a particular type of vesicles released by most cells in the body, which are known to carry an endogenous pool of miRNAs[87]. Exosomes have been shown to herald proteins that prevent their clearance by monocytes and have been proven to be more effective than fully artificial vesicles[88]. Moreover, there is increasing evidence indicating that exosomes released by the cells are endowed with signals capable of directing them to specific target tissues[89]. Thus, careful selection of the pool of exosomes to be used for the transport of miRNA mimics or anti-miRs

may potentially ensure the delivery of the therapeutic molecules to the appropriate target cells.

As outlined above, miRNAs are powerful cellular regulators and attractive therapeutic targets. Pharmacological tools that efficiently modulate the level of these small non-coding RNAs promise to become precious weapons to fight diabetes and its long term complications. However, at present the risk of severe side effects remains unacceptable to envisage the treatment of a chronic disease such as diabetes. Before these new powerful tools can integrate the arsenal for the treatment of diabetic patients, researcher will need to identify better strategies to insure the selective delivery of these pharmacological principles to the appropriate cells.

Article highlights box

- microRNAs are important regulators of gene expression and a subset of them controls the function of insulin-secreting cells and insulin target tissues.
- Accumulating evidence points to an involvement of microRNAs in the development of diabetes and its long term complications.
- Restoration of the level of specific microRNAs can successfully prevent and/or treat diabetes or its associated complications.
- We already dispose of molecules permitting to modulate the level or the activity of selected microRNAs *in vivo*.
- Experiments are underway to develop innovative strategies allowing a specific targeting of these molecules to relevant cells and reducing the potential side effects.

Table 1) Examples of successful oligonucleotide-based approaches to treat diabetes and its complications

The indicated miRNA mimics or anti-miRs were injected *in vivo* to improve insulin resistance in type 2 diabetes models or to attenuate common diabetic complications such as nephropathy, retinopathy or impaired wound healing.

Clinical process	Treatment	Effect	Reference
Insulin resistance	anti-miR-103/107	Decrease	[40]
	anti-Let-7	Decrease	[49]
	anti-miR-181a	Decrease	[65]
Diabetic nephropathy	anti-miR-21	Decrease	[66]
	anti-miR-192	Decrease	[67]
Diabetic retinopathy	anti-miR-195	Decrease	[68]
	miR-200b mimic	Decrease	[59]
Wound healing	anti-miR-26	Increase	[69]
	miR-27b mimic	Increase	[61]

Figure legend

Fig.1) Currently explored strategies to modulate the level of miRNAs in selected cells

A) Commonly used approaches to regulate the activity of miRNAs in selected tissues involve the use viral vectors (AAV or lentiviruses) engineered to produce either miRNA precursors or miRNA decoy molecules. The choice of cell-specific promoters permits to restrict the expression of the miRNA regulators to the relevant tissues.

B) Another strategy to control the level of miRNAs in a tissue-dependent manner is to couple miRNA mimics or anti-miRs to aptamers capable of binding specifically to the target cells.

C) Cell-specific delivery of miRNA mimics or anti-miRs could also be achieved by encapsulating these molecules in liposomes bearing antibodies or receptor ligands that bind to proteins present at the surface of the target cells. Alternatively, miRNA mimics or anti-miRs could be introduced in exosomes capable of delivering their cargo to the target cells.

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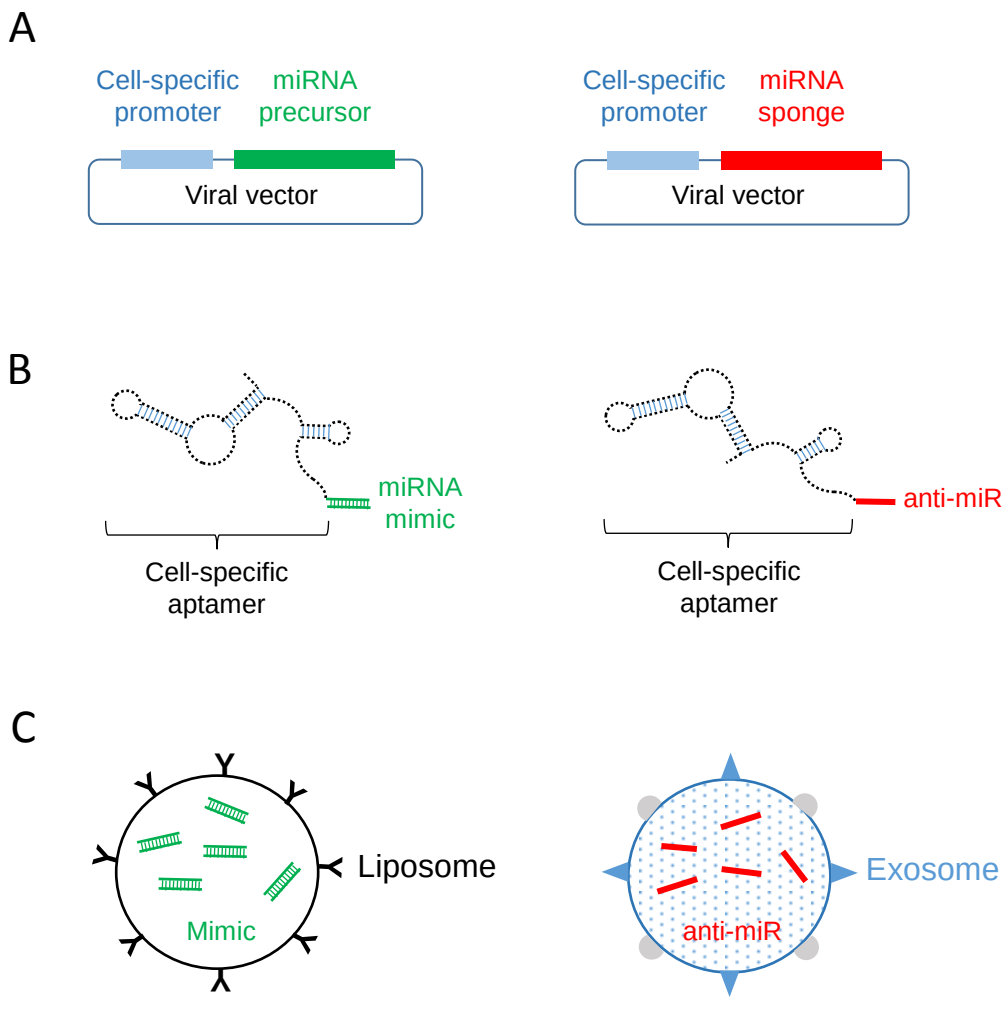
Fig.1


A
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