# MicroRNAs in **B**-Cell Biology, Insulin Resistance, **Diabetes and Its Complications**

Selene L. Fernandez-Valverde, Ryan J. Taft, and John S. Mattick

icroRNAs (miRNAs) are small 19-23 nucleotide RNA molecules that act as regulators of protein expression in eukaryotic cells by inducing the translational arrest and degradation of messenger RNAs (1). They are potent drivers of differentiation and development (1), and their dysregulation has been linked to many diseases. Here, we present an overview of the known and proposed roles and effects of miRNAs in type 1 and type 2 diabetes (T1D and T2D), focusing on  $\beta$ -cell biology, insulin resistance, and diabetes complications. Specifically, we discuss miRNAs in  $\beta$ -cell biology, altered expression of miRNAs in adipose tissue in response to obesity, and miRNA dysfunction in organs and tissues that may be affected in later stages of the disease. Additionally, we propose a set of research directions that may yield novel diagnostic and therapeutic approaches for this chronic illness.

T2D is characterized by hyperglycemia resultant from impaired insulin secretion and/or impaired insulin action in peripheral tissues (2). T2D constitutes one of the greatest pandemics of our time, with 220 million people currently diagnosed (3), and 366 million people expected to be affected by 2030 (4). A number of lines of evidence support a key role for pancreatic  $\beta$ -cell dysfunction in T2D (in addition to T1D), in which it is the major pathology. For example, recent genome-wide association studies have strongly implicated genes involved in insulin secretion as etiological factors in the development of T2D (5).

A role for miRNAs in T2D was first established in 2004 by Poy et al. (6) who showed that miR-375 is directly involved in the regulation of insulin secretion. This study was one of the first to demonstrate that a miRNA could be tightly linked to a disease phenotype. In recent years, dozens of additional miRNAs have been identified as components of pathways triggered by, or contributing to, the pathology of both T1D and T2D (Table 1). Because of the multifactorial and polysystemic nature of this disease and the increased interest in miRNAs, it seems likely that many more miRNAs, and perhaps other small regulatory RNA species, will be identified as factors in diabetes. This will undoubtedly lead to a greater understanding of the genetic basis of the disease and provide novel diagnostic, prognostic, and treatment alternatives.

See accompanying perspective, p. 1832.

# miRNAS IN β-CELL BIOLOGY

Pancreatic  $\beta$ -cells play a fundamental role in glucose homeostasis, releasing insulin in response to glucose levels in the bloodstream. Insulin then triggers glucose uptake in its target tissues, such as the liver, kidney, skeletal muscle, and cardiac muscle. Absence or malfunction of β-cells leads to diabetes due to lack of insulin producing cells (T1D), or to the inability to increase insulin levels to sufficiently stimulate glucose uptake in the face of insulin resistance (T2D).

In T1D, the lack of insulin is primarily caused by the absence or destruction of pancreatic  $\beta$ -cells, which is driven by developmental errors or immune malfunction, respectively. A large suite of miRNAs has been implicated in pancreas (and therefore  $\beta$ -cell) development including miR-15a/b, miR-16, miR-195 (7), miR-503, miR-541, miR-214 (8), miR-9 (9), miR-124a (10), miR-7 (9,11), miR-376 (9) and miR-375 (9,12), among others. There remains a need for detailed studies of the role of these miRNAs in diabetes, but it is clear that mutations or misexpression of these species could lead to  $\beta$ -cell pathologies (see below).

Likewise, miRNAs have been implicated in the autoimmune destruction of  $\beta$ -cells, also leading to T1D. Recently, Hezova et al. (13) measured changes in miRNA expression in regulatory T cells (T-reg cells) of T1D-affected individuals. These cells are of special interest as they are critical regulators of autoimmune disease. They found that miR-510 was significantly upregulated, and miR-191 and miR-342 were significantly downregulated in adult peripheral T-reg cells of diabetic patients compared with healthy individuals (13). miR-342 is also known to show altered expression profiles in hematological disease (14,15). These observations suggest a role for these miRNAs in the autoimmune destruction of B-cells.

The role of miRNAs in the function of  $\beta$ -cells in T2D patients has been extensively studied but is not yet fully understood, as exemplified by miR-375. In adult  $\beta$ -cell islets miR-375 levels are decreased when high levels of glucose are available (6). Low levels of miR-375 induce insulin secretion by de-repression of its targets Mtpn (6,16)and PDK1 (17,18), while overexpression of miR-375 attenuates proliferation and insulin gene transcription while reducing glucose-induced insulin secretion (16,18,19). Indeed, ectopic expression of miR-375 in diabetic pancreatic β-cells results in increased susceptibility to fatty acid induced apoptosis (16). Consistent with these studies, high levels of miR-375 are present in pancreatic islets of obese diabetic mouse models (ob/ob) (20) and T2D affected individuals (21). Additionally, when miR-375 is deleted in ob/ob mice, they develop a marked decrease in  $\beta$ -cell mass, which results in a severe insulin-deficient diabetes not found in *ob/ob* mice (20). Overall, it is becoming clear that miR-375 targets a suite of genes that negatively regulate cellular growth and proliferation (20), and that aberrant loss of this miRNA leads to dramatic reduction of  $\beta$ -cell

From the Institute for Molecular Bioscience, University of Queensland, St Lucia, Queensland, Australia.

Corresponding author: John S. Mattick, j.mattick@imb.uq.edu.au. Received 9 February 2011 and accepted 12 April 2011.

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# TABLE 1

miRNAs involved in diabetes

miRNA	Tissue	Relevance to diabetes	References
miR-375	Pancreas	Expressed in pancreatic development. Regulates insulin secretion in $\beta$ -cells and increases their death by lipoapoptosis, as it regulates this cell viability and proliferation. Upregulated in $\beta$ -cells of T2D patients. Its deletion causes	(6,8,9,12,16,18–21,69)
miR-29(a/b/c)	Adipose	Induced by high glucose and high insulin.	(31,32,70)
miR-143	Adipose	Participates in adipocyte differentiation and is induced in adipogenesis and downregulated in obesity.	(41,42)
miR-9	Pancreas, cardiac muscle	Expressed in pancreatic development. Impairs insulin secretion in $\beta$ -cells and is upregulated in cardiomyocytes of STZ-induced diabetic mice.	(9,22)
miR-124a	Pancreas	Upregulated by glucose. Regulates the insulin exocytosis pathway, causing exaggerated insulin release when no glucose is available but reduced glucose induced insulin secretion	(10,23,24)
miR-195	Pancreas, liver	Expressed in pancreatic development and unregulated in liver of diabetic rats	(7,32)
miR-192	Kidney	Induced by transforming growth factor-β and highly expressed in glomeruli of STZ-induced diabetic mice. Targets SIP1.	(59)
miR-222	Adipose	Upregulated in response to high glucose in adipose tissue of diabetic rats	(32)
miR-126	Pancreas, skeletal muscle	Expressed in pancreatic development. Upregulated in skeletal muscle of GK rats and in livers of <i>ob/ob</i> mice compared with STZ mice	(8,56,71)
miR-133a	Cardiac and skeletal muscle	Overexpressed in rabbit diabetic heart, where it induces prolongation of QT interval. Downregulated in cardiac hypertrophy in mouse and human hearts and in hearts of STZ- induced diabetic mice. Also reduced in human skeletal muscle in T2D. High fasting glucose associates with lower expression of this miRNA.	(45,46,48,53,54,72)
miR-296	Pancreas	Expressed in β-cell islets and upregulated by glucose.	(18)
miR-96	Pancreas	Negatively regulates insulin exocytosis through upregulation of granuphilin.	(23)
miR-34a	Pancreas, liver	Increases in $\beta$ -cells in response to palmitate, making them more susceptible to death by apoptosis and inhibiting nutrient-induced insulin secretion. Upregulated in the livers of STZ-induced diabetic mice. Found in the bloodstream and can differentiate between nondiabetic and early T2D patients.	(23,56,73,74)
miR-146b	Pancreas	Contributes to increased apoptosis of β-cells. Expression induced by cytokines and sodium palmitate.	(72,74)
miR-657	Liver	Regulates insulin-like growth factor 2 receptor, and variants in its regulation site (changes in regulation) give predisposition to diabetes.	(75)
miR-30d	Adipose	Upregulated in presence of high glucose, upregulates insulin gene transcription	(24)
miR-320	Cardiac vascular endothelium	Upregulated in GK rats with impaired angiogenesis.	(76)

Continued on facing page

#### TABLE 1 Continued

miRNA	Tissue	Relevance to diabetes	References
miR-103	Pancreas, liver	Overexpression accelerates adipogenesis. Reduced in response to TNF-α. Downregulated in obesity. Upregulated in liver of <i>ob/ob</i> mice (vs. STZ-induced diabetic mice) and diabetic rats.	(32,41,56)
miR-107	Pancreas, adipose	Overexpression accelerates adipogenesis. Reduced in response to TNF-α. Upregulated in β-cells in presence of high glucose.	(24,41)
miR-1	Cardiac and skeletal muscle	Regulates cardiac arritmogenic potential. Upregulated by high glucose in cardiomyocytes, where it accelerates apoptosis. High levels found in ventricle of diabetic patients. Impaired insulin response in skeletal muscle of T2D patients. Significantly downregulated in the heart of STZ-induced diabetic mice.	(45,51,53,55,77)
miR-223	Heart	Upregulated in the insulin-resistant heart, where it increases glucose uptake through increase of Glut4.	(78)
miR-125(a/b)	Liver, vascular tissue	Upregulated in liver of hyperglycemic rats. Increase of this miRNA results in a proinflammatory diabetic phenotype in vascular smooth muscle cells.	(31,79)
miR-27(a/b)	Adipose	Impairs human adipocyte differentiation and targets peroxisome proliferator–activated receptor γ. Upregulated in adipose tissue of diabetic rats and by glucose in 3T3 adipocytes.	(32,34)
miR-216a, miR-217	Kidney	Highly expressed in kidney and upregulated by transforming growth factor-β. Activates Akt signaling through targeting of PTEN.	(60,80)
miR-122	Liver	Suppression in liver results in reduced fatty acid accumulation. Downregulated in liver of STZ- induced diabetic mice.	(56,57)
miR-320	Adipose, vascular endothelium	Highly upregulated in insulin-resistant adipocytes. Targets p85, leading to increased insulin resistance in adipocytes. Upregulated in myocardial microvascular cells in GK rats, where it impairs angiogenesis.	(33,76)
miR-21	Pancreas, liver	Upregulated by nuclear factor-κB and fatty acids in liver, leading to downregulation of its target PTEN. Induced by interleukin-1β and TNF-α in pancreatic islets. Expression increased in rats on high-fat diet and in liver of T2D patients. Overexpression reduces maximal glucose- induced insulin release in β-cells.	(58,74)
miR-206	Cardiac and skeletal muscle	Upregulated in skeletal muscle of diabetic and prediabetic patients. Upregulated by high glucose in cardiomyocytes. Accelerates cardiomyocyte anoptosis.	(48,55)
miR-93	Vascular endothelium	Downregulated by high glucose through	(81)
miR-30a*	Adipose	Downregulated in T2D individuals, independent	(68)
miR-181d	Liver	Most effective miRNA at reducing intracellular lipid content of hepatocytes.	(82)

mass, leading to low levels of insulin, hyperglycemia, and thus diabetes.

At least three additional miRNAs play a critical role in insulin exocytosis in  $\beta$ -cells. For example, miR-9 positively regulates glucose-induced insulin secretion in  $\beta$ -cells by directly repressing Onecut-2, the granuphilin (synaptotagmin-like 4) repressor, a protein that significantly enhances

basal, but strongly inhibits K<sup>+</sup>-induced insulin secretion (22). Likewise, miR-96 also negatively regulates insulin exocytosis by targeting synaptotagmin-like 4 (23), and reduces levels of Noc2, which impairs  $\beta$ -cell ability to respond to secretagogues (23). The third well-described  $\beta$ -cell miRNA, miR-124a, intriguingly, appears to link  $\beta$ -cell and neural biology through the exocytosis pathway. miR-124a is upregulated in  $\beta$ -cells in the presence of glucose (24), but was first described as a brain-specific miRNA implicated in neurogenesis (25). High levels of miR-124a in  $\beta$ -cells lead to exaggerated insulin release at low-glucose concentrations but reduced glucose-induced secretion (24). This is likely to the result of targeting of Foxa2, which in turn downregulates Sur-1, Kir6.2, and Pdx1, the latter of which directly regulates the expression of the insulin gene (10). miR-124a also decreases levels of Rab27a and Noc2 and upregulates Snap25, Rab3a, and Synapsin1a, facilitating tight regulation of the insulin exocytosis pathway (23).

Interestingly, two other miRNAs, miR-7 (26) and miR-375 (12), are expressed both in brain and in  $\beta$ -cell islets. Both  $\beta$ -cells and neurons share similar secretion mechanisms and are responsive to signals in the bloodstream including glucose and insulin. Taking into account the fact that insulin stimulates glucose metabolism in the brain, mainly in the cerebral cortex (27), it is likely that miRNA expression in the brain is affected by diabetes, which could therefore have profound neurologic consequences.

### miRNAS IN OBESITY AND ADIPOSE TISSUE

Obesity, hyperlipidemia (elevated levels of blood lipids), and insulin resistance (reduced glucose uptake in response to the insulin signal) are strongly associated with the onset of T2D (28). Obesity itself is characterized by adipocyte dysfunction with abnormalities in adipokine secretion and in energy metabolism. Adipogenic abnormalities lead to an adipocyte phenotype distinct from the "ideal" which stores excess energy benignly in the triglyceride droplet. Among other downstream effects, this leads to an accumulation of fat in ectopic sites, such as liver, muscle, pancreas, and kidneys (29). It also leads to the secretion of chemoattractants that promote macrophage infiltration of adipose tissue, leading to inflammation and excessive release of free fatty acids into the bloodstream (29,30).

miRNAs in adipose tissue are strongly dysregulated in response to obesity-induced molecular changes and environmental signals. For instance, the expression of the miR-29 family is induced by hyperglycemia and hyperinsulinemia in adipose tissue (31), and miR-29a is highly upregulated in 3T3-L1 adipocytes in response to high glucose (32). Likewise, miR-320 increases insulin sensitivity of insulin resistant adipocytes (33) and miR-27b impairs human adipocyte differentiation (34). These miRNAs target conserved core cell-regulatory pathways that are affected both locally and systematically by obesity and by diabetes generally. Indeed, miR-320 targets p85, which plays a critical role in cell growth by increasing Akt phosphorylation and thus the level of Glut4 (33). Similarly, miR-29 targets  $p85\alpha$  in HeLa cells, and is thus likely to affect Akt signaling in adipocytes (35). Additionally, miR-27b targets peroxisome proliferator-activated receptor  $\gamma$  (34), the nuclear receptor targeted by thiazolidinediones, an insulin-sensitizing agent used for treating T2D (36). Just as miRNAs are diagnostic of broad changes in cancer (37,38), they also reflect the degree of disturbance in diabetes.

As mentioned above, obesity triggers macrophage infiltration and cytokine release in adipose tissue. This is closely followed by changes in miRNA expression, which in turn affect lipid levels and adipogenesis. Indeed, many cytokines, such as tumor necrosis factor (TNF)- $\alpha$ , interfere with insulin signaling and inhibit adipogenesis (39,40) and several miRNAs that are induced during adipogenesis are downregulated in obesity (41). For example, inhibition of miR-143 has been shown to regulate adipocyte differentiation and results in reduced adipogenesis (42). Similarly, miR-103 and miR-107 have been shown to accelerate adipogenesis (43), and are predicted to target pathways that regulate cellular acetyl-CoA and lipid levels (44). Levels of miR-143, miR-103 and miR-107 are reduced after one-day treatment with TNF- $\alpha$  in adipocytes (43), suggesting that macrophage infiltration and cytokine release are a contributing factor to reduced adipogenesis in obesity. The connection between miRNA expression and cytokine exposure may eventually be leveraged into treatment options for morbidly obese diabetic patients.

#### miRNAS IN DIABETES COMPLICATIONS

Tissues adversely affected by diabetes may include cardiac and skeletal muscle, liver, kidney, and endothelium. Hyperglycemia and hyperlipedemia damage these tissues causing conditions such as fatty liver, stroke, kidney failure, neuropathy, and blindness. Not surprisingly, the expression of miRNAs is altered in these tissues in diabetic individuals.

A precondition for the onset of T2D is insulin resistance of skeletal muscle. Abnormally circulating fatty acids accumulate in skeletal muscle and disrupt important signaling pathways (29). Normally, insulin activates the transcription of sterol regulatory element–binding protein 1, which represses transcription of miR-1 and miR-133 by inhibiting muscle specific factor myocyte enhancer factor (MEF) 2C (45,46). The repression of miR-133a and miR-1 in response to insulin levels is impaired in T2D patients (45), which contributes to impaired muscle function (47). Indeed, a recent study has shown that the expression of miR-133a is strongly downregulated in this tissue in T2Daffected individuals (48).

The miRNAs miR-1 and miR-133 also play a primordial role in normal cardiac function and cardiogenesis (49,50). In the normal heart, cardiomyocytes exposed to high levels of glucose develop hypertrophy and, similar to skeletal muscle, have low levels of miR-133a (51). Indeed, glucoseinduced cardiomyocyte hypertrophy is associated with increased levels of MEF2A and MEF2C (52). In diabetes, however, the relationship between MEF2A/C is impaired. miR-133 is in fact overexpressed in the rabbit diabetic heart, where it induces prolongation of the QT interval, a known phenotype of T2D (53). However, and surprisingly, both miR-1 and miR-133a are downregulated in the hearts of mice with insulin-deficiency induced by streptozotocin (STZ), and in cardiac-hypertrophy and heart failure (54). This strongly suggests that the relationship between miR-133 and miR-1 and cardiomyocte responses to insulin and glucose is complex. Indeed, a recent study has shown that these miRNAs are upregulated in cardiomyocytes after high-glucose exposure (55), which accelerates cardiomyocyte apoptosis (55), a key factor in the development of diabetic cardiomyopathy. These observations suggest that miR-1 and miR-133 are excellent candidates for further functional studies. Unraveling their seemingly contradictory behavior will undoubtedly shed light on the complicated biology underpinning muscle cell responses to physiological stimuli.

In the late stages of diabetes, liver and kidney function may be impaired, and this is reflected in several abnormal miRNA expression profiles. For example, inhibition of

a miRNA crucial for liver function, miR-122, which is downregulated in the livers of STZ-induced diabetic mice (56), results in low plasma cholesterol levels, increased hepatic fatty acid oxidation, and decreased hepatic fatty acid and cholesterol synthesis rate (57). miR-21 also plays an important role in hepatocytes, where it is induced by nuclear factor- $\kappa B$ , leading to the downregulation of phosphatase and tensin homolog (PTEN), a protein that inhibits Akt activation (58). High-fat diets result in the upregulation of miR-21 in rats (58), and liver biopsies of obese human patients also show an increase in the expression of miR-21 and a decreased level of PTEN expression in comparison with normal controls (58). In the diabetic kidney, tranforming growth factor- $\beta$  and miR-192 induce expression of miR-216a and miR-217 (59,60), which leads to activation of Akt through targeting of PTEN (60).

A tissue that has recently come to attention in diabetes is vascular endothelium, which changes in response to diabetic inflammatory signals. There is a strong negative correlation between miR-126 levels and the onset of diabetic vascular complications (61). However, this miRNA is significantly increased in patients suffering of coronary artery disease (62). Interestingly, delivery of this miRNA by apoptotic bodies protects against diet-induced atherosclerosis (62). This indicates that miR-126 can be used as both a biomarker for early detection of vascular complications of diabetes, and as a possible RNA-based therapeutic for diabetes-induced atherosclerosis.

## FUTURE CHALLENGES AND POSSIBILITIES

There are many areas of research that remain unexplored in relation to miRNAs and diabetes. One is the interplay between the brain and insulin target tissues. It is known that the brain is fueled by glucose and, despite previous reports that this tissue is insulin insensitive, recent articles have shown insulin-induced glucose uptake in specific brain regions (27). Indeed, the similarities between  $\beta$ -cell and neuronal exocytosis systems are remarkable (see above). Due to the fact that the brain is responsible for behavioral control, it is likely that it reacts directly to the availability or depletion of glucose in the bloodstream by regulating caloric uptake. miRNAs have already been shown to carry out important regulatory functions in the brain, and therefore a study focusing on the miRNA changes in brain in response to hyperglycemia and hyperlipidemia is essential for a complete systemic understanding of diabetes.

It is now clear that the miRNA profile of insulin resistance tissues changes years before the onset and/or diagnosis of T2D. Indeed, a study by Zampetaki et al. (61) has shown that a plasma signature of five miRNAs (miR-15a, miR-29b, miR-126, miR-223, and miR-28-3p) can accurately differentiate patients with a high likelihood of developing diabetes from healthy controls. In a review, Regazzi (63) proposed that these miRNAs might not only be indicators of early disease onset but are also responsible for its progression and thus a good target for early intervention. One possibility is that these miRNAs actually act distant from their sites of biogenesis, and are transmitted from one tissue to another (64). The recent observation of miR-150 transmission through the bloodstream and uptake by target endothelial cells (65) makes this possibility likely. If diabetes-related miRNAs are trafficked in trans, it may be possible to interfere in the progression of the disease with minimally invasive RNA therapeutics.

Because of the high genetic predisposition of some individuals to develop T2D, identification of genetic variants that alter the levels of key miRNAs may become a clinically powerful tool, undoubtedly aided by the increasing affordability of personal genomic sequencing. We predict that the availability of this technology will provide clinicians with an invaluable tool to identify at-risk individuals, to prompt their patients to take preventive action, and, eventually, to prescribe tailored therapeutics (66). It will also provide a baseline for beginning to rigorously identify environmental factors that promote epigenetic variations, particularly for genetically susceptible individuals.

It is important to emphasize the need for integrating current and future miRNA studies into existing resources like the Beta Cell Biology Consortium (http://www.betacell.org/). Many recent genome-wide studies have provided a snapshot of the miRNA content of different tissues involved in this disease, including the developing pancreas (67) and subcutaneous adipocytes of T2D patients (68). However, it is difficult to integrate this information if it is not summarized in a central repository. As highlighted by Gallagher et al. (48), even though the community hoped to gain insight into miRNA mechanisms of action by studying them in isolated model systems, it is now clear that this information must be centralized and integrated with clinical data in order to obtain the insights necessary to conquer this illness.

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