The involvement of microRNAs in Type 2 diabetes

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Abstract

T2D (Type 2 diabetes mellitus) is a major health issue that has reached epidemic status worldwide. T2D is a progressive metabolic disorder characterized by reduced insulin sensitivity, insulin resistance and pancreatic β-cell dysfunction. Improper treatment of T2D can lead to severe complications such as heart disease, stroke, kidney failure, blindness and nerve damage. The aetiology and molecular mechanisms of T2D are not fully understood, but compelling evidence points to a link between T2D, obesity, dyslipidaemia and insulin resistance. Although T2D seems to be strongly linked to environmental factors such as nutrition and lifestyle, studies have shown that genetic factors, such as polymorphisms associated with metabolic genes, imprinting, fetal programming and miRNA (microRNA) expression, could also contribute to the development of this disease. miRNAs are small 22–25-nt-long untranslated RNAs that negatively regulate the translation of mRNAs. miRNAs are involved in a large number of biological functions such as development, metabolism, immunity and diseases such as cancer, cardiovascular diseases and diabetes. The present review examines the various miRNAs that have been identified as being potentially involved in T2D, focusing on the insulin-sensitive organs: white adipose tissue, liver, skeletal muscle and the insulin-producing pancreatic β-cells.

Introduction

T2D (Type 2 diabetes mellitus) has now reached epidemic proportions worldwide. In 2009, in the U.K. alone, 2.6 million individuals were considered diabetic (Diabetes in the UK 2010: Key Statistics on Diabetes; http://www.diabetes.org.uk/Documents/Reports/Diabetes_in_the_UK_2010.pdf) and 90% of these patients were suffering from T2D. T2D is a progressive metabolic disorder characterized by reduced insulin sensitivity, insulin resistance in tissues such as skeletal muscle, liver and adipose tissue, combined with pancreatic β-cell dysfunction, resulting in systemic hyperglycaemia [1]. Improper treatment of T2D can lead to severe complications such as heart disease, stroke, kidney failure, blindness and nerve damage [2].

Although the aetiology of this disease is not fully understood, there is compelling evidence that insulin resistance plays a major role in its development. Indeed, cross-sectional studies demonstrated that insulin resistance is the best predictor for the development of T2D and that insulin resistance can be present 10–20 years before the onset of T2D [3]. The molecular mechanisms leading to insulin resistance have not been elucidated completely, but a compelling number of studies have established a link between insulin resistance, dyslipidaemia and obesity. It has been shown that excessive NEFA (non-esterified ‘free’ fatty acid) mobilization from adipose tissue leads to plasma NEFA increase, ectopic deposition of triacylglycerols in muscle and liver and insulin resistance. Increased lipid deposition in pancreatic β-cells is associated with β-cell dysfunction and accelerated apoptosis [4]. Thus deregulation of adipose tissue function and lipid metabolism are central to the development of insulin resistance and β-cell dysfunction, two major components of T2D.

miRNAs (microRNAs) are small 22–25-nt-long non-coding RNA molecules that negatively regulate translation of target mRNAs. miRNAs normally bind to the 3′-UTR (untranslated region) of their target mRNA through imperfect base pairing, leading to translation inhibition and/or mRNA degradation [5]. Over 500 miRNAs have been found in the human genome [6], and it has been estimated that they could regulate 74–92% of all protein-encoding mRNAs [7]. Considering the complex level of gene-expression regulation conferred by miRNAs, it comes as no surprise that miRNAs are involved in a plethora of biological functions such as cell growth and proliferation, development, differentiation, organogenesis, metabolism, immunity and diseases such as cancer, cardiovascular diseases and diabetes [8].

The present review examines the various miRNAs that have been identified as being potentially involved in T2D, focusing on the insulin-sensitive organs: WAT (white adipose tissue), liver, skeletal muscle and the insulin-producing pancreatic β-cells. Many studies have characterized the global
expression pattern variation of miRNAs in these organs affected by T2D, but few have identified the functional implications of these changes, mostly due to the unavailability of methods designed to identify the biological targets of a large number of miRNAs. We focus mainly on the miRNAs with validated targets and their possible involvement in T2D.

### miRNA and adipose tissue function during T2D

WAT is the main site of lipid and triacylglycerol storage in mammals and is an important regulator of whole-body energy homeostasis [9]. Adipocytes are insulin-sensitive cells that act as a ‘sink’ for fatty acids, storing lipids that would otherwise be detrimental if present in too high a concentration in the plasma or in ectopic organs. Recent advances in adipose research have established that a net positive energy balance and dysfunctional adipocytes lead to abnormal fat storage and mobilization. These phenomena form a critical link between obesity and insulin resistance [10–12].

Various studies have identified many miRNAs as being regulated in the adipose tissue from a rat model of T2D and a human with T2D, illustrated in Table 1. Two studies have shown the up-regulation of miR-222, miR-27a, miR-29a, miR-335 and miR-125a which have functional and biological relevance in the WAT of T2D rats [13–15]. Interestingly, the expression of three of these miRNAs (miR-222, miR-27a and miR-29a) were shown to be transcriptionally up-regulated in cultured 3T3-L1 adipocytes when cultured under high-glucose conditions [14]. The authors suggest that increased expression of these miRNAs could be involved in the initial cellular response of adipocytes to hyperglycaemia [14]. This hypothesis is supported by a study showing that miR-29 is up-regulated in insulin-resistant 3T3-L1 adipocytes [16]. This study also demonstrated that overexpression of the miR-29 family (miR-29a, miR-29b and miR-29c) in 3T3-L1 adipocytes blocks insulin-stimulated glucose uptake by inhibiting insulin signalling via the Akt pathway [16]. Another miRNA, miR-125a is up-regulated in the WAT of T2D rats as well as in insulin-resistant 3T3-L1 adipocytes [10]. Although none of the miR-125a targets has been validated *in vivo* or *in vitro*, bioinformatics analysis reveal several predicted target miRNAs involved in glucose metabolism [14]. Among them is the gene for prostaglandin E synthase 2 (PTGES2) which has been reported to contain an SNP (single nucleotide polymorphism) linked to T2D in humans [17,18]. miR-335, whose expression is also increased in the WAT of GK (Goto–Kakikazi) rats, has been shown to be involved in adipocyte differentiation and maturation and to be increased in the WAT of obese mice [19]. miR-335 expression is increased as cultured pre-adipocytes differentiate into adipocytes, and the rise in expression of adipose differentiation markers [aP2 (adipocyte fatty-acid-binding protein 2), PPARγ (peroxisome-proliferator-activated receptor γ) and FAS (fatty acid synthase)] is concomitant with the rise in miR-335 expression [19]. This phenomenon could be linked to the increase in size and number of adipocytes observed in conditions of high-energy intake leading to obesity, enabling the WAT to store more lipids and fatty acids [19].

### miRNA involvement in glucose and fat uptake in the liver of T2D individuals

The liver is the main site for gluconeogenesis in the body. This organ is also responsible for the production and transport of cholesterol and fatty acids. Both of these processes are inhibited by insulin in healthy individuals and become deregulated in instances of insulin resistance and diabetes [20]. Table 2 illustrates the miRNAs that have been shown in the literature to be transcriptionally modulated in the liver of T2D GK rats. The targets of these miRNAs have

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>miRNA</th>
<th>Expression</th>
<th>Reference(s)</th>
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<td>miR-125a</td>
<td>Up</td>
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<td>Up</td>
<td>[13]</td>
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<td>miR-222</td>
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<tr>
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<td>miR-30a&quot;</td>
<td>Down</td>
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been not validated in the context of T2D, but studies of these miRNAs in other biological contexts suggest that they may be functionally relevant in insulin resistance and T2D pathogenesis [21–25].

For example, miR-221 and miR-222 have been shown to directly bind the 3′-UTR of PTEN (phosphatase and tensin homologue deleted on chromosome 10) and inhibit its translation in cultured hepatocellular carcinoma cells [21]. Liver-specific KO (knockout) of PTEN has been shown to have two different effects in lipid and glucose metabolism. PTEN-KO in mice liver results in increased fatty acid synthesis, accompanied by hepatomegaly and a fatty liver phenotype. PTEN also leads to increased insulin-stimulated glucose uptake in the liver and improved systemic glucose tolerance [22]. Therefore overexpression of miR-221 and miR-222 in the liver of T2D rats could contribute to the lipotoxicity observed in diabetic liver while exerting an insulin-sensitizing effect to compensate for systemic hyperglycaemia.

Microarray based technologies have also identified two miRNAs which could contribute to fatty liver and associated complications: miR-27b and miR-335. miR-27b expression is down-regulated in the liver of T2D rats [13]. miR-27b can bind the 3′-UTR of PPARγ mRNA and induce its degradation [23]. The destabilization of PPARγ in 3T3-L1 adipocytes by miR-27b blocks their differentiation into lipid-storing adipocytes [24]. It has been shown that the expression of PPARγ, as well as other adipogenic genes, was increased in humans and mice with a fatty-liver phenotype [25]. The lowered expression of miR-27b in diabetic liver could be in part responsible for the fatty liver phenotype by increasing PPARγ protein abundance. PPARγ would then be able to exert its adipogenic effect in the liver cells. As mentioned above, miR-335 is an miRNA involved in lipid metabolism. It has been shown that miR-335 is up-regulated not only in the liver of T2D rats, but also in the liver of two strains of obese mice (ob/ob and KKAy) [19]. miR-335 could be another contributor to the fatty-liver phenotype and associated complications.

### miRNAs in the adaptation of the skeletal muscle to insulin resistance and T2D

Muscle tissue is the primary site of glucose uptake postprandially, with skeletal muscle accounting for approx. 75% of insulin-dependant glucose removal from the plasma [26]. Skeletal muscle insulin resistance is an early feature in T2D pathogenesis and is a direct risk factor for the development of cardiovascular disease [27]. Table 3 shows the variant miRNAs in both insulin-resistant human and spontaneously diabetic rat muscle. miR-24, which is down-regulated in the diabetic muscle, was shown to target directly p38 MAPK (mitogen-activated protein kinase) [28]. The protein expression of MAPK is up-regulated concomitantly with the decrease in miR-24 expression in GK rat muscles. Interestingly, p38 MAPK is responsible for the activation of MEF2 (myocyte-enhancer factor 2), a muscle-specific transcription factor responsible for the transcription of the insulin-responsive GLUT4 (glucose transporter 4) [29] and its activity is also increased under high-glucose conditions [30]. These data suggest that a decrease in miR-24 could activate the p38 MAPK pathway and contribute to the adaptation of muscle tissue to higher glucose tolerance than under normal conditions by increasing insulin-dependant glucose uptake [28]. Similarly to miR-24, the down-regulation of miR-126 could contribute to adaptive increased glucose uptake in skeletal muscle. One of the validated targets of miR-126 is p85β, one isoform of the regulatory sub-unit of PI3K (phosphoinositide 3-kinase): exogenous expression of miR-126 decreases p85β protein expression and represses the PI3K/Akt pathway in vitro [31]. PI3K/Akt is a pathway essential for GLUT4 translocation to the membrane in skeletal muscle, so a decrease in miR-126 and an increase in PI3K activity could contribute to an increased glucose uptake. miR-24 and miR-126 do not seem to participate in the pathogenesis, but seem to be involved in the adaptation of muscle tissue to increased glucose levels.

Identification of the targets and characterization of the
activity of the remaining 19 miRNAs identified in the two microarray-based screens (Table 3) might yield miRNAs involved directly in the rise of insulin resistance and the development of T2D.

**miRNA involvement in pancreatic β-cell insulin production and apoptosis in obesity and T2D**

The pancreatic β-cells are specialized cells responsible for the global insulin secretion in response to elevated glucose levels. Defects in the development or in the homeostasis of β-cells, such as chronically elevated levels of NEFAs during obesity, results in a reduction in insulin content, stunted insulin secretion (non-stimulated and glucose-stimulated) and increased apoptosis [32,33]. miR-375 is essential for β-cell development and function; miR-375 KO mice have a lowered pancreatic α- and β-cell mass, exhibit fasting and non-fasting hyperglycaemia and greatly reduced insulin secretion [34]. Functional studies of miR-375 in cultured insulin-secreting MIN6 and primary β-cells reveal that this miRNA is essential for insulin secretion. Inhibition of miR-375 impairs exocytosis of insulin-containing intracellular vesicles due to aberrant regulation of the miR-375 target myotropin [35]. Interestingly, the levels of miR-375 in pancreatic islets have been found to be reduced in obese BTBR-ob/ob mice as well as in spontaneously diabetic GK rats [36,37], suggesting that miR-375 down-regulation plays a major role in the pathogenesis of T2D in islets. Another candidate miRNA in the pathogenic pathway to β-cell dysfunction.
and T2D is miR-34a. NEFAs induce miR-34a expression, and, correspondingly, its expression levels are elevated in the islets of obese mice [36]. It has been demonstrated that miR-34a is involved in NEFA-induced apoptosis in primary cultured β-cells, probably by inhibition of the anti-apoptotic gene Bcl-2 [38,39]. It was also shown that miR-34a can directly inhibit the translation of VAMP2 (vesicle-associated membrane protein 2), a SNARE (soluble N-ethylmaleimide-sensitive fusion protein-attachment protein receptor) protein involved in exocytosis. VAMP2 inhibition could lead to a lowered insulin secretion by β-cells under high-fat conditions [39]. It is possible that miR-34a overexpression in the islets of obese mice inhibits insulin secretion and participates in the lipotoxic effect induced by ectopic fat accumulation observed in obesity and the metabolic syndrome, leading to defective β-cell function and eventual cell death.

Conclusions

miRNAs are involved in a large number of biological and physiological functions and have been linked to the pathogenesis and pathophysiology of several diseases, including cancer, atherosclerosis, cardiomyopathies and diabetes. Although many studies have shown changes in the pattern of expression of various miRNAs, very few target genes and pathways affected by these changes have been identified. T2D is a complex disease, bringing into play many different organs and conditions contributing to the pathogenesis of this condition. As the present review shows, miRNAs are emerging as major contributors to the development of T2D. The interplay of miRNAs, the genes they control and the different affected organs highlights the complexity of this disease. Further studies are required to identify targets of known variant miRNAs. Refinement of bioinformatics and large-scale gene-validation techniques will hopefully enable researchers to understand T2D and unravel new ways of treating this disease.

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References


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