

MicroRNAs in Diabetes: Tiny Players in Big Disease

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Key Words

MicroRNA • Diabetes • Insulin • Lipid • Glucose • Diabetic complications

Abstract

MicroRNAs (miRNAs) are a novel group of universally present small non-coding RNAs that have been implicated in wide ranging physiological processes and thereby are critical in the manifestation of diverse diseases. Since their discovery as developmental regulators in *C.elegans*, they have come a long way and are currently associated with normal and diverse pathophysiological states including Parkinson's syndrome, cardiac hypertrophy, viral infection, diabetes and several types of cancer. Of special significance is their involvement in diabetes, an area in which several emerging reports point to the fact that these small RNA species could be special and critical in this complex disease and they or their specific inhibitors may be exploited as targets for therapeutic intervention. The stable nature of these miRNAs over mRNAs is an added advantage of them being projected for the same. This review focuses on and discusses the current diabetic epidemic and the potential role(s) of these miRNAs in various

physiological processes that lead to the diabetic phenotype with an objective of better understanding the emerging mechanisms of these small molecules in the development and progression of diabetes and its complications.

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Introduction

MicroRNAs (miRs or miRNAs) comprise a novel group of small (~22 nucleotide) single stranded non-coding RNAs that are ubiquitously present in plants and animals and act in a sequence specific manner to regulate gene expression at the post transcriptional level by cleavage or translational repression of their target mRNAs. *lin-4* and *let-7* were the first identified microRNAs (miRNAs) in *C.elegans* as regulators of the developmental timing in the nematode and subsequently, going by numbers, as many as 866, 350 and 627 microRNAs have been annotated in the human, rat and mouse respectively till date and this collectively has been cataloged in the miRNA

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1015-8987/09/0236-0221\$26.00/0

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registry (<http://microrna.sanger.ac.uk>, V 12.0 September, 2008). In higher eukaryotes, almost 1-3% of the genome codes for miRNA genes and as high as 30% of the genes are believed to be regulated by these small RNA species [1-4]. Primarily these are transcribed as long RNA precursors (referred to as pri-miRNAs) that are processed in the nucleus by the RNase, Drosha, which chops off the stem loop to give rise to the subsequent ~70 nucleotide intermediate, the pre-miRNA. Exportin-5 and Ran-GTP export these small duplex pre-miRNA species into the cytoplasm where they are excised by another enzyme, Dicer, to form the functionally active ~22 nucleotide mature miRNA that becomes part of the RNA induced silencing complex (RISC). In the cytoplasm, one of the strands is eliminated and the other remaining mature miRNA strand, in animals, functions through imperfect base pairing to the 3' untranslated region (3'UTR) of their target mRNAs and subsequently inhibits translational initiation (Figure 1). Interestingly, miRNAs display a spatiotemporal pattern of expression and function and therefore are crucial in the regulation of various biological processes in various stages. It is therefore not surprising that they have been implicated in several diseases like cancer [5-8], cardiovascular diseases [9-11], neurological disorders [12, 13], viral infection [14-16], asthma [17] and diabetes [18-20].

Unlike in the case of cancer, not much insight has been obtained regarding the contribution of microRNAs in diabetes and the picture that stands as of today is just the beginning towards understanding the complex mechanisms that culminate into the disease phenotype and its complications. In the midst of the current diabetic epidemic, this review will discuss in detail the current status of the potential roles that these microRNAs play in the initiation and progression of diabetes and its complications and provide insights into novel mechanism(s) of the disease pathogenesis. Considering the complexity and interplay of several factors that culminate in the manifestation of diabetes, unraveling the roles that microRNAs might contribute in these phenomena offers significant information in the area of diabetes research.

The global epidemic of Diabetes

Diabetes, the deadly global health problem, has reached epidemic proportions and is expected to touch a whopping devastating number of 366 million by the year 2030 [21]. Most of this explosion is predicted to being

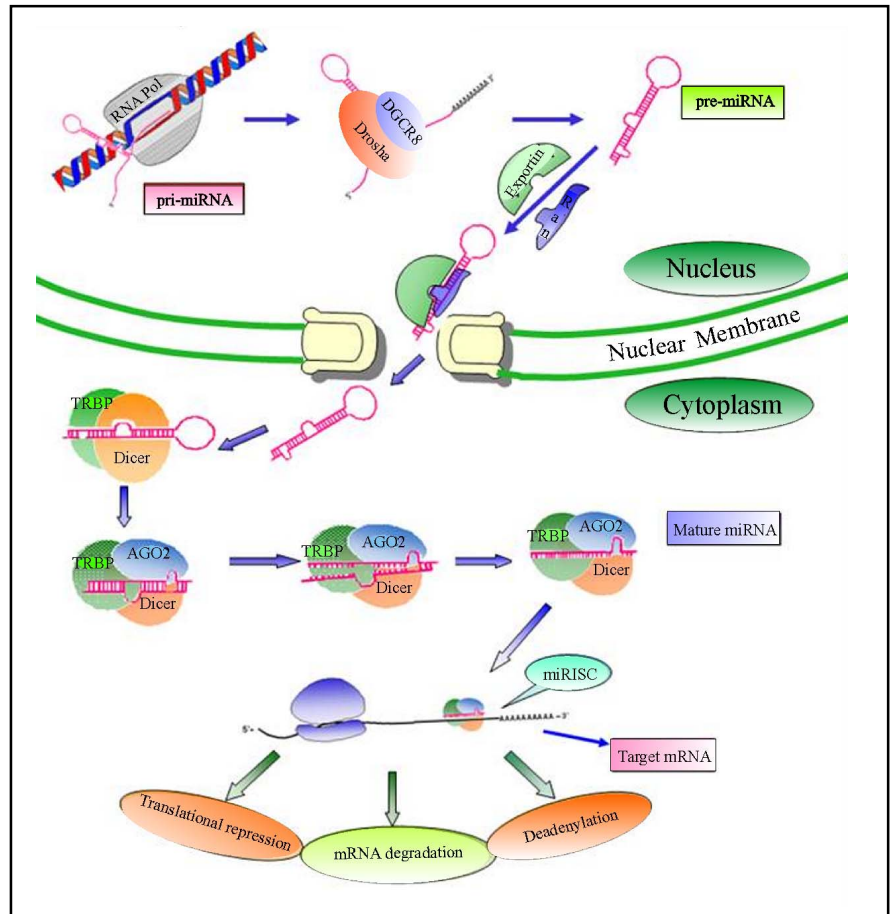
contributed by developing countries, mainly India and China. Rapid changes in urbanization, industrialization and globalization have, on the one hand, opened up new avenues towards increased socio-economic prosperity but on the other, accompanied by an escalating tendency towards physical inactivity and obesity, have gifted us with a plethora of metabolic disorders. Concurrent with the soaring rates of obesity there has been a simultaneous surge in the incidence of insulin resistance and type 2 diabetes at an alarming rate leading researchers to adopt the term “diabesity” to imply obesity associated diabetes. Insulin resistance is a persistent fundamental finding in diabetic patients and it usually manifests itself years before diabetes sets in and is therefore quite often considered a predictor for the onset of diabetes. The term “insulin resistance” refers to resistance to either endogenously produced or exogenously administered insulin mediated glucose uptake in the skeletal muscle and adipocyte and impaired suppression of insulin mediated inhibition of hepatic glucose output; all resulting from impaired insulin signaling in insulin target tissues, mainly the adipose, liver and skeletal muscle. Such consistent insulin resistance also culminates in impaired pancreatic function that adds on to the existing metabolic dysregulation in diabetes.

The discovery of microRNAs and subsequent reports illustrating their role(s) in regulating glucose and lipid metabolism have opened up a novel mode of fine-tuning genes that control diverse facets of metabolic regulation.

MicroRNAs and glucose metabolism

Maintenance of appropriate levels of circulatory glucose levels results from a balance between normal insulin secretion and action. Dysregulation at any step of this fine tuning is responsible for the initiation of Type 1 diabetes and insulin resistance that culminates in Type 2 diabetes. Apart from the various mechanistic regulators of insulin secretion and action, microRNAs have also emerged as novel regulators of these phenomena and hence appropriately referred to as “ribo-regulators of glucose homeostasis” [22]. Along these lines, a major player that emerged as a significant mediator of insulin release and thereby of glucose homeostasis is the pancreatic islet specific microRNA, miR-375. It is one of the earliest microRNAs to be identified as possessing a validated functional role in the pancreas where it negatively regulates glucose-stimulated insulin release in a calcium independent manner and its antagomirs (small

Fig. 1. Micro-RNA biogenesis and mechanism of action. The primary transcripts of miRNA i.e. pri-miRNAs are transcribed usually by RNA Polymerase II from varied genomic locations. The pri-miRNAs are subsequently processed in the nucleus by Droscha, a type III RNase and DGCR8 (DiGeorge syndrome Critical Region gene 8), a double stranded RNA binding protein to give a 60-70 nucleotide long stem-loop structure known as pre-miRNAs. These pre-miRNAs are then exported into the cytoplasm by a Ran-GTP dependent nuclear transport receptor, Exportin 5 where they are further processed into 21-23 nucleotide long miRNA duplex by the type III RNase, Dicer and its interacting partner, TRBP (TAR RNA Binding Protein). One of the strands of this miRNA duplex along with Dicer, TRBP and a member of the Argonaute protein family (AGO2) assembles into the miRNA Induced Silencing Complex (miRISC). Mature miRNAs then regulate gene expression by guiding the miRISC to their target complementary mRNA. Regulation may be mediated by various mechanisms namely repression of translation, mRNA cleavage or deadenylation.



synthetic chemically engineered oligonucleotide that is used to silence endogenous miRNAs) revert back normal insulin secretion [19]. From a set of its specific predicted targets that included Vti1a (t-SNAREs yeast homologue 1A that is critical in insulin vesicle biogenesis and recycling), Mtpn (myotrophin), MAPK14 (p38 mitogen-activated protein kinase), Slc16A2 (monocarboxylic acid transporter member 8) and Mxi1 (Max interacting protein 1 with a role in β -cell differentiation), all with a potential role in β -cell function and insulin secretion, the authors found that overexpression of miR-375 led to significant reduced levels of the Mtpn and Vti1a protein; however transfection with 2'-O-me-375 (2'-O-methyl oligoribonucleotide that inhibits the miRNA) could increase only the levels of Mtpn with no effects on the levels of Vti1a [19]. The 3'UTR of Mtpn harbors a binding site for miR-375 that when bound inhibits Mtpn expression that is withdrawn when the binding site is mutated to reduce the complementarity between the microRNA and the Mtpn mRNA. Functionally Mtpn is involved in modulation of the actin network that affects membrane docking and fusion [18, 22-23]. This strongly correlates to insulin vesicle exocytosis in the pancreas. Additionally

inhibition of Mtpn with siRNA also attenuates insulin release. All these indicate towards a direct sturdy role of miR-375 and its target, myotrophin in insulin release from the pancreas that ultimately determines glucose homeostasis within the body.

Quite interestingly in a later study miR-375 was identified as the most abundant intra-islet miRNA [24]. Recently, miR-375 has also been reported to target PDK1 in the pancreatic islet cell [25]. Its elevated expression in the pancreatic islets of diabetic Goto-Kakizaki rats indicates towards its role in diabetes. Using INS-1E insulinoma cells and rat primary islets, it was observed that miR-375 directly binds to the 3'UTR of PDK1 and inhibits its protein level. PDK1 in the presence of phosphatidylinositides generated by PI3K, activates Akt/PKB. Glucose stimulation of insulin gene expression via PDX-1 also involves the PI3K pathway and a recent experiment in this connection [25] unravels a novel angle of regulation of this pathway wherein miR-375 modulates glucose mediated stimulatory effect on insulin gene expression by targeting PDK1. Earlier it was reported that β -cell specific ablation of PDK1 induces diabetes accompanied with a reduction in β -cell mass [26]. Along

with these reports of the roles of miR-375 in pancreatic function, its presence and significance in the pancreas is also substantiated by the fact that its knockdown is accompanied by severe morphological defects in the pancreatic islets in zebrafish [27]. miR-375, therefore, appears to be the most well studied as far as the regulation of insulin release and glucose homeostasis is concerned.

Another microRNA, miR-9 has been reported as a strong candidate and regulator of insulin exocytosis from the pancreas [28]. The pancreatic exocytotic machinery for insulin involves the participation of several proteins that are under direct and/or indirect control of several factors. Elevated levels of miR-9 inversely correlated with glucose stimulated insulin release. This effect of miR-9 on insulin release was preceded by elevated levels of the Rab GTPase effector, Granuphilin/Slp4 [29-30] and this is regulated by the direct miR-9 target, Onecut2 (OC2) that inhibits the expression of Granuphilin/Slp4. While overexpression of OC2 downregulated Granuphilin transcription, its silencing by RNAi replicated the effects of miR-9 on granuphilin expression and insulin exocytosis. All these indicate miR-9 to be explicitly involved in insulin exocytosis from the pancreas. In a later study, using miRNA microarray, Baroukh et al., 2007 [31] found that miR-124a strongly correlated with mouse pancreatic development suggesting its role in β -cell differentiation. Looking for predicted miR-124a targets, Foxa2 emerged and was subsequently validated as the one with an identified role in pancreatic β -cell development. Overexpression of miR-124a inhibited and anti-miR-124a could withdraw this inhibition on Foxa2 and its downstream target, Pdx-1. Under identical conditions, mRNA levels of other significant regulators of pancreatic development and function namely, Kir-6.2 and Sur-1 also depicted identical patterns of expression though this appears not to be a direct effect. It can be said quite conclusively that such miR-124a regulation of Foxa2 and subsequently of Pdx-1, Kir-6.2 and Sur-1 suggests a central role of this miRNA in the fine-tuning of targets that together comprise a major fraction of determiners of global pancreatic development and function. Very recently, by employing an identical microarray approach, Tang et al. found 61 glucose regulated miRs from a total of 108 miRs in the mouse insulinoma cell line, MIN6 [32]. Of these, most of the miRs were upregulated and only few that included miR-296, miR-184 and miR-160 were downregulated. miR-30d that was significantly elevated here was subsequently found to mediate glucose stimulated insulin gene expression while inhibition of this microRNA attenuated this effect.

miR-30d overexpression or inhibition by itself, however, did not have any effect on insulin gene transcription. The authors thereby suggest that miR-30d and its targets may be potential regulators of insulin gene expression.

A recent report [20] appropriately documents the altered miRNA pattern that contributes to free fatty acid (FFA)-induced pancreatic β -cell dysfunction. Microarray analysis revealed several miRNAs to be altered by palmitate in MIN6B1 cells and after appropriate validation and replication studies, only miR-34a and miR-146 were analysed for their further role in the pancreas. Supportively, their levels were also elevated in the islets of diabetic db/db mice that parallels the elevated plasma free fatty acid concentrations. Looking beyond these alterations, it was found that miR-34a is allied to p53 activation that is an inducer of apoptosis in several diseases [33] and also to Bcl2 inhibition [34], especially in the pancreas they are known to be involved in apoptosis of insulinoma cell lines [35-36]. FFA mediated regulation of p53 via miR-34a, is therefore a novel mode of regulation of pancreatic apoptosis initiated by FFA. Within the islets as well, miR-34a affects hormone secretion by targeting VAMP2, a vesicle protein that is involved in insulin exocytosis. The other miRNA that was identified by Lovis et al., 2008 [20] was miR-146 that acts by targeting IRAK1 and TRAF6, both of which are involved in pancreatic β -cell death [37-40]. These miRNAs, therefore underlie some of the negative effects of free fatty acids on pancreatic function and survival that mimics the state of obesity associated Type 2 diabetes.

Another miRNA highly elevated in the skeletal muscle of diabetic GK rats is miR-29 that has been implicated in inhibition of insulin action. Adenovirus-mediated overexpression of this miRNA in 3T3-L1 adipocytes significantly repressed insulin stimulated glucose uptake that was accompanied by inhibition of insulin stimulated Akt activation. However, the total levels of the Akt protein were not downregulated by miR-29 overexpression indicating that Akt is not the direct target gene of miR-29 and the effects of miR-29 on insulin action could involve other mediators [41]. Another significant intermediate, Insulin Receptor Substrate-1 (IRS-1) is a major mediator of insulin signaling and its mutation or dysfunction has been associated with diabetes [42-44]. Although in a different context, miR-145 has been recently identified to target and downregulate the IRS-1 (Insulin Receptor Substrate 1) protein in human colon cancer cells [45] and this targeting has elaborate effects on the growth and proliferation of these cells. Considering the role that IRS proteins play in insulin signaling and thereby on

glucose homeostasis, it may be worthwhile to undertake in depth studies to unravel the role of this miRNA in insulin action, if any.

MicroRNAs and lipid metabolism

It has now been established beyond doubt that alterations in lipid metabolism contribute to insulin resistance and diabetes. Abnormalities of triglyceride storage and lipolysis in insulin-sensitive tissues are an early manifestation of conditions characterized by insulin resistance. Increased free fatty acid (FFA) flux from adipose tissue to nonadipose tissue, resulting from abnormalities of fat metabolism, participates in and amplifies many of the elementary metabolic derangements that are notable traits of the insulin resistance syndrome and type 2 diabetes [46]. The precise biochemical mechanisms whereby fatty acids and cytosolic triglycerides exert their effects resulting in the diabetic phenotype remain poorly understood. With the discovery of microRNAs and emerging evidences of their regulation of lipid metabolism, a new paradigm that was until now not completely unknown is gradually being exposed. Initial studies in this regard began with the identification of miR-14 as a regulator of fat metabolism in *Drosophila melanogaster* [47]. miR-14 null animals had increased levels of triglycerides and diacylglycerol that reverted back on increasing the copy numbers of the microRNA. Another microRNA involved in energy homeostasis in *Drosophila* is miR-278 [48] and miR-278 mutants in spite of having elevated insulin production capacities depict increased circulatory glucose levels indicating a loss of insulin responsiveness. Being prominently expressed in the fat, its involvement in the regulation of fat metabolism is not really unexpected and of the mRNAs that miR-278 targets, *expanded* was the most noteworthy because of the elevated levels of its mRNA in miR-278 mutants. *Expanded* loss-of-function mutants cause tissue overgrowth [49-50] in parallel with increased miR-278 expression. miR-278 mutants exhibit a lean phenotype indicating towards its involvement in maintaining a balance between fat accumulation and utilization. Around the same time, Esau et al., 2006 [51] revealed the role of the liver specific miR-122 as a significant regulator of hepatic lipid metabolism. In normal mice, inhibition of miR-122 with antisense oligonucleotides led to an increase in hepatic fatty acid oxidation accompanied with a decreased rate of fatty acid and cholesterol synthesis in the liver. More importantly the

circulatory cholesterol levels were also reduced indicating that miR-122 inhibition may be a significant module for lowering plasma cholesterol levels that is elevated in several metabolic diseases. In an obese mouse model miR-122 inhibition not only lowered plasma cholesterol levels but also significantly improved liver steatosis and the status of several hepatic lipogenic enzymes specifically phosphomevalonate kinase. Such a role of miR-122 in the liver is also substantiated by an earlier report [52] wherein the authors have used antagomirs against miR-122 and concluded that genes of the cholesterol biosynthetic pathway are the most affected by miR-122 and *in vivo* antagomir inhibition of this microRNA significantly reduced circulatory cholesterol levels.

The miRNA paralogs, miR-103 and miR-107 have recently been reviewed [53] with a prediction of their involvement in metabolism. Of these absolutely conserved vertebrate intronic miRNAs that exist within the pantothenate kinase (PANK) gene, miR-103 genes encode for two mature miRNAs, miR-103-1 and miR-103-2 while the miR-107 gene encodes miR-107 that differs from miR-103 by a single nucleotide. The host gene, PANK catalyses the rate limiting step of pantothenate phosphorylation during the generation of Coenzyme A (CoA) that is a critical cofactor of several enzymes involved in diverse metabolic pathways. Although there is no experimental report as of now regarding the role of these miRNAs in regulating lipid metabolism; bioinformatics predictions suggest a possible role similar to their host gene, PANK, in regulation of acetyl CoA and lipid metabolism. Such a coordinated and symbiotic function between the host gene and the microRNA is quite expected considering the co-relatable expression patterns between them [54].

When it comes to lipid metabolism, the adipose tissue emerges as the most critical organ and considering the parallel escalating numbers of obesity and diabetes, this tissue has of late surfaced as a significant mediator of this complex disease. It therefore is even more thrilling to study the regulation of adipogenesis by microRNAs and in an effort in this direction, Esau et al., 2004 [55] using antisense oligonucleotides studied the role of miRNAs in adipocyte differentiation. Using a human adipocyte model system, the authors profiled the miRNA pattern between preadipocytes and differentiated adipocytes. From the set of differentially regulated miRNAs, miR-143 was singled out particularly since its elevated expression levels paralleled with adipocyte differentiation and inhibition of miR-143 with an antisense oligonucleotide inhibited the same. While hunting around

for the targets of this microRNA, the authors reported that ERK5/BMK1 could be one of the possible mediators of the link between miR-143 and adipocyte differentiation and it may be involved in maintaining a balance between proliferation and differentiation of adipocytes [55]. Although the authors did not completely dissect out the direct or indirect association between miR-143 and ERK5, they did conclude the possibility of exploiting miR-143 as a potential target for therapeutic intervention for obesity and metabolic diseases.

MicroRNAs and diabetic complications

Almost all forms of diabetes are invariably characterized by end stage specific pathological complications in the renal glomerulus, peripheral nerve and the retina. Another significant long-term diabetic complication is hypertension and heart valve defects which later on manifest as cardiac hypertrophy that is characterized by thickening of the myocardial wall and reduction of the ventricular chambers. Just as microRNAs are critical in the development and progression of diabetes, currently emerging reports also associate altered levels of a range of miRNAs with these diabetic complications.

miR-133 is one of the most abundant microRNAs present in the adult cardiac and skeletal muscle in mammals where they are critical in regulating myogenesis. miR-133 was one of the first miRNAs reported to be overexpressed in the hearts of diabetic rabbits and this was accompanied by a parallel increase in the expression of serum response factor (SRF) [56]. Serum response factor (SRF) is a member of the MADS (MCM1, AGAMOUS, DEFICIENS, SRF) box family of nuclear transcription factors that interacts as a dimer with a 10 bp AT-rich sequence on the DNA known as the serum response element (SRE) [57-58]. It plays an important role in cardiac development and function and regulates the expression of a wide variety of inducible genes by various stimuli ranging from growth factors to changes in intracellular calcium flux [59]. More specifically, SRF has also been implicated in the regulation of genes encoding non-contractile cardiac proteins, including the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2) and the Na/Ca^{2+} exchanger (NCX1) [60-61]. Further support for an important role for SRF in cardiac function comes from transgenic experiments in rodent model systems demonstrating that cardiac-specific dysregulation of SRF expression induces cardiac hypertrophy and cardiomyopathies in postnatal animals and this mimics

those that are observed during the initial development of congestive heart failure, indicating that SRF is involved in cardiac pathogenesis [62-64]. The increase in miR-133 levels in the diabetic heart was also accompanied with a decrease of ERG (*ether-a-go-go* related gene) and I_{kr} (rapid delayed rectifier K^+ current) protein levels. The increase in the levels of SRF in the diabetic heart is invariably accompanied with a prolonged QT (an indicator of the cardiac electrical activity) syndrome, a potentially dangerous situation that may lead to cardiac arrest [65]. All these effects could be reversed using miR-133 specific antisense oligonucleotides. Such a decrease in HERG (human ERG) protein levels possibly is responsible for repolarisation, the observed QT prolongation and the associated arrhythmias in diabetic hearts.

Apart from these, alongwith miR-1, miR-133 also determines the pathogenesis of cardiac hypertrophy where both these miRs are significantly downregulated. In a rat model of cardiac hypertrophy, hyperpolarisation-activated cyclic nucleotide gated channels encoded by HCN2 and HCN4 were considerably increased accompanied by significant reductions of miR-1/miR-133 levels [66]. HCN channels generate I_p (hyperpolarisation activated current) which contributes to the genesis of the cardiac pacemaker activity. Four different HCN genes have been identified [67]. HCN1 is the most rapidly acting channel, HCN4 the slowest with HCN2 and 3 possessing intermediate kinetics [68]. HCN4 is the most highly expressed in the SA (sino-auricular) node and HCN2 expression is prominent in the atrium, ventricle and SA node. Overexpression of miR-133/miR-1 significantly inhibited the increase in HCN2 and HCN4 levels. Elevated levels of HCN2 and 4 are hallmarks of arrhythmia [69-70] and subsequently the hyperpolarisation activated current (I_p) is strikingly increased in animal models of cardiac hypertrophy and heart failure [71-73]. In fact, miR-1 has been shown to be overexpressed in individuals with coronary heart disease (CAD) and its targeted overexpression in normal hearts manifests an identical phenotype associated with arrhythmias [74]. Mechanistically this microRNA targets KCNJ2 (that encodes the K^+ channel subunit Kir2.1) and GJA1 (encoding connexin 43) that consequently slows down the cardiac conduction resulting in depolarization of the cytoplasmic membrane. All these effects could be attenuated in the presence of an antisense miR-1 inhibitor thereby indicating a role of this microRNA in cardiac physiology.

A very recent article on miRNA related myocardial dysfunction as observed in diabetes was reported by Wang

et al. [75]. Abnormal expression and signaling of many angiogenic factors are some of the many impaired parameters of diabetes and several cardiovascular diseases correlate to insufficient myocardial angiogenesis that is mediated by these abnormal angiogenic factors. By employing a miRNA microarray, these authors report that of all the miRs altered in diabetic myocardial microvascular endothelial cells (MMVECs) as compared to normal MMVECs, miR-320 emerged as a potential mediator with a predicted target list that includes several angiogenic factors and their receptors namely VEGF, IGF-1, IGF-1R and FGF [75] that are significant mediators of diabetic cardiomyopathy [76-77]. This revelation by Wang et al. [75] of elevated levels of miR-320 in diabetic MMVECs was also accompanied by decreased proliferation and migration rates that amazingly reverted back in the presence of the miR-320 inhibitor. Such a correlation between elevated levels of miR-320 and decreased IGF-1 and IGF-1R levels possibly underlies impaired angiogenesis in diabetes. All these indicate that although the current literature regarding these aspects is at a very nascent stage, miRs are critical in the proper functioning of the heart and thereby implicated in cardiac pathophysiology.

A very significant diabetic complication is that of the kidney where the membrane of the glomerulus shows extreme thickening and gets hypertrophied [78] possibly due to accumulation of extracellular matrix (ECM) proteins namely collagen 1 α 1 and 2. The ECM proteins are an integral part of the capillary basement membrane and mesangial matrix and they majorly include various types of collagens, laminin, fibronectin, and proteoglycans [79]. A very strong underlying factor behind the accumulation of these ECM proteins as is observed in a diabetic kidney is the transforming growth factor β 1 (TGF- β). The diversely bared and still being discovered regulatory roles of microRNAs in the pathogenesis of various diseases and the almost exclusive presence of at least five microRNAs in the kidney [80] undoubtedly indicate towards their involvement in kidney function and disease. A recent article has depicted the role of miR-192 in the kidney and in the pathogenesis of diabetic nephropathy [81]. Using microarray analysis, it was found that collagen 1 α 1 mRNA is increased by TGF β in mouse mesangial cells with a concomitant decrease in the mRNA levels of E-box repressors, δ EF-1 and Smad-interacting protein 1 (SIP1). While looking for the possible roles of miRs in these phenomena, the authors found that miR-192 levels were elevated by TGF β in these cells and interestingly, SIP1 is a validated target of miR-192 [81].

Both SIP1 and δ EF-1 are repressors of Coll1 α 2 expression and this repression is withdrawn under diabetic conditions initiated by TGF β . Since TGF β elevates the levels of miR-192 and downregulates SIP1, a target of miR-192, the authors concluded that the observed increases in collagen synthesis and accumulation observed in a diabetic kidney was due to the elimination of the SIP1 repression of collagen expression due to increased miR-192 levels. Such correlations were also observed in a streptozotocin induced diabetic mouse model as well as transgenic db/db diabetic mice. Kidney glomeruli in both these induced and transgenic diabetic species depicted elevated levels of TGF- β 1, collagen 1 and 2 and miR-192. All these observations suggest that small non-coding miRs, in this case miR-192 and their inhibitors, could possibly be targets of diabetic nephropathy and other associated diabetic complications.

Another matrix protein that is excessively accumulated in the diabetic kidney is fibronectin. Fibronectin, a large glycoprotein consisting of two similar polypeptide chains, is a key component of the mesangial matrix [82]. It may exist in a soluble dimeric form or as oligomers of fibronectin or a highly insoluble fibrillar form in the extracellular matrix. The latter form has been shown to modulate various biological processes such as cell adhesion, migration, and differentiation [83]. In a recent article, Wang et al., [84] reported that in cultured human and mouse mesangial cells exposed to high glucose and transforming growth factor β as well as in a mouse diabetic nephropathic model, miR-377 was consistently upregulated. In a computational study, fibronectin did not emerge as a direct predicted target of miR-377 but two proteins namely p21-activated kinase and superoxide dismutase, which enhanced fibronectin production surfaced as miR-377 targets. Experimentally too, an increase of miR-377 led to reduced levels of these two proteins. So, although indirectly, elevated levels of miR-377 in turn increases fibronectin levels that accumulate in the kidney matrix and this emerges as phenotype of diabetic nephropathy.

Basically, four main hypotheses are associated with hyperglycemia induced diabetic complications, namely increased polyol pathway flux, increased advanced glycation end product (AGE) formation, activation of protein kinase C (PKC), and increased hexosamine pathway flux [85]. Advanced glycation end products (AGEs) act via their receptors (RAGEs) and interact and modify several intracellular proteins and other extracellular matrix components that then depict altered functions. Another well studied ligand that interacts with

RAGE is the proinflammatory peptide, S100b that belongs to the S100/calgranulin family [86-87] and these interactions are critical in inflammation and diabetic atherosclerosis [87-88]. Mechanistically along these lines, binding of S100b to its receptor significantly inhibited the expression of miR-16 in human THP-1 monocytic cells that consequently altered the mRNA stability of the inflammatory gene, cyclooxygenase-2 (COX-2) by binding to its 3'UTR [89]. An interesting protein that modulates this interaction is the heterogeneous nuclear ribonuclear protein K (hnRNPK) that binds to the COX-2 promoter in the nucleus. Exposure to S100b mimics the diabetic milieu and this displaces the nuclear hnRNPK that translocates to the cytoplasm and interacts with the 3'UTR and prevents the binding of miR-16. Such an intracellular crosstalk between microRNAs and RNA binding proteins may underlie the acute regulation of diverse genes particularly those related to inflammation under diabetic conditions. This attains significance considering the fact that studies over the couple of years has revealed a strong association of diabetes and inflammation.

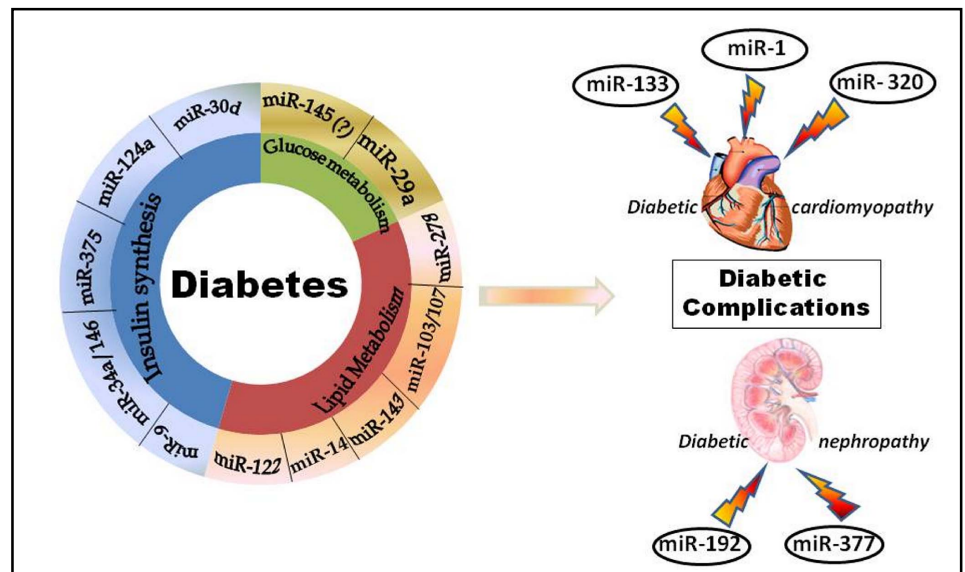
The Clinical Perspective

The discovery of miRNAs and revelation of their involvement in diverse facets of diabetes manifestation has modernized the otherwise traditional way in which researchers have been focusing on until the exciting world of miRNAs opened up. For a disease as complex as diabetes, identification of specific clinical biomarkers has invariably been a challenge towards revolutionizing the prognosis and diagnosis of this chronic metabolic disease. There has always been a hunt for potential and sensitive clinical biomarkers that could be exploited to detect the development and progression of diabetes at an early stage and at least delay if not inhibit the onset of late stage diabetes. Recent studies have increasingly shown the presence of RNA in the serum [90-92] although its stability has always been of extreme concern to researchers of biomarker discovery. The discovery of miRNAs has been a breakthrough in this regard considering their remarkable stability and presence in huge amounts in the serum and plasma [93]. These qualities may be exploited through various practicable detection methods to monitor the progression of insulin resistance and diabetes. In fact, this aspect of miRNAs has been touched upon and a very recent preliminary work has been published by Chen et al. [93] where the authors found an altered serum profile of miRNA expression in diabetic patients that

constitutes the diseased signature of potential first class biomarkers for diagnosis of diabetes. Circulatory miRNAs are resistant to endogenous RNase activity and enter the circulation from diseased tissues [94] and their unique aberrant expression profile that is encountered in diabetes as described earlier are indicative of the possible future use of miRNAs as new strategies for targeting diabetes.

Apart from taking advantage of circulating miRNAs as potential biomarkers for identification of the precise stage of insulin resistance and diabetes, the altered expression profiles of miRNAs in the pancreas and insulin target tissues in diabetes could be probable targets for treating this complex disease. Overexpression or inhibition of specific miRNAs could be the future tool for targeting diabetes [95]. The most significant challenge in this regard appears to be the delivery of appropriate sequence specific molecules to precise targeted sites and to address issues regarding the potential non-specific effects on non-targets [2]. Relatively at a stage of infancy, there are certain reports that detail the role(s) of varied delivery agents for delivering miRNAs and siRNAs (short interfering RNAs) *in vivo*. While adenoviruses and lentiviruses have been successfully used to deliver siRNA in *in vivo* and *in vitro* systems [96-99], this approach was effectively employed to deliver miR-122 and its antagomirs into mice followed by their *in vivo* analyses [52]. The antagomir was quite effective in removing miR-122 and this effect could be maintained for as long as 23 days with a concomitant decrease of serum cholesterol levels by almost 44 percent. Several lipid based delivery agents are also shaping up as excellent delivery agents of these small RNA species. Wolfrum et al. [100] while taking advantage of this, have successfully delivered siRNA conjugates (with high and low-density lipoproteins) to precise targets including the liver, gut and kidney. However, the fact that on an average one microRNA targets several genes adds additional levels of complexity to the clinical perspective as far as miRNAs are concerned. This automatically implies that the concept of specificity of miRNA therapy needs to be given a thought. Since miRNAs exhibit a spatiotemporal pattern of expression and action, their use as a therapeutic agent calls for, if need be, development of appropriate strategies based on these unique characteristics. The field of miRNAs still has a long way to go and several more miRNAs and their functions remain to be decoded. Yet they assure of a high potential that their field of targeted delivery, amidst some practical hurdles, holds for future investigation with a promise towards a powerful clinical breakthrough in diabetes therapy.

Fig. 2. Summarized features of the known role(s) of miRNAs in cellular functions. Various miRs have been identified to be critical in diverse metabolic processes, dysregulations of which lead to diabetes and its complications. miR-145: miR-145 has not been reported in connection with diabetes but since it targets IRS-1, it may be a possible candidate to be investigated in this regard.



Conclusions

MicroRNAs belong to a recently identified class of small non coding RNAs that have been widely implicated in the fine-tuning of several physiological processes and thereby associated with the pathogenesis of several diseases. Although there are several microRNAs and their predicted targets that have been indexed in the miR database, only few have been validated. Considering that each miR can target several genes and each gene can also be regulated by several miRs [4], the regulatory story of miRs becomes even more complicated. The pathogenesis of a disease as complex as diabetes adds on to the complexity of these studies. Yet emerging evidences suggest that miRs play significant roles in insulin production, action and secretion and also in diverse aspects of glucose and lipid metabolism (Figure 2). Most importantly, microarray studies have highlighted an altered profile of microRNA expression in insulin target tissues in *in vitro* and *in vivo* diabetic models. All these indicate that microRNAs are critical in the pathogenesis and progression of diabetes and its complications and appropriate therapeutic intervention targeted towards their altered levels may offer novel valuable tools for the treatment of a metabolic disease as complicated as diabetes.

Abbreviations

MTPN (Myotrophin); PDK1 (Pyruvate Dehydrogenase Kinase, isozyme 1); OC2 (One Cut homeobox 2); Foxa2 (Forkhead Box A2); IRAK1 (Interleukin-1 Receptor-

Associated Kinase 1); TRAF6 (TNF Receptor Associated Factor 6); SRF (Serum Response Factor); HERG (human ether-a-go-go-related potassium channel protein); ERK5 (mitogen-activated protein kinase); HCN (Hyperpolarization Activated Cyclic Nucleotide-Gated Potassium Channel); KCNJ2 (Potassium inwardly-rectifying channel, subfamily J, member 2); GJA1 (Gap Junction Protein, alpha 1); IGF1 (Insulin-like Growth Factor 1); SIP1 (Survival of motor neuron protein interacting Protein 1); SOD (Superoxide Dismutase); COX2 (Cyclooxygenase 2); Akt (v-akt murine thymoma viral oncogene homolog); Pdx1 (Pancreatic and Duodenal Homeobox 1); PANK1 (Pantothenate kinase 1); PI3K (Phosphatidylinositide 3-Kinase); VEGF (Vascular Endothelial Growth Factor Precursor); IGF-1R (Insulin-like Growth Factor 1 Receptor); FGF (Fibroblast Growth Receptor).

Acknowledgements

We are thankful to Professor Samir K. Brahmachari for his constant inspiration and all the necessary support. We are grateful to Drs. Munia Ganguli and Vibha Taneja for giving their valuable inputs that have been very helpful in enhancing the scientific clarity of this article. We sincerely acknowledge the help of Mr. Saurabh Vig for his help in making the illustrations associated with this article. AKP and PA acknowledge the financial support from the Council of Scientific and Industrial Research (CSIR), New Delhi, India (NWP 0036) and KK is thankful to the University Grants Commission (UGC), New Delhi, India for financial assistance.

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