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MicroRNAs and Diabetic Complications

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Abstract

Both Type 1 and Type 2 diabetes can lead to debilitating microvascular complications such as retinopathy, nephropathy and neuropathy, as well as macrovascular complications such as cardiovascular diseases including atherosclerosis and hypertension. Diabetic complications have been attributed to several contributing factors such as hyperglycemia, hyperlipidemia, advanced glycation end products, growth factors and inflammatory cytokines/chemokines. However, current therapies are not fully efficacious and hence there is an imperative need for a better understanding of the molecular mechanisms underlying diabetic complications in order to identify newer therapeutic targets. microRNAs (miRNAs) are short non-coding RNAs that repress target gene expression via post-transcriptional mechanisms. Emerging evidence shows that they have diverse cellular and biological functions and play key roles in several diseases. In this review, we explore the role of miRNAs in the pathology of diabetic complications and also discuss the potential use of miRNAs as novel diagnostic and therapeutic targets for diabetic complications.

Diabetic Complications

The prevalence of diabetes in the United States is increasing at a rapid rate and current estimates from the American Diabetes Association reveal that approximately 8.3% of the population have diabetes. Furthermore, nearly 79 million people have signs of insulin resistance and pre-diabetes which suggest an increased risk for future diabetes development, while nearly 1.9 million new cases of diabetes were reported in people aged 20 years and older in 2010 alone (American Diabetes Association, http://www.diabetes.org/diabetes-basics/diabetes-statistics/).

Diabetes, defined by elevated fasting blood sugar levels, increases the risk for many serious health problems. Type 1 and Type 2 diabetes can have devastating effects on the vasculature leading to microvascular complications such as retinopathy (that can lead to blindness), nephropathy (that can result in end stage renal disease or renal failure) and painful neuropathy (that can lead to amputations). Diabetes is also a leading cause of macrovascular complications usually associated with cardiovascular diseases such as coronary artery disease, atherosclerosis, hypertension and stroke (1,2). Furthermore, microvascular complications like diabetic nephropathy are closely associated with accelerated rates of cardiovascular disease and atherosclerosis. The increased incidence of these complications in the diabetic population has been attributed to several pathological factors such as hyperglycemia, hyperlipidemia, advanced glycation end products (AGEs), growth factors and inflammatory cytokines/chemokines (3,4). Most diabetic complications, if left untreated, can be life threatening and greatly reduce the quality of life. Furthermore, in some patients, complications seem to progress despite glycemic control, a phenomenon termed metabolic memory (5,6). Hence there is an imperative need for a better understanding of the molecular mechanisms underlying the accelerated rates of complications under diabetic conditions in

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order to develop more effective therapies. Here we discuss the emerging role of microRNAs as new players in the development of various diabetic complications.

MicroRNAs

MicroRNAs (miRNAs) are endogenously produced short non-coding RNAs of about 20–22 nucleotides that have been shown to play an important role in modulating mammalian gene expression and therefore regulate several key cellular functions (7–12). miRNAs were discovered in the early 1990s in a nematode, *Caenorhabditis elegans*, and the first identified miRNA, lin-4 was found to play a critical role in controlling developmental timing by downregulating its target, lin-14, due to the antisense complementarity (13,14). miRNAs can inhibit the expression of their target genes via post-transcriptional mechanisms (9,15,16). It is estimated that there are over 1000 human miRNAs and that about 60% of the human protein-coding genes can be targeted by miRNAs (9) and thus these small molecules can have profound effects on the functions of numerous proteins. Although miRNAs were discovered only about 20 years ago, they have made an enormous impact in our understanding of gene regulation at the post-transcriptional level, and numerous studies have now documented their roles in cellular functions such as differentiation, growth, proliferation and apoptosis.

miRNAs are transcribed by RNA Polymerase II into primary transcripts (Pri-miRNAs) in the nucleus. These Pri-miRNAs are relatively long (up to several kilo bases) and may contain one or more hairpin-like structures. A microprocessor complex consisting of the ribonuclease (RNase III) endonuclease, Drosha, and its binding partner, DGCR8, bind to the hairpin structures in Pri-miRNAs and process them to precursor miRNAs (Pre-miRNAs), which are about ~ 70-nucleotides and have a stem loop structure (15,17). Pre-miRNAs are then exported to the cytoplasm by Exportin 5 where they are recognized and cleaved by another RNase III enzyme, Dicer, in collaboration with TAR RNA binding protein (TRBP) to generate the mature miRNA duplex comprising ~22 nucleotides (15,17). One strand of the duplex is selected to be loaded into the RNA-induced silencing complex (RISC), while the other gets degraded. RISC is a multiprotein complex that contains Argonaute proteins and the mature miRNA which guide the RISC to recognize the target mRNAs through sequence complementarity. Depending on the complementarity, the target mRNA is either cleaved (perfect complementarity) or its translation is inhibited (imperfect complementarity to the 3′UTRs) or destined for degradation in processing bodies (P-bodies) (12,15,17).

Since miRNAs can modulate key physiological processes and pathophysiological disease states, there is an imperative need to determine the identity of the miRNAs and their targets associated with diabetic complications as this would provide a new window of opportunity to identify new biomarkers and therapeutic targets. While several studies have shown that miRNAs may play a role in diabetes itself and in vascular complications unrelated to diabetes (18–22), this review is focused primarily on miRNAs related to diabetic complications (especially diabetic retinopathy, diabetic nephropathy and cardiovascular diseases).

MicroRNAs in Diabetes Complications

Diabetic Retinopathy (DR)

DR is a very common microvascular complication of diabetes and also one of the leading causes of blindness in the United States (23,24). A few recent studies have demonstrated the role of miRNAs in DR. Kovacs et al. performed the first in-depth miRNA-expression profiling analysis of miRNAs in the retina and retinal endothelial cells (RECs) from a Streptozotocin (STZ) induced diabetic rat model 3 months post diabetes onset (25). Among

the differentially expressed miRNAs, interestingly they found that key NF-kB responsive miRNAs (such as miR-146, miR-155, miR-132 and miR-21) were upregulated in the diabetic RECs, and also that key vascular endothelial growth factor (VEGF) responsive miRNAs and the p53-responsive miR-34 family were upregulated in both the retinas and RECs of the diabetic rats. Furthermore, due to the negative feedback regulation of miR-146 on NF-kB activation in endothelial cells, they suggest that miR-146 could serve as a potential therapeutic target for DR through NF-kB inhibition. In a more recent study, Feng et al. reported that miR-146a is decreased in endothelial cells treated with HG and also in retinas from diabetic animals (STZ injected rats and db/db mice) (26). Furthermore, they demonstrated that under these conditions the expression of fibronectin (a miR-146a target that can contribute to hypertrophy and fibrosis) was increased due to decreases in miR-146a levels, which in turn was mediated by increases in the co-activator p300. In another study, McArthur et al. reported that miR-200b was downregulated in endothelial cells treated with high glucose (HG) and in retinas of STZ-induced diabetic rats (27). They also validated VEGF as a direct target. miRNA-mimic treatments in vitro in endothelial cells, or in vivo (intravitreal injection) could ameliorate diabetes induced increases in VEGF mRNA and protein levels. Conversely, miR-200b antagomirs could increase VEGF production, thus providing further understanding of the role of this miRNA in the pathogenesis of DR. Silva et al. examined the role of miR-29b and its potential target RAX (an activator of the pro-apoptotic PKR signaling pathway), in the apoptosis of retinal neurons related to the pathogenesis of DR (28). They observed that miR-29b and RAX were localized in the retinal ganglion cells and the cells of the inner nuclear layer of the retinas from normal and STZ-induced diabetic rats. Their results suggest that miR-29b upregulation at the early stages of diabetes in this model may have protective effects against apoptosis of the retinal cells by the PKR pathway. Intravitreal injections of key miRNAs such as miR-29b and miR-200b could be developed as translational approaches for the treatment of DR.

Diabetic Nephropathy (DN)

Several studies to date have demonstrated a role for miRNAs in diabetic nephropathy (DN), a severe microvascular complication that can lead to end-stage renal disease and painful dialysis. Increased expansion (hypertrophy) and accumulation of extracellular matrix (ECM) proteins such as collagen (fibrosis) in the glomerular mesangium along with glomerular podocyte dysfunction are major features of DN (29,30). DN is associated with increased expression of transforming growth factor-β 1 (TGF-β) which is a potent inducer of these fibrotic events and renal dysfunction (30–33). We observed that a group of miRNAs (miR-192, miR-200b/c, miR-216a and miR-217) were increased in mouse renal mesangial cells (MMC) treated with TGF-β, and in glomeruli of mouse models of diabetes [type 1(STZ-induced) and type2 (db/db)] (31,34–36). Our studies showed that miR-192 regulates Collagen type I alpha2 (Col1a2) and Col4a1 genes as well as downstream miRNAs, miR-216a/217 and miR-200b/c, by targeting the E-box repressor, Zeb1/2. In addition, we found that the miR-216a/miR-217 cluster activates Akt kinase by targeting Pten in MMC treated with TGF-β and induces hypertrophy in MMC (36). miR-216a also regulates Col1a2 gene through mechanisms involving inhibition of the RNA binding protein Ybx1 (34). Recently we found that miR-200b/c are also involved in collagen expression and TGF-β auto-regulation by targeting Zeb1 in MMC (35). Together these cascades of miRNAs are initiated from miR-192 which is up-regulated by TGF-β and under diabetic conditions in the mesangium, and contribute to major features of DN such as glomerular hypertrophy and ECM accumulation via key signaling and gene regulation mechanisms (35–37).

Other miRNAs have also been implicated in renal fibrosis associated with DN. miR-377 was found to induce fibronectin (ECM protein) expression in MCs *via* downregulation of manganese superoxide dismutase and p21-activated kinase (38). In this study, the authors

also found that HG treatment of the MCs increased the expression of miR-192. Wang et al. reported that miR-192 levels were also increased in kidneys of STZ-injected type 1 diabetic mice fed with a high fat diet and this was accelerated in FXR knockout mice (39). miR-93 levels were reported by Long et al. to be lower in glomeruli of diabetic db/db mice compared to control mice, and also in HG treated podocytes and renal microvascular endothelial cells (40). The parallel increase in the expression of VEGF-A suggested that it was a direct target of miR-93. More recent work by these authors showed that miR-29c as well as miR-192 and miR-200b were upregulated in glomeruli from db/db mice, and in endothelial cells and podocytes treated with HG (41). Using extensive in vitro and in vivo approaches, they demonstrated that the decrease in miR-29c activates Rho kinase by targeting Spry1 and contributes to DN (enhanced ECM accumulation and podocyte apoptosis). On the other hand, recently Wang et al. reported that members of the miR-29 family were downregulated, along with increases in collagens I, III, IV(validated miR-29 family targets), in diabetic ApoE−/− mice kidneys and in proximal tubule epithelial cells, podocytes and MC treated with TGF-β (42). The differences in these two studies with miR-29 were attributed to the different animal models of diabetes used and types of cell stimulations. In addition, a report suggested that decreased miR-192 levels was associated with severity of DN and fibrosis in diabetic patients, although normal levels of miR-192 in healthy kidneys were not provided(43). miR-192 also regulates E-cadherin gene expression in tubular epithelial cells through Zeb1/2 and Wang et al. reported decreased levels of miR-192 in diabetic ApoE−/− mice as well as in TGF-β treated tubular and other renal cells (44). It therefore appears that the nature of the animal models used as well renal cell-specific responses could play a key role in the regulation of miRNAs in the kidney. In another study, Wang et al. showed that miR-200a targets TGF-β2 in cultured proximal-tubular epithelial cells, creating another circuit in the TGF-β response since TGF-β increased TGF-β2 expression via decreases in miR-200a in these cells (45). miR-21 has attracted a lot of attention and Dey et al. reported that miR-21 is increased in the renal cortex of the OVE26 type 1 diabetic mice and activates mTOR signaling related to DN pathogenesis by targeting Pten (46). On the other hand, another study found that miR-21 levels were decreased in db/db mice and that its over-expression could inhibit the proliferation of MC and decrease the 24 hour urine albumin excretion rate in the diabetic mice (47). miR-25 was also reported to be decreased in diabetic rat kidneys and in MC treated with HG (48). These authors found that miR-25 targets Nox4 which has been shown to promote oxidant stress associated with the pathogenesis of DN. Thus, decrease of miR-25 can induce Nox4 and contribute to the development of DN in rats. Taken together, several miRNAs have now been identified that may promote or inhibit the progression of DN. As discussed below, these miRNAs could be evaluated as potential therapeutic targets for DN in the future.

Limb Ischemia

Limb ischemia due to poor circulation and endothelial dysfunction is another complication of diabetes. Caporali et al. found that miR-503 expression levels were upregulated in endothelial cells (ECs) under HG culture conditions and ischemia associated cell starvation. Blocking miR-503 by either decoy or antisense oligonucleotides (oligos) improved functional capacities of ECs in vitro (49). They validated CCNE1 and cdc25A as direct targets of miR-503 whose levels were downregulated in HG conditions. The expression levels of miR-503 were also increased in ischemic limb muscles of STZ diabetic mice. Furthermore, the delivery of an adenovirus based miR-503 decoy to the ischemic muscles of diabetic mice in vivo corrected the impairment of post-ischemic angiogenesis in these mice. Notably, muscle samples obtained from human diabetic patients had 0000000000000000elevated levels of miR-503 levels that were inversely correlated with cdc25 protein levels. This data suggest that miR-503 could be a potential therapeutic target for diabetic patients affected with critical limb ischemia.

Diabetic Cardiovascular Complications and Cardiomyopathy

Evidence shows that hyperglycemia leads to aberrant cell signaling by activating several inflammatory and fibrotic pathways in vascular cells that can lead to increased cardiovascular complications which include coronary arterial disease (CAD), stroke, hypertension, and atherosclerosis (50–53). In addition, diabetic cardiomyopathy is a complication that can lead to heart failure. Shan et al. have shown that HG treated rat neonatal cardiomyocytes and rat myocardium itself have increased levels of miR-1 and miR-206 through modulating serum response factor (SRF) and the MEK1/2 pathway (54). They also demonstrated that miR-1/miR-206 negatively regulate heat shock protein-60 (Hsp60), thereby contributing to the HG mediated apoptosis in cardiomyocytes. Katare et al. showed that miR-1 was increased in STZ-injected type 1 diabetic mice during the progression of cardiomyopathy and could target Pim-1 which has anti-apoptotic properties (55). In addition, anti-miR-1 upregulated Pim-1 and promoted cardiomyocyte survival under HG conditions. Wang et al. examined the expression of miRNAs in normal and diabetic myocardial microvascular endothelial cells (MMVEC) from type 2 diabetic GK rats and examined their role in impaired angiogenesis associated with diabetic ischemic cardiovascular disease (56). They found that miR-320 is upregulated in the MMVEC from GK rats and is associated with impaired angiogenesis while miR-320 inhibitor improved the angiogenesis activity in vitro and also increased levels of IGF-1 protein. Hence key miRNAs such as miR-320 can be targeted as novel therapeutic approaches role for impaired angiogenesis in diabetes. Care et al. observed decreased expression levels of miR-133 in mouse models of cardiac hypertrophy and human myocardial hypertrophy (57). They also demonstrated that miR-133 targets RhoA (a GDP-GTP exchange protein) and Cdc42 (a signal transduction kinase) regulating cardiac hypertrophy. Inhibition of miR-133 in vitro by decoy sequences or in vivo by infusion of antagomirs led to marked and sustained cardiac hypertrophy while overexpression inhibited cardiac hypertrophy. Feng et al. also showed that miR-133a was decreased in a model of cardiomyocyte hypertrophy in STZ injected diabetic mice and this was related to hypertrophy since it could target key growth related genes MEF2A/C, IGF-1R, SGFK1 (58). In another evaluation of diabetic cardiomyocyte hypertrophy, miR-373 was reported to be decreased in the hearts of STZ treated diabetic mice and in rat cardiomyocytes treated with HG, and could target MEF2C (59). miR-373 was also downregulated by HG via p38 mitogen activated protein kinase (MAPK) signaling. Recently, Greco et al. reported a dysregulation of several miRNAs (miR-34b/c, miR-199b, miR-210, miR-650and miR-223) in left ventricle biopsies obtained from diabetic heart failure patients relative to non-diabetic heart failure, while miR-216a was increased in both groups (60). Bioinformatic analyes of the predicted targets of these miRNAs showed enrichment of heart failure and cardiac dysfunction related genes. Studies from various groups have demonstrated the role of key miRNAs such as miR-29, miR-30 and miR-21 in cardiac fibrosis related to myocardial infarction and heart failure due their regulation of fibrotic genes such as collagens and connective tissue growth factor (61–63). It is possible that these miRNAs may also be involved in diabetic heart disease.

Several diabetic vascular complications including atherosclerosis have been associated with enhanced inflammation. Increased expression of inflammatory genes in vascular cells and inflammatory cells like monocytes has been observed under diabetic conditions in vitro and in vivo, and recent studies have also suggested an involvement of epigenetic mechanisms (5,6,64). Some reports have demonstrated a role for miRNAs in regulating inflammatory genes under diabetic conditions. Shanmugam et al. demonstrated the distinct roles of heterogeneous nuclear ribonuclear protein K (hnRNPK) and miR-16 in RNA stability of the proinflammatory cyclo-oxygenase (COX-2) gene in monocytes. Based on several lines of experimental evidence, they reported that miR-16 can bind to the 3′UTR of COX-2 to destabilize it, while S100b, a proinflammatory ligand for the receptor for advanced glycation

end products (RAGE), could enhance COX-2 expression by downregulating miR-16 levels. These studies from our group also showed a cross-talk between the DNA/RNA binding protein hnRNPK and miR-16 (65). Additional studies from our group examined the role of miRNAs in the increased inflammatory gene expression and dysfunctional behavior of vascular smooth muscle cells (VSMC) derived from type 2 diabetic db/db mice relative to those cultured from genetic control db/+ mice (66,67). There was a significant increase in the levels of miR-125b in the diabetic db/db VSMC compared to db/+. Interestingly, miR-125b could target and downregulate Suv39h1, a histone methyltransferase that can mediate epigenetic histone H3-lysine-9 trimethylation (H3K9me3), a chromatin mark associated with repressed genes. Along with the increases in miR-125b levels, there was a reciprocal decrease of Suv39h1 protein levels in the db/db VSMC as well as aortas of the db/db mice relative to control db/+ mice. Chromatin immunporecipitation assays showed a parallel decrease in histone H3K9me3 levels at the promoters of key inflammatory genes interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1). Furthermore, transfection of miR-125b mimics into normal db/+ MVSMC conferred a diabetic phenotype of decreased SUV39h1 and H3K9me3, increased expression of MCP-1 and IL-6, as well as enhanced monocyte binding. These studies reveal a role for miR-125b in the epigenetic regulation of inflammatory genes in diabetic db/db mice. They also reveal a novel cross-talk between miRNAs and epigenetic histone lysine methylation that can lead to the de-repression of pathological genes under diabetic conditions and thereby contribute to the progression of diabetic complications (67). In another recent study, we showed that miR-200b levels were also increased in the VSMC from type 2 diabetic db/db mice and could enhance the expression of inflammatory genes by also targeting and inhibiting a transcriptional repressor Zeb1, which is a negative regulator of certain inflammatory genes through E-boxes in their promoters (68). A recent report used small RNA sequencing strategies and expression profiling to identify Angiotensin II-regulated miRNAs in VSMC and demonstrated their roles in signaling and vascular dysfunction (69).

Relations between miRNAs, genetics and epigenetics of diabetic complications

Complications like DN appear to have some element of genetic predisposition and numerous efforts including Genome Wide Association Studies (GWAS) in specific clinical cohorts have led to the identification of some candidate genes and sequence variants (70). However, the functional and pathological role(s) of these genes and variants have not yet been clearly established and hence the specific involvement of inherited susceptibility seems more complicated or rather small (70). Recent studies have suggested that epigenetics may also play a significant role (5,6). Given that miRNAs have epigenetic mechanisms of action, they should also be considered in the equation. Importantly, there could be novel cross talk mechanisms between these multilayers consisting of miRNAs, epigenetics and genetics. As indicated earlier, genes with epigenetic functions in chromatin may be directly targeted and downregulated by miRNAs and this could affect chromatin remodeling and gene expression. miR-125b was upregulated in VSMC of diabetic mice relative to control mice, and could target the chromatin histone H3K9 methyltransferase Suv39h1 leading to increased expression of inflammatory gene expression in the diabetic cells (66,67). Thus, by downregulating key cellular repressive factors, miRNAs can act as riboregulators to promote epigenetic de-repression mechanisms in the chromatin which lead to the induction of genes associated with the pathology of diabetic complications.

While several single nucleotide polymorphisms (SNPs) have been reported to be associated with various diseases, very few have been directly shown to correlate with disease incidence. Furthermore, to-date most genetic studies have evaluated SNPs in the coding regions of genes. On the other hand increasing evidence suggests that SNPs in non-coding

regions, including promoters and enhancers and miRNA seed regions, might play significant roles. Since miRNAs (non-coding RNAs) can affect gene expression by targeting of the 3′UTRs of target genes, if any sequence variations were present around the miRNA processing sites, or in the mature miRNA seed sequence, this would greatly alter the miRNA biogenesis as well as its functions and target genes (71,72). This could alter the repertoire of tissue and cellular gene expression profiles which in turn would affect disease susceptibility (71,72). Thus it is worth paying more attention to SNPs and variations in miRNA seed sequences as they could have more functionality.

Apart from genetic predisposition, additional factors and especially environment and epigenetics may provide that "second chromatin hit" to confer functionality to convert disease associated SNPs to disease causing SNPs. This was illustrated in a recent study comparing epigenomic histone modification profiles in blood monocytes of type 1 diabetic patients versus normal volunteers which demonstrated significant differences in the levels of histone H3K9 acetylation at enhancer regions of two MHC locus genes DRB1 and DQB1 known to be highly associated with type 1 diabetes (73). Another study compared DNA methylation in saliva samples from diabetic patients with end stage renal disease versus those without diabetic nephropathy. Interestingly, some of the differentially methylated candidate genes were previously reported to harbor variants associated with kidney disease (74). Several efforts are currently ongoing to evaluate epigenetic changes (such as DNA methylation) in archived genomic DNA, as well as miRNA profiles in tissue and blood cell RNA from large clinical cohorts of diabetic patients with various complications. Since genetic data from these patients are already available in most cases, integration of datasets from these multiple layers of the genome using high throughout sequencing, experimental validation as well as in silico/systems biology approaches aided by advanced genomic, transcriptomic, epigenomic and computational methods, will no doubt yield significant new information. These new ventures will greatly advance our knowledge to accelerate the discovery of much needed new therapies for diabetic complications.

miRNAs as biomarkers of diabetic complications

There have been several recent advances in technologies for miRNA detection in cells, tissues and biofluids, including sensitive quantitative PCRs, microarrays and high throughput deep sequencing. As such, there is tremendous potential and interest in developing miRNAs as sensitive biomarkers for human diseases and tissue injury (75–79). Since miRNAs are relatively stable and easily quantified in a non-invasive manner in plasma and urine, they might fulfill the critical need for biomarkers for the early detection of diabetic complications which could greatly facilitate the clinical management of long term outcomes. Recent reports showed that circulating miRNAs in blood can be sensitive biomarkers for cancer, tissue injury and heart failure (75–79). miRNA levels have been studied in the urinary sediment of patients with IgA nephropathy (80). Circulating miRNA levels were also evaluated in chronic kidney diseases (81,82). Furthermore, since there are reports of changes in miRNAs such as miR-1, miR-133, miR-125b, miR-200b, miR-206, miR-503 in models of diabetic cardiovascular and heart diseases, miR-146 and miR-29b in DR, and miR-192, miR-200b/c, miR-216a, miR-217, miR-29b/c, miR-377 in DN, as shown in Table1, these miRNAs may potentially be worth examining as biomarkers to detect early stages of the associated diabetic complications. In particular, it is likely that renal, vascular and blood cell-associated miRNAs can be detected in urine and serum samples due to various transport mechanisms. However, it should be noted that circulating miRNAs do not always correlate with tissue levels. Notwithstanding, overall, the future holds great promise for the evaluation of circulating miRNAs in biofluids as diagnostic biomarkers of diabetic complications.

Translational Approaches: miRNAs as therapeutic targets for diabetic complications

Preventing or slowing down the progression of complications is a major goal in the clinical management of diabetes. miRNA targeting alone or in combination with conventional and currently used drugs could be a new opportunity in this connection. Recent advances in the synthesis and chemistry of nucleic acids have allowed us to establish more efficient methods to inhibit specific miRNAs in vitro and in vivo. Locked nucleic acid (LNA) modified anti-miRNAs (antimiR) have been shown to be very effective for inhibiting miRNAs (36,83,84). We recently demonstrated specific and efficient reduction of miR-192 in vivo in mouse kidneys injected with LNA-modified antimiR-192 (LNA-antimiR-192) (36). This miR-192 inhibitor also reduced downstream miRNAs (miR-216a, miR-217 and miR-200 family) and functional indices of renal fibrosis and hypertrophy, namely collagens and TGF-β 1 and Akt activation in these mice, similar to the effects in cultured MMC (35,36). Furthermore, we recently demonstrated that injection of LNA-antimiR-192 into STZ-injected type 1 diabetic mice ameliorated renal fibrosis (85) demonstrating the translational potential of such anti-miRNA therapies for diabetic renal disease. Interestingly, low doses paclitaxel (a cancer drug) ameliorated fibrosis in the remnant kidney model by down-regulating miR-192 (86). Reduced rates of DN progression were also noted in db/db mice injected with 2′-O-methyl antisense oligos targeting miR-29c (41). Adeno-associated virus vectors and miRNA sponges have been considered as in vivo delivery systems for miRNAs (55,87,88). Taken together, along with the discussion above and the information in Table 1, several miRNAs appear to be upregulated in target cells and tissues related to the development of diabetic complications. Thus, inhibitor antisense LNA oligos or antagomirs (in other appropriate delivery vehicles) against key miRNAs that are increased in diabetic complications could be developed as new therapeutic approaches for the prevention or treatment of such complications. As Table 1 shows, several miRNAs are also reported to be downregulated under diabetic conditions. Injections with mimics of these miRNAs would be an option for the associated complications, although it is technically more complicated due to the inherent instability of functional mature miRNAs. Importantly, since the up- or down-regulation of miRNAs in vivo could also be related to defensive or other adaptive mechanisms, miRNA based therapies should be carefully designed only after documenting the in vivo functional role of the specific miRNA being targeted.

Closing remarks

In this review, we have discussed not only the significance of miRNAs in diabetic complications but also the complexity in the tissue and cell-specific expression and functions of specific miRNAs in the same complication. This could be related to the cell-type specific patterns, different model systems and animals studied, time of sampling, or the severity of the complications in the models studied. Furthermore, one miRNA can potentially target several genes. These discrepancies and facts reflect the challenges of miRNA-based therapeutics. However, this is a dynamic and rapidly advancing field and some clinical trials with anti-miRNAs are already ongoing indicating that miRNA targeting holds much promise for the future. As we learn more about the molecular mechanisms by which these small, yet powerful, gene regulatory molecules operate in vitro and in vivo, we will be able to device better ways to exploit them as non-invasive biomarkers, as well as better in vivo delivery methods for miRNA based therapies for diabetic complications.

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Table 1

miRNAs implicated in diabetic complications

miRNAs in complications	Targets	Cell types & animal models	Expression	Ref.
Diabetic retinopathy				
miR-29b	Rax	STZ rat	Increase	(28)
miR-146	Irak1, Traf6	STZ rat, Retinal EC	Increase	(25)
miR-146a	Fibronectin	STZ rat, db/db mice, HUVEC	Decrease	(26)
miR-200b	VEGF	STZ rat, HUVEC	Decrease	(27)
Diabetic nephropathy				
miR-21	Pten	OVE26 mice	Increase	(46)
miR-21	Pten	db/db mice, MC	Decrease	(47)
miR-25	Nox4	STZ rat, MC	Decrease	(48)
miR-29c	Spry1	db/db mice, EC, podocytes	Increase	(41)
miR-29	Collagens	ApoE−/− mice, PTEC	Decrease	(42)
miR-93	Vegf	STZ mice, db/db mice, EC, podocytes	Increase	(40)
miR-192	Zeb1/2	STZ mice, db/db mice, MC	Increase	(31,34–36,38,39,41,85,89)
miR-192	Zeb1/2	ApoE−/− mice, PTEC	Decrease	(43,44)
miR-200a	Tgfb2	ApoE−/− mice, PTEC	Decrease	(45)
miR-200b/c	Zeb1	STZ mice, db/db mice, MC	Increase	(35,41)
miR-216a	Ybx1	STZ mice, db/db mice, MC	Increase	(34)
miR-216a/217	Pten	STZ mice, db/db mice, MC	increase	(36)
miR-377	Pak1, Sod1/2	STZ mice, MC	Increase	(38)
Cardiovascular diseases				
miR-1	Pim1	STZ mice, Cardiomyocytes	Increase	(55)
miR-1, miR-206	Hsp60	STZ rat, Cardiomyocytes	Increase	(54)
miR-16	Cox-2	THP-1 monocytes	Decrease	(65)
miR-125b	Suv39h1	db/db mice, VSMC	Increase	(67)
miR-133	Rho-A, Cdc42	Cardiac hypertrophy	Decrease	(57)
miR-133a	Mef2A/C, Sgk1, Igf1R	STZ mice	Decrease	(58)
miR-200b	Zeb1	db/db mice, VSMC	Increase	(68)
miR-320	Igf-1	GK rat, MMVEC	Increase	(56)
miR-373	Mef2C	STZ mice, cardiomyocytes	Decrease	(59)
miR-503	Ccne1, Cdc25A	STZ mice, HUVEC, HMVEC	Increase	(49)

EC, endothelial cells; HUVEC, human umbilical vein endothelial cells; VSMC, vascular smooth musclecells; STZ, streptozotocin; MVEC, microvascular endothelial cells; MMVEC, myocardial MVEC; HMVEC, human MVEC; MC, mesangial cells; PTEC, proximal tubular epithelial cells.