Review Article

MicroRNAs as newer therapeutic targets: A big hope from a tiny player

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ABSTRACT

MicroRNAs (miRNAs) are a novel group of universally present small noncoding endogenous RNAs that regulate gene expression and protein coding by base pairing with the 3′ untranslated region (UTR) of target mRNAs. So they have been associated with several physiological processes and play an important role in the manifestation of diverse diseases. miRNAs expression is associated with the normal and diverse pathophysiological state including cardiac hypertrophy, neurodegenerative diseases, diabetes and its complication, and cancer because individual miRNAs are associated with the regulation of the expression of multiple target genes. Modulating the expression of a single miRNA can influence an entire gene network and thereby modify complex disease phenotypes. From recent studies, it has been confirmed that miRNA has a potential physiological role in various body systems. But in some specialized condition over expression of miRNA within the cytoplasm also leads to some pathological condition in the body. Here, we summarize the roles of miRNAs in various pathological conditions and consider the advantages and potential challenges of miRNA-based therapeutic approaches compared to conventional drug-based therapies.

Key words: Antagomere, cardiovascular, cancer, diabetes, microRNAs, target genes

INTRODUCTION

Mature miRNAs are a class of naturally occurring, small noncoding RNA molecules, about 21–25 nucleotides in length. miRNAs are partially complementary to one or more messenger RNA (mRNA) molecules, and their main function is to downregulate or upregulate gene expression, including translational repression, mRNA cleavage, and deadenylation. They were first described in 1993 by Lee and colleagues,[1] and the term microRNA (miRNA) was given in 2001.[2] Thousands of miRNAs have been identified in various organisms through random cloning and sequencing or computational prediction. In this study, we reviewed the potential impact of miRNAs in different pathological conditions and miRNAs-based therapeutic approaches.

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BIOGENESIS AND MECHANISM OF MicroRNA

In the last decade, the complex picture of gene regulation was extended by the discovery of miRNAs. MiRNAs are short, approximately 22-nucleotide-long noncoding RNAs which involved in a number of evolutionarily conserved regulatory pathways.[3] They are transcribed by both RNA polymerase II and III as evidenced by miRNA transcripts, termed Pri-miRNAs, that are caped at the 5′ end and polyadenylated at the 3′ end.[4] The maturation of small RNAs is mainly guided by two RNA type-III endonucleases, named Drosha and Dicer.

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Drosha initiates the processing of the pri-miRNA transcript in the nucleus by cleaving it at the bottom of its stem loop.[5] Recently, it has been showed that Di George Critical Region 8 (DGCR8), a RNA-binding protein that acts together with Drosha, is essential for the biogenesis of miRNAs and seems to be solely important in processing miRNAs.[6] The pre-miRNA is then exported into the cytoplasm, which is mediated by the nuclear transport receptor, exportin-5.[7] Once in the cytoplasm, pre-miRNAs are subsequently processed by Dicer into the short 22-nucleotide mature miRNA duplexes.[8] These duplexes are then incorporated into the RNA-induced silencing complex (RISC), and based on the thermodynamic properties; one strand is eliminated whereas the other one will remain in the complex.[9,10] Several proteins of the argonaute family are associated with the RISC complex, of which argonaute-2 was shown to be responsible for mRNA cleavage.[11] miRNAs will then mediate their effect on gene expression by annealing to the 3′-untranslated region (UTR) of targeted genes, resulting in mRNA degradation or the repression of translation. Recent studies suggest the role of processing bodies (P-bodies) in the mode of repression as these structures represent an accumulation of translationally repressed messenger ribonucleoproteins.[12] [Figure 1].

MicroRNAs INVOLVED IN DIFFERENT PATHOLOGICAL CONDITIONS

Cardiac hypertrophy and heart failure

Cardiac hypertrophy is a common pathological condition due to a number of cardiovascular diseases, such as hypertension, ischemic heart disease, valvular diseases, and endocrine disorders. Cardiac hypertrophy often leads to heart failure in humans and is a major determinant of mortality and morbidity in cardiovascular diseases. miRNAs regulate the differentiation and growth of cardiac cells, and so it has been hypothesized that miRNAs play important roles in cardiac hypertrophy and heart failure. In 2005, a Japanese research group determined the expression profile of miRNAs in the kidney and heart of salt-sensitive hypertensive rats.[13]

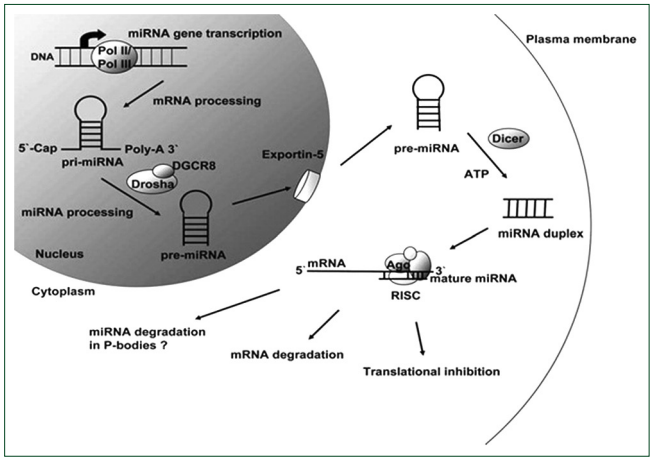

Figure 1: MicroRNA biogenesis

Expression of microRNAs in the cardiovascular system

One of the most important characteristic of miRNAs is tissue-specific expression. Indeed, one miRNA may be highly expressed in one tissue, but may have no or low expression in other tissues.[14] Identifying a miRNA signature is therefore an essential prerequisite to study the biological functions of this class of molecules in the cardiovascular system. Microarray analysis design is an important tool to detect the majority of mammalian miRNAs. Recently, Cheng and his colleagues determined the miRNA signature in both heart and artery samples.[15] Overall, out of 180 mature miRNAs arrayed, 140 were found in normal rat carotid arteries, whereas 157 mature miRNAs out of 233 arrayed were found in normal mouse hearts.[16] The miRNA signature in the mouse heart has also been demonstrated by three other independent studies.[14,17,18] The tissue-specific expression profiles indicate that the physiological functions of miRNAs in each tissue could be unique; identifying these miRNA signatures and clarifying their physiological functions could be important for future studies.

Role of microRNAs in cardiovascular system

MiR-1 was identified in 2005, to play a key role in cardiomyocyte differentiation. MiR-1 is specifically expressed in cardiac precursor cells, and the miR-1 gene is a direct transcriptional target of muscle differentiation regulators, including serum-response factors (SRF), myogenic differentiation factor D (MyoD), and myocyte-enhancing factor 2 (Mef2). Correspondingly, excess miR-1 in the developing heart leads to a decreased pool of proliferating ventricular cardiomyocyte.[19] These results suggest that miR-1 genes modulate the effects of critical cardiac regulatory proteins to control the balance between differentiation and proliferation during cardiogenesis. Overexpression of miR-23a, miR-23b, miR-24, miR-195, or miR-214 induced hypertrophic growth of cultured cardiomyocytes, whereas overexpression of miR-150 or miR-181b caused a decrease in cardiomyocyte cell size.[20] A recent study also shows that miRNAs are aberrantly expressed in cultured neonatal hypertrophic cardiomyocytes that are stimulated by angiotensin II (AngII) or phenylephrine (PE). Modulation of upregulated miR-21, *via* antisense-mediated depletion (knockdown), has a significant negative effect on cardiomyocyte hypertrophy *in vitro*.[16] While overexpression of downregulated miR-1, *via* adenovirus-mediated gene transfer, is sufficient to prevent hypertrophic growth of cardiac myocyte. The cellular effects of miRNAs in the heart have been confirmed further both *in vitro* and *in vivo*.[21,22,23] In a recent study of the potential roles of miRNAs in vascular smooth muscle cell (VSMC) proliferation apoptosis, the author found that depletion of miR-21, which is upregulated in proliferative VSMCs, can decrease cell proliferation and increase cell

apoptosis in a dose-dependent manner in cultured rat aortic VSMCs. Therefore, it is reasonable to hypothesize that miR-21 possesses a proliferative and anti-apoptotic effect on VSMCs. The mechanisms responsible for these effects are unclear, but a preliminary study suggests that phosphatase and tensin homology deleted on chromosome 10 (PTEN) and Bcl-2 might be involved.[24] The role of miRNAs in cardiac development has been well described in several studies. MiR-1 is specifically expressed in cardiac and skeletal muscle of embryonic mice, and this expression is controlled by several key heart transcription factors such as SRF and Mef2. Overexpression of miR-1 results in thin-walled ventricles, heart failure, and developmental arrest at embryonic day 13.5, due to a significant decrease in the number of cycling myocardial cells. In addition, overexpression of miR-1 decreased the level of heart and neural crest derivatives expressed protein 2 (Hand2) without changing its mRNA level, suggesting that Hand2 is a target of miR-1 during heart development. The essential role of miRNAs in cardiovascular development is demonstrated indirectly in Dicer-deficient mice that lose miRNAs which leads to impairment of both heart and vessel development targeted deletion of the muscle-specific miRNA miR-1-2 also implicates miRNAs as key players in cardiovascular development. Consistent with the above animal studies, miRNAs also play an important role in heart development.[25-27]

MicroRNAs in hypertrophic phenotypes

Many diseases generate abnormal hemodynamic loads on the heart; in response, the heart increases its mass. Cardiac hypertrophy is thus the phenotypic end point that has been the most studied in relation to miRNAs of the heart to date. In animal models of hypertrophy, whole arrays of miRNAs have been reported to be upregulated, downregulated, or unchanged with respect to normal heart.[16,28-32] The overlap between the sets of miRNAs to be involved is partial, which may reflect in part the differences in the techniques and models used. However, some miRNAs have been more frequently reported as deregulated in the same direction than others, indicating the possibility that these miRNAs might have common roles in hypertrophy pathogenesis. For example, miR-1, miR-133, miR-29, miR-30, and miR-150 have often been found to be downregulated whereas miR-21, miR-23a, miR-125, miR-195, and miR-199 have often been found to be upregulated with hypertrophy. Interestingly, in cultured cardiomyocytes, the forced expression of individual miRNA is found to be upregulated with the stimulation of cardiomyocyte growth which is sometimes sufficient to induce hypertrophy, whereas inhibition of miRNA is found to be downregulated during hypertrophy which could blunt increases in cardiomyocyte size. For a few miRNAs, these results have been replicated *in vivo* studies. For example, miR-195, found to be upregulated in a model of stress-induced hypertrophy, was sufficient to provoke pathological cardiac growth when over expressed in transgenic mice;[17] similarly, knockdown of miR-133, a miRNA found to be downregulated in enlarged hearts, was sufficient to induce hypertrophy in wild-type mice.[22] Some recent studies reported that miR-21 regulates the extracellular signal-regulated and mitogen activated protein (ERK–MAP) kinase signaling pathway in cardiac fibroblasts, which has impacts on the global cardiac structure and function. miR-21 levels are increased selectively in fibroblasts of the failing heart, augmenting ERK–MAP kinase activity through inhibition of sprouty homologue 1 (Spry1). This mechanism regulates fibroblast survival and growth factor secretion, apparently controlling the extent of interstitial fibrosis and cardiac hypertrophy. *In vivo* silencing of miR-21 by a specific antagomir in a mouse pressure-overload-induced disease model showed reduction in cardiac ERK–MAP kinase activity, inhibition of interstitial fibrosis, and attenuation of cardiac dysfunction. These findings reveal that miRNA can contribute to myocardial disease by an effect in cardiac fibroblasts. Further miR-21 was validated as a disease target in heart failure and established the therapeutic efficacy of miRNA in a cardiovascular disease setting.[33] [Table 1]

MicroRNA as a therapeutic target in cardiac disorder

miR-21 regulates gene signaling pathways that are active in heart failure and a new experimental drug was able to silence these pathways and prevent heart failure in mice. Dr. Thomas Thum and his team have tried to discover and make commercial use of MiRNA-based therapeutics. Now it has been confirmed that miR-21 is over-expressed in the failing human heart and affected the structure and working of heart muscle through regulation of a gene signalling pathway involved in responding to stress. They also targeted miR-21 and prevented heart failure in laboratory mice by using a new experimental drug called antisense oligonucleotide. Furthermore, they showed that giving mice anti-miR-21 after established heart failure appeared to reverse some of the damage caused by the condition. This is the first study to clearly demonstrate

Table 1: Pathological/physiological role of some important microRNAs in the cardiovascular system

Micro RNA	Target gene or protein	Pathological/physiological change
miR-1	SRFs, MyoD, Mef2	Cardiomyocyte differentiation
miR-21	PTEN, Blc-2	Proproliferative and anti-apoptotic effect on VSMCs
miR-195	NA	Stress-induced hypertrophy
miR-21	NA	Overexpression in heart failure
miR-208	NA	Cardiac hypertrophy, heart failure, and myocardial infarction

NA: Not applicable

therapeutic efficacy for targeting miRNAs in an animal model of human disease. This study has revealed a key role for miR-21 in regulating a major stress-response pathway in the failing heart. Administration of anti-miR-21 led to a striking effect in preventing and treating cellular, morphologic, and functional features of heart failure in a well-established animal model.[33]

Neurodegenerative diseases

"Neurodegeneration" is a term that has been used to refer to differing topics. Abundant nerve cell death occurs in the course of normal brain development, and many pediatric neurological diseases are characterized by pathological degeneration of neurons and/or muscles. This review is focused upon the human-specific neurodegenerative diseases (NDs) that afflict mainly the elderly, particularly trinucleotide repeat diseases, Alzheimer's disease (AD) and the synucleinopathies, such as Parkinson's disease (PD). In the course of NDs, neurons lose their connections and die prematurely. However, there are some general ideas that are relevant at least circumstantially to how miRNA biochemistry may interact with the pathogenesis of NDs.[34]

Most prevalent subtypes of NDs, such as AD and PD, are inherited in a manner termed "sporadic" influenced by alleles with limited genetic penetrance, or are caused by genetic and/or environmental influences as yet uncharacterized.[35,36] These facts may shift the focus of ND genetic studies away from "traditional" genes which constitute only approximately 1% to 2% of human DNA, and toward the other approximately 50% of transcribed DNA about which we are mostly ignorant.[37,38] Some biochemical pathways that are upregulated during normal brain development, but downregulated in normal adulthood, are then aberrantly upregulated again in the course of NDs. These include pathways involved in cell–cell signaling, cell division, neuroplasticity, and apoptosis.[39] For example, the developmentally upregulated phosphorylation of microtubule-associated protein tau (MAPT) is upregulated in AD neurofibrillary pathology.[40] RNA is labile in even tightly controlled circumstances. In the course of some NDs, brain RNAs become pathologically altered. These changes include aberrant RNA oxidation, RNA degradation, altered RNA splicing, and ribosomal changes which cause mRNA translational frame-shifting abnormalities.[41,42] Using AD as an example, the time from patients' clinical diagnosis to death is typically approximately 8 years.[43] However, the underlying pathological processes of AD occur over many decades. Persons at risk for developing AD in their seventh or eighth decade already show brain metabolic abnormalities.[44] In the case of AD, the most prevalent hypothesis is the "amyloid cascade hypothesis".[45] Neuritic amyloid plaques and neurofibrillary tangles are a major histopathological hallmark for AD.

Changes in microRNA expression associated with neurodegenerative disorders

The current data that explicitly pertain to miRNAs and neurodegeneration must be characterized as preliminary, reflecting that our understanding of miRNAs is still in its infancy. However, recent studies have begun providing important glimpses into the functions of miRNAs in neuroprotection and neurodegeneration. These studies have been performed across a variety of cells and organisms [Table 2].

MicroRNA as a therapeutic target in NDs

The hypothesis that miRNAs could be involved in neurodegenerative disorders is intriguing and experiments performed in the mouse demonstrate that the miRNA network is necessary for neuronal survival.[46] Recent studies performed in humans support the idea that changes in miRNA expression profiles or miRNA target sequences could contribute significantly to risk for major neurodegenerative diseases such as AD and PD. MiRNAs seem to participate directly in the regulation of expression of AD-related genes involved in Aβ (amyloid beta) production. In this regard, miRNA research seems to be particular promising for the understanding of the very prevalent and poorly understood sporadic forms of AD and possibly PD. The challenge now is to address the role of specific miRNAs in biological models and expand the clinical studies. The search for disease-associated SNPs influencing miRNA function is also under way.[47,48] For instance, naturally occurring antisense RNA transcripts of the β-secretase 1 (BACE1) gene are altered in the AD brain and could participate in the regulation of BACE1 expression and Aβ production.[49] Another small RNA species, BC200 (brain-specific dendritic), is altered in the AD brain and possibly implicated in the

Table 2: Studies reporting changes in miRNA profiles in neurodegenerative disorders

Pathology	Tissue examined	Reported changes	Potential targets and/or signaling pathways
AD	Brain: CA1 region	Increased miR-9, miR-138 and miR-125b	NA
AD	Brain: superior and middle temporal cortex	Decreased miR-107, and possibly miR-103 in MCI and AD; decreased miR-23b in AD	BACE1/β-secretase
AD	Brain: hippocampus, medial frontal gyrus, cerebellum	Decreased miR-9 in AD Braak stage 5–6 in all regions; increased miR-29a, miR-29b-1 in AD Braak stage 5–6 in medial frontal gyrus	Insulin resistance; innate immunity
AD	Brain: temporal cortex	Decreased miR-29b-1, miR-29a and miR-9	BACE1/ β-secretase
PD	Brain: midbrain, cerebellum, cerebral cortex	Decreased miR-133b	Pitx3

MCI: Mild cognitive impairment; NA: Not applicable

synaptodendritic degeneration of neurons.[50] Thus, the study of miRNAs and possibly other small RNAs opens a new and intriguing area of research in neurodegenerative diseases. It is now clear that miRNAs, and also other noncoding RNAs, provide a novel and exciting layer of complexity to molecular neuronal biology. In addition, it can be safely predicted that we will see an exponential increase in publications in the years to come, which will bring forward novel insights into this recently discovered field of research.

DIABETES MELLITUS

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.[51] 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 toward increased socio-economic prosperity but on the other hand, accompanied by an escalating tendency toward 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. The discovery of miRNAs 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 as ribo-regulators of glucose homeostasis

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, miRNAs have also emerged as novel regulators of these phenomena and hence appropriately referred to as "ribo-regulators of glucose homeostasis".[52] 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 miRNA, miR-375. It is one of the earliest miRNAs 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 revert back normal insulin secretion.[53] From a set of its specific predicted targets that included Vti1a (vesicle transport through interaction 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. 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.[53] 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 miRNA and the Mtpn mRNA. Functionally Mtpn is involved in modulation of the actin network that affects membrane docking and fusion.[54,55] This strongly correlates to insulin vesicle exocytosis in the pancreas. All these indicate toward 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. Recently, miR-375 has also been reported to target 3′-phosphoinositide-dependent protein kinase-1 (PDK1) in the pancreatic islet cell. Its elevated expression in the pancreatic islets of diabetic Goto-Kakizaki rats indicates toward its role in diabetes. Glucose stimulation of insulin gene expression *via* PDX-1 also involves the PI3K pathway and a recent experiment in this connection unravels a novel angle of regulation of this pathway wherein miR-375 modulates glucose-mediated stimulatory effect on insulin gene expression by targeting PDK1.[56] Another miRNA, miR-9, has been reported as a strong candidate and regulator of insulin exocytosis from the pancreas.[57] The pancreatic exocytosis 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 Granuphilin/Synaptotagmin-like protein 4 (Slp4)[58,59] and this is regulated by the direct miR-9 target, Onecut2 (OC2) that inhibits the expression of Granuphilin/Slp4. All these indicate miR-9 to be explicitly involved in insulin exocytosis from the pancreas. In a later study, using miRNA microarray, it has been found that miR-124a strongly correlated with mouse pancreatic development suggesting its role in β-cell differentiation.[60] Looking for predicted miR-124a targets, fork head box protein A2 (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. 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[61] and also to Bcl2 inhibition[62] especially in the pancreas they are known to be involved in apoptosis of insulinoma cell lines.[63,64] Free fatty acids (FFA)

mediated regulation of p53 *via* miR-34a are therefore a novel mode of regulation of pancreatic apoptosis initiated by FFA. Within the islets as well, miR-34a affects hormone secretion by targeting vesicle-associated membrane protein 2 (VAMP2), involved in insulin exocytosis. The other miRNA, miR-146, that acts by targeting interleukin-1 receptor-associated kinase 1 (IRAK1) and TNF receptor associated factor 6 (TRAF6), both of which are involved in pancreatic β-cell death.[65,66] 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 by inhibition of insulin-stimulated Akt (protein kinase B) 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.[67] 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.[68] Although in a different context, miR-145 has been recently identified to target and downregulate the IRS-1 protein in human colon cancer cells[69] and this targeting has elaborate effects on the growth and proliferation of these cells. It may be worthwhile to undertake in depth studies to unravel the role of this miRNA on insulin action.

MicroRNAs and lipid metabolism

It has now been established beyond doubt that alterations in lipid metabolism contribute to insulin resistance and diabetes. Abnormal triglyceride storage and lipolysis in insulin-sensitive tissues are an early manifestation of insulin resistance. Increased 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.[70] 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 miRNAs 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*.[71] MiR-14 knockout animals had increased levels of triglycerides and diacylglycerol that reverted back on increasing the copy numbers of the miRNA. Another miRNA involved in energy homeostasis in *Drosophila* is miR-278[72] and miR- 278 mutants in spite of having elevated insulin production capacities depict increased circulatory glucose levels indicating a loss of insulin responsiveness. Around the same time, Esau *et al*. (2006) revealed the role of the liver specific miR-122 as a significant regulator of hepatic lipid metabolism.[73] In normal mice, inhibition of miR-122 with antisense oligo nucleotides 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 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 miRNA significantly reduced circulatory cholesterol levels.[74] 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 miRNA, the authors reported that extracellular signal-related protein kinase 5/big mitogen-activated protein kinase 1 (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.[75] 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 cardiac hypertrophy, renal glomerulus, peripheral nerve and the retina.

Cardiac complication

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 miRNAs 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 miRNAs 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).[76] 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. 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.[77] 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 repolarization, the observed QT prolongation and the associated arrhythmias in diabetic hearts. 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, that of all the miRNAs 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 vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF-1), insulin-like growth factor receptor (IGF-1R), and fibroblast growth factors (FGF) that are significant mediators of diabetic cardiomyopathy.[78,79] This revelation by Wang *et al*. 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 IGI-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, miRNAs are critical in the proper functioning of the heart and thereby implicated in cardiac pathophysiology.[80]

Renal complication

A very significant diabetic complication is that of the kidney where the membrane of the glomerulus shows extreme thickening and gets hypertrophied possibly due to accumulation of extracellular matrix (ECM) proteins namely collagen. 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.[81] A very strong underlying factor behind the accumulation of these ECM proteins as is observed in a diabetic kidney is the transforming growth factor β1 (TGF-β). Yin *et al*. have discovered exclusively presence of at least five miRNA in kidney which undoubtedly indicate toward their involvement in kidney function and disease.[82] A recent article has depicted the role of miR-192 in the kidney and in the pathogenesis of diabetic nephropathy. Using microarray analysis, it was found that collagen $1\alpha_1$ miRNAs is increased by TGF-β in mouse mesangial cells with a concomitant decrease in the miRNAs levels of δ elongation factor 1 (δEF-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.[83] Both SIP1 and δEF-1 are repressors of Collagen $1\alpha_2$ 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. All these observations suggest that small noncoding 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. It may exist in a soluble dimeric form or as oligomers of fibronectin or a highly insoluble fibrillar form in the extracellular matrix. In a recent article, Wang *et al*. 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 mi R-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 a phenotype of diabetic nephropathy[84] [Table 3].

CANCER

Croce and his colleagues, for the first time, reported a link between miRNAs and cancer by mapping the genomic locus of miR15 and miR16 to chromosome 13q14, a region deleted in majority of B-cell chronic lymphocytic leukemias (B-CLL).[85] Since then, studies in a variety of human tumors have shown that miRNAs are frequently associated with sites of chromosomal instability or amplification.[86] Furthermore, many recent experimental and clinical studies have revealed that the aberrant expression of miRNAs is associated with the stage, progression, and metastasis of cancers.[87] It has been shown that miRNAs can function as tumor promoters (oncomirs) or tumor suppressors (anti-oncomirs).[88]

Oncogenic microRNAs (oncomirs)

The "oncomirs" promote tumor development by negatively inhibiting tumor suppressor genes and/or genes that control cell differentiation or apoptosis. Oncomirs are significantly over expressed in various tumors because of gene amplification, epigenetic mechanisms, or transcriptional dysregulation.[88] MiR-17-92 cluster is a typical example, which is located at chromosome 13q31, a region amplified in lung and other malignancies.[89] Myelocytomatosis (Myc)-induced upregulation of miR-17-92 cluster has been shown to enhance

Table 3: Role of some important microRNA in DM and its complication

microRNA	Target gene or protein	Pathological/physiological role
miR-375	Vti1a, Mtpn, MAPK14, Slc16A2, Mxi1	β-cell function and insulin secretion
	PDK1	Glucose stimulation of insulin secretion
miR-9	Onecut2 (OC2)	Regulator of insulin exocytosis from the pancreas
miR-124a	Foxa2, Pdx-1	Pancreatic development, β-cell differentiation
miR-34a	VAMP2	Insulin exocytosis
	p53	Apoptosis of insulinoma cell lines
miR-146	IRAK1, TRAF6	Pancreatic β-cell death
miR-29	Akt	Inhibition of insulin action
miR-145	IRS-1	Mediator of insulin signaling and its mutation or dysfunction, growth and proliferation of β-cell
miR-133	SRF	Regulating myogenesis, overexpressed in the hearts of diabetic
miR-320	VEGF, IGF- 1, IGF1R, FGF	Diabetic cardiomyopathy
miR- 192	SIP1, βEF-1	Diabetic nephropathy
miR-377	p21-activated kinase, superoxide dismutase	Enhanced fibronectin production which lead to diabetic nephropathy
miR-14	NA	Regulator of fat metabolism
miR-122	NA	Lowering plasma cholesterol, hepatic lipid metabolism, liver steatosis
miR-143	ERK5/BMK1	Adipocyte differentiation

NA: Not applicable

tumorigenesis and angiogenesis[90] among other oncogenic miRNAs; miR-21 has been shown to promote apoptosis through activation of caspases in human glioblastoma cells. [91] In breast cancer cells, silencing of miR-21 inhibited cell growth *in vitro* and *in vivo* by causing downregulation of Bcl-2 and induction of apoptosis.[92] Among the experimentally validated targets of miR-21 are programmed cell death protein 4 (PDCD4), bone morphogenetic protein receptor II (BMPRII) and leucine-rich repeat flightless-interacting protein 1 (LRRFIP1).[93-95] MiRNA expression profiling has reported increased expression of miR-155 in various cancers. Its expression was significantly correlated with poor survival in pancreatic cancer patients.[96] In another study, transfection of anti-miR-155 oligonucleotides into pancreatic cancer induced the expression of tumor suppressor protein 53-induced nuclear protein and enhanced apoptosis.[97] MiR-372 and miR-373 are two additional examples of oncogenic miRNAs that are shown to promote cell proliferation and tumor development by neutralizing p53-mediated cyclin-dependent kinases (CDK) inhibition, possibly through direct targeting of the tumor suppressor gene Serine/threonine-protein kinase (LATS2)[98] [Table 4]. MiR-221 and miR-222 (miR-221/222) are frequently upregulated in various types of human malignancy including glioblastoma. Recent studies have reported that miR-221/222 regulate cell growth and cell cycle progression by targeting p27 and p57. However the underlying mechanism involved in cell survival modulation of miR-221/222 remains elusive. Further research is likely to add many more miRNAs to the growing list of oncomirs.

Table 4: Some important oncomirs and its physiological/pathological role

MicroRNA	Target gene or protein	Pathological/physiological role
miR16 and miR 15	NA	B-cell chronic lymphocytic leukemias
miR-17-92 cluster	NA	Lung and other malignancies
	Myc	Tumorigenesis and angiogenesis
miR-21	Pdcd4, BMPRII, and LRRFIP1	Promote apoptosis through activation of caspases
miR-155	TP53INP1	Overexpression in pancreatic cancer
miR-372 and miR-373	LATS2	Cell proliferation and tumor development

NA: Not applicable

Tumor suppressor microRNAs (anti-oncomirs)

There are several miRNAs have been identified as tumor suppressors, namely miR-17-5p, miR-29, miR-34, and miR-127.[88,99] Indeed, the earliest miRNAs identified to be tumor-associated (miR-15 and miR-16) were from this class,[100] which are now experimentally shown to possess anti-oncogenic activity. MiR-15/16 induces apoptosis by negatively regulating the expression of anti-apoptotic gene, *BCL2*.[101] In another study, miR-16 is shown to suppress the growth of prostate cancer cells by regulating the expression of CDK1 and CDK2, which are associated with cell cycle control and proliferation.[102] It has also been demonstrated that miR-15a and miR-16-1 cluster in prostate cancer cells target CCND1 (encoding cyclin D1) and Wingless Type 3A (WNT3A), and thus impact survival, proliferation and invasion.[103] Among the most tumor suppressor miRNAs are part of let-7 family. Expression of let-7 miRNAs is downregulated in various cancers and they are good candidates as diagnostic and prognostic biomarkers.[104] Expression of let-7 is frequently decreased in lung cancer and forced expression of let-7 in A549 lung adenocarcinoma cell line inhibited cancer cell growth.[99] MiR-34a is another miRNA in this category that participates in the p53 tumor suppressor network and have been shown to be directly transactivated by p53. Overexpression of miR-34a induces apoptosis and alters the expression of several genes related to cell-cycle progression, apoptosis, DNA repair, and angiogenesis.[105] MiR-34a inhibited human pancreatic cancer tumor-initiating cells and restored the tumor-suppressor function of p53 in p53-deficient human pancreatic cancer cells.[106]

Therapeutic strategies for cancer with microRNAs

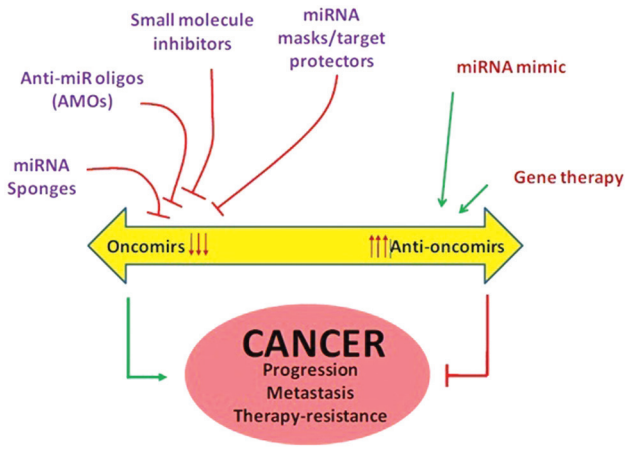

Figure 2: MiRNA-based therapeutic strategies against cancer

Inhibiting the function of oncomirs by use of anti-miR oligonucleotides (AMOs), small molecule inhibitors, miRNA sponges and miRNA masks/target protectors, and promoting the activity of anti-oncomirs through gene therapy or delivery of miRNA mimics can serve as novel therapeutic options against cancer. In consideration of the fact that miRNAs involve in tumor initiation, progression, and metastasis, their targeting is expected to emerge as an effective therapeutic option for cancer treatment. Plausible approaches for therapy would include achieving "gain" or "loss" of miRNA functions in the cancer cells [Figure 2]. Since many miRNAs have been identified to impart tumor suppressive effects, restoring their expression (endogenously or exogenously) may yield therapeutic effects.

CONCLUSION

Emergence of miRNAs as a new class of gene regulators and their proven role in different diseases' progression has opened new avenues for therapeutic discovery. Interest in miRNAs is now more than ever, and the literature is getting enriched rapidly with reports on novel miRNAs, their validated gene targets, and the development of miRNA-based therapeutics. The realization of a miRNA-based therapeutic approach in clinics; however, may still be far from sight and several hindrances pertaining to the stability, specificity, and delivery of short oligonucleotides need to be overcome. Nonetheless, phase I clinical trial with antimiR-122 for treatment of hypolipidemia has already been initiated based on exciting preliminary data in non-human primates. With increasing interest, further research in miRNA functions and technological advancements, miRNA-based therapeutics may create a paradigm-shift in medicine and pharmaceutical industry

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