Chapter

Therapeutic Implication of miRNA in Human Disease

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Abstract

MicroRNAs (miRNAs) are a class of short non-coding RNA molecules that are involved in development and diseases. Early studies are focusing on the miRNA profile as a biomarker in disease. As discovery of human miRNAs increased in the setting of disease, the research focus was gradually shifted towards miRNA therapeutic strategy for diagnostic and treatment of disease. Increasing evidences suggest that miRNAs are the next important class of antisense therapeutic molecules, which have significant advantage over antisense such as siRNAs because miRNAs are naturally occurring endogenous molecules. Aberrant alteration of the endogenous miRNAs has been linked to the development of certain diseases. Correcting these altered miRNAs by their mimics or inhibitors has been developed as potential therapeutic approaches. Some of the miRNA-based therapeutics are processed in preclinical and clinical trial for treatment hepatitis C, liver cancer, and other diseases. Currently, the major focus in the development of miRNA-based therapeutics is how to increase the miRNA stability and optimize delivery systems for specific disease with minimal off-target effect. This chapter will first overview the miRNA biogenesis, patho- and physiologic function, and regulation of miRNA molecules. Then, we discuss the miRNA-based potential therapeutic approaches and implication in disease.

Keywords: miRNA, function, disease biomarker, therapeutics

1. Introduction

MicroRNAs (miRNAs) belong to a family of small non-protein-coding RNAs with a single strand of 18–25 nucleotides that regulate multiple target genes at the post-transcriptional level. Functionally, miRNAs bind to 6–8 bp seed sequences in the 3' Untranslated Region (3' UTR) of targets mRNA and induce mRNA degradation or repression of protein translation. The term "non-coding RNA" is commonly defined a group of RNA that does not encode a protein. With a rapid advancement of molecular technology, many new classes of noncoding RNA have been founded. Among those noncoding RNAs, miRNA has attracted considerable attention because its endogenous origin and its role in the regulation of gene makes it more likely target for drug discovery and potential biomarker for specific disease.

miRNA research is a relatively new topic, with research ranging back for the past 25 years; it has its beginnings in its detection in *C. elegans* in 1993 and its detection in humans in 2000 [1]. Their use in transgenic mice in 2005 to

eventually efficacy studies of modified inhibitors of miRNAs in primates in 2010 illustrates the explosion of research surrounding miRNAs in just 5 years. There are now over 2000 miRNAs that have been discovered in humans and it is believed that they collectively regulate one third of the genes in the genome [2]. miRNAs have been linked to many human diseases and are being pursued as clinical diagnostics and as therapeutic targets, showing promise in many fields, ranging from cancer therapy to cardiac disease, to even suggestions as a potential biomarker for numerous diseases and treatment responses. This chapter will briefly discuss the miRNAs biogenesis, their function, regulation, and implication in disease, then discuss the miRNA-based therapeutic strategies, their therapeutic implication in diseases, and some of the current clinical trials involving miRNAs.

2. miRNA biochemical synthesis

miRNAs are encoded in the genomes (inter or intragenic) and are transcribed from genes located in nuclear DNA; however, such genes are not eventually translated into protein [3]. These transcribed genes are typically longer than the eventual gene product miRNA and undergo much post-transcriptional modification between initial transcription and the functional miRNA end-product. After initial transcription of the DNA sequence, the miRNA sequence contains a reverse-complement base pair segment that forms a double stranded RNA hairpin loop. The entire DNA transcript, including the double stranded RNA loop, constitute the primary miRNA structure (called pri-miRNA). Pri-miRNA is usually several kilobases long and has local stem loop structures. The primary transcripts undergo further processing in the nucleus. The ribonucleases Drosha and DiGeorge syndrome critical region gene 8 (DGCR8) complex are mainly involved in the pri-miRNA processing, which is cleaved at the stem of the hairpin structure and generates a hairpin intermediate of about 70–100 nucleotides, called pre-miRNA.

The pre-miRNA is then transported out of the nucleus to the cytoplasm for further processing to become mature miRNA. There are nuclear pore complexes in the nuclear membrane where the pre-miRNA can be transported out of the nucleus by means of the RanGTP-dependent nuclear transport receptor exportin 5. In the cytoplasm, the pre-miRNA is processed by another ribonuclease, Dicer to create a mature miRNA. The mature miRNA is a double-stranded miRNA of variable length (~18–25 nucleotides). After the generation of mature miRNA duplex by Dicer, the miRNA duplex is incorporated into an Ago family protein complex, which generates an effector complex. Then one strand of the miRNA is degraded, whereas the other strand remains bound to Ago as mature miRNA (guide strand). After strand separation, the guide strand or mature miRNA is incorporated into an RNA-induced silencing complex (RISC). After loading, the miRNA promotes the RISC to its target mRNA and induces mRNA degradation or translational repression (see **Figure 1**).

3. Functions of miRNAs

The general function of miRNA is oriented towards gene silencing [3, 4]. miRNAs specifically recognize mRNA and downregulate gene expression by one of the two post-transcriptional mechanisms: (1) translational repression and (2) mRNA cleavage. The determinant of the regulatory mechanism process is mainly dependent on the degree of

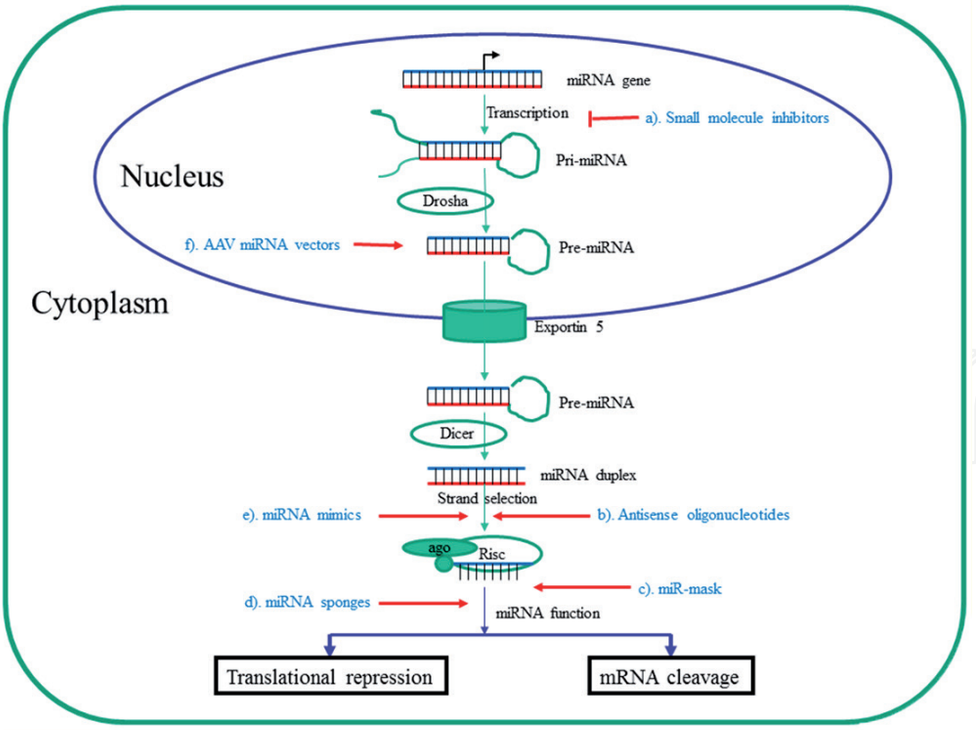

Figure 1.
miRNA biogenesis, function, and strategies for miRNA-based therapies. miRNA is transcribed from miRNA gene via RNA polymerase II as pri-miRNA and cleaved by Drosha complex in the nucleus. The resulting precursor miRNA (pre-miRNA) is exported to the cytoplasm via exportin 5 complex. In the cytoplasm, Dicer complex cleaves pre-miRNA to form mature miRNA duplex. The strand is separated and the functional strand is loaded into the RISC complex. The function of miRNA is depending on the complementarity of the seed region of mature miRNA to the 3'UTR of the target mRNA gene, either undergoing mRNA cleavage or translational repression. Strategies for miRNA-based therapies: improving miRNA in disease can be achieved by the following approaches: (a) Small molecule miRNA inhibitors can regulate miRNA expression at the transcriptional level. (b) Antisense oligonucleotides can bind to the target miRNA and induce degradation effect. (c) The miR-mask oligonucleotides are synthetic oligonucleotides complementary to the 3' UTR target mRNA that compete with endogenous miRNA for its target. (d) The miRNA sponges are oligonucleotide constructs with multiple complementary miRNA binding sites to the target miRNA. (e) The miRNA mimics are synthetic miRNAs which can restored the downregulated miRNA expression. (f) The AAV miRNA vectors are a group of adenovirus-associated vectors that have been inserted genes coding for miRNAs and they are used for restoring downregulated miRNA expression.

miRNA–mRNA complementarity. If there is a high degree of complementarity between the miRNA and mRNA, it will enable the Ago-catalyzed degradation of target mRNA sequences through the mRNA cleavage mechanism process. However, if there is a low degree of degree of miRNA–mRNA complementarity, a central mismatch will omit degradation and promotes the translational repression mechanism.

The exact mechanism for translational repression by miRNA is still not fully understood. However, recent studies suggest that, as the miRNA is incorporated into a RISC [3, 4], the associated protein silencing complex can either repress translational mechanisms typically associated with ribosomal translation, or induce deadenylation of the 3′ poly-A protective posttranscriptional mRNA modification, thought to be involved in repression via the mRNA 5′ terminal cap. The mechanism for mRNA degradation is mainly involved in endonucleolytic cleavage, which is facilitated by Argonaute cleavage proteins. It has been shown that when miRNAs have a high degree of sequence complementarity, then target mRNA degradation processes are facilitated through Ago protein slicer activity [2, 3]. miRNA typically binds to the 3′ untranslated region (3' UTR) in mRNA that follows the translation termination codon. The mechanism of miRNA translation inhibition requires partial sequence match, whereas the mechanism of miRNA-mediated mRNA degradation requires a near-perfect complementary match (see **Figure 1**).

4. Regulation of miRNAs

There are multiple levels of regulation of miRNA expression [5]. Those regulatory mechanisms mainly include transcriptional and post-transcriptional mechanisms, as well as effects of endogenous and exogenous compounds on the miRNA expression.

4.1 Transcriptional regulation

Similar to protein-coding genes, miRNA genes can also be regulated through transcription level. The promoters of miRNA genes are controlled by transcription factors (TFs). Many TFs regulate miRNA gene expression through positive or negative mechanism in a tissue-specific or developmental-specific manner. For instance, MYC inhibits expression of tumor suppressor miRNA-15a, which promote MYC-mediated tumorigenesis [6]. On the other hands, MYC can stimulate expression of miR-9 in neuroblastoma cells, resulting in regulation of E-cadherin and cancer metastasis [7]. It has shown that p53 enhances the expression of miR-34 and miR-107 families, which induce cell cycle arrest and apoptosis [8]. In addition to regulate by TFs, the expression of miRNA can be regulated by methylation of the promoter. Most of the miRNA promoter region has certain CpG islands. For example, promoter hypermethylation of genes such as miR-132, miR-34b/c, miR-218-1/2, and miR33b have been associated with or denote a poor prognosis of various cancers [9, 10]. In addition, the changes in DNMT1 and DNMT3b DNA methyltransferases lead to alter the miRNAs (miR-148a, miR-34b/c, miR-9 and let-7) gene promoter methylation status, resulting in regulation of their gene transcription levels [11]. Furthermore, it has reported that miR-210 is highly induced by hypoxia in various cancer cell lines [12], whose expression is not only regulated by the transcription factors hypoxia-inducible factor-1 (HIF-1), but also regulated by DNA demethylation mechanism in neural progenitor cells under both normoxia and hypoxia [12].

4.2 Post-transcriptional regulation

Post-transcriptional regulation has emerged as another important mechanism in define the miRNA expression pattern, which mainly involves the processing of the miRNA after transcription. On the post-transcriptional level, the expression of microRNAs can be downregulated due to changes in the activity of key miRNA biogenesis enzymes, such as Dicer and Drosha. Dicer and Drosha generally operate in complexes with double-stranded RBP partner (such as TRBP and DGCR8). Both the levels and activity of all of these proteins are subject to regulate the accumulation of miRNAs. For example, a decrease in TRBP leads to Dicer destabilization and pre-miRNA processing defects [9, 13]. In addition, recent studies have also demonstrated that post-translational changes in the Ago family of protein could cause significant changes in miRNA expression profiles [9, 13].

Another important contributor to miRNA levels is the stability of miRNA, which depends on the stage of development or cell type involved. It has been discovered that some proteins can bind miRNAs and affect their half-life [9, 14]. For example, HuR, a member of the ElaV family of RNA-binding proteins has been shown to suppress the inhibitory effect of miRNAs [14]. On the other hands, it has been shown that overexpression of Argonaute proteins can decelerate miRNA degradation and increase miRNA stability [9, 15].

4.3 Endogenous and xenobiotics regulation

The presence of physiological and pathological conditions have been demonstrated to regulate miRNA expression. Previous studies have shown that steroid hormones can regulate miRNA expression [16, 17]. Treatment with estradiol or tamoxifen has been shown significant changes of different miRNAs expressions in patients with breast cancer and in various breast cancer cell lines [16, 17]. In addition, corticosterone also can directly regulate miRNAs expressions [18]. Rats given chronic corticosterone showed significant behavioral disorder associated with differential regulation of 26 miRNAs in the rat prefrontal cortex [18]. Off interesting, those altered miRNAs that were modulated by corticosterone have binding sites for glucocorticoid receptor element (GRE), which were either simple, composite or tethering type within the 1-kb upstream of the transcription start site. These finding suggests that binding with GRE is a common regulatory mechanism of miRNA regulation by corticosterone.

In addition to endogenous regulation, xenobiotics can affect miRNA expression. Increasing evidence from *in vivo* and *in vitro* experiments have showed that specific carcinogen could differentially alter certain miRNAs expressions [19]. Chronic treatment with benzene in mice had showed significant changes in certain miRNAs expressions [20]. Aflatoxin B1, a genotoxic carcinogen, has been reported that it can affect the profile of miRNA expression in different animal models and cell lines [21, 22]. Therefore, miRNAs can serve as biomarkers of toxicity of carcinogen agents and may be useful for early cancer diagnosis. Furthermore, harmful life styles such as alcohol consumption and tobacco smoking can impair miRNAs expressions [23, 24]. Cigarette smoking can change microRNA profile in many human organs and induces the change of plasma miRNA expression profiles in healthy subjects [25, 26]. It has been reported that 24 miRNAs were up-regulated and 11 miRNAs were down-regulated in plasma from smokers [26]. In addition, the serum miRNA profiles in nonsmokers, smokers, and lung-cancer patients were significantly different [24].

5. Clinical implication of miRNAs

MicroRNAs are becoming well recognized as their expression levels are changed in different diseases such as cancer, liver disease, coronary heart ischemic disease, and metabolic disease. Some miRNAs are increased, whereas others are decreased in a specific disease, creating a signature miRNA pattern that could serve as a biomarker or molecular therapeutic target for a particular disease. For example, in the case of cancers, the overexpressed miRNAs are commonly considered as oncogenes. On the other hand, some of the miRNAs are expressed very low levels in certain cancerous cells and usually prevent tumor development. These groups of miRNAs are called as tumor suppressor miRNAs. Let-7 is one of these tumor suppressor miRNAs [27]. The miRNAs have remarkable potential in the clinical arena because they can be detected in the blood, serum, tissues (fresh and formalin-fixed paraffin-embedded), and fine-needle aspirate specimens. Recently, novel in situ hybridization techniques have been described to detect miRNAs in tissues, which enables direct miRNA and histomorphologic correlation [28]. The clinical implications of miRNA use in medicine are present in both diagnostic and therapeutic approaches. In miRNA-based diagnostics, miRNA expression profiling has been evaluated as a reliable diagnostic biomarker for differentiating between normal and tumor specimens [29, 30]. Ali et al. have reported that the expression of

let-7c, let-7f, and miR-200c were significantly decreased in pancreas cancer patients whereas miR-486-5p and miR-451 were significantly increased in those patients compared with the normal people, which suggests that these miRNAs can be served as signature biomarkers for pancreas cancer [30]. Additionally, circulating miRNAs can be employed in diagnostic strategies to detect for alterations associated with either disorder affliction or treatment response. In the miRNA-based therapeutics, the miRNA expression is altered in different diseases and it is now feasible to correct miRNA expression by injecting miRNAs similar to the use of antisense mRNAs and RNAi. For example, because the activation of onco-miRNA genes could cause development of cancer, artificial antisense miRNAs could be synthesized and used to inhibit their targeted miRNAs to treat or prevent the cancer.

6. miRNA therapeutics: strategies

There are at least two main strategies to target miRNA expression for prevention and potential treatment of disease. The first strategies is the use of oligonucleotides or virus-based constructs to either directly block the expression of a disease-associated signature miRNA or to directly substitute for the loss of expression of the miRNA. The second strategies is indirectly employing drugs to alter miRNA expression by targeting their transcription and processing. Blocking miRNA expression can be achieved by the use of antisense oligonucleotides, miRNA sponges, miRNA-mask and small RNA inhibitors. Restoring downregulated miRNA expression can be achieved by using synthetic miRNA (miRNA mimic) or by inserting genes coding for miRNA into viral constructs. At the transcriptional level, small-molecule miRNA inhibitors can be employed to prevent the transitions from DNA transcript to pri-miRNA and pre-miRNA. Antisense oligonucleotides can be employed at the mature miRNA level to induce degradation or revert the mature miRNA into a duplex form with the antisense oligonucleotide. At the functional level, miRNA masks can bind complementarily to the 3' UTR region of target mRNA, competing for bindings with endogenous miRNAs for the specific target. miRNA sponges can be employed to bind target miRNA via complementary mRNA binding sites, decreasing expression levels of target miRNAs (see **Figure 1**).

6.1 Antisense oligonucleotides (AMO)

Antisense inhibition of miRNA function has been an important tool for uncovering miRNA biology and potential therapeutics [31]. Synthetic oligonucleotides can be used therapeutically when miRNA dysregulation contributes to pathophysiology. These oligonucleotides are known as anti-miRNA oligonucleotides (miRNA inhibitors). To improve functional potency and to provide protection against nuclease degradation, they are often chemically modified [31, 32]. An ideal modification should increase binding affinity to the extent that specificity is compromised and should be non-toxic. There are four most common oligonucleotide modifications: (1) 2'-O-methyl groups, (2) phosphorothioate, (3) locked nucleic acid (LNA) anti-miRNA constructs, and (4) N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine (ZEN).

2'-O-methyl groups are the first generation of AMOs. 2'-O-methyl modifications can help AMOs to increase nuclease resistance and facilitate binding affinities to miRNA by the addition of an O-methyl group to the 2'-C atom. Phosphorothioate, compared to the 2'-O-methyl analogs, bonds at both the 3′ and 5′ ends to prevent nuclease degradation, and a 3′ cholesterol tail to help with cell uptake [33]. These modifications help the AMO to penetrate into tissues and

organs and significantly increase their half-lives in the target tissues. However, the first generation of AMOs have relative low potencies to be effective in animal model [34]. Second generation AMOs contain other modifications at the 2′ sugar position. Locked nucleic acid (LNA) modifications which are bicyclic nucleic acids that tether the 2'O to the 4'C via methylene bridge locking sugar into a 3′ endo conformation have been shown to have the best binding affinity and nuclease resistance. This group of AMO has been widely used in experimental animals [35–37]. Currently, the most advanced miRNA targeting therapy is SPC3649 (miravirsen), which is a locked nucleic acid-modified oligonucleotide antagonizing miR-122. This is the first miRNA-targeted drug to enter human clinical trials [38]. Despite LNA modifications have higher binding affinity, these modifications can lead to off-target effects which may cause toxicity *in vivo* [35]. Recently, a new compound called N,N-diethyl-4-(4-nitronaphthalen-1-ylazo)-phenylamine (ZEN), when is included at each end of the AMO, led to increased binding affinity to the miRNA and inhibited exonuclease degradation. Recent studies have shown that this group of AMOs (ZEN-AMOs) have higher potency and less toxicity than LNA-AMOs [39].

6.2 miRNA sponges

miRNA sponges are transcripts that contain multiple (typically 4–10 separated by a few nucleotides) tandem-binding sites to a miRNA of interest and are transcribed from mammalian expression vectors. The use of miRNA sponges in mammalian cells was introduced by Ebert and colleagues [40]. miRNA sponges have been found to occur naturally as long non-coding RNA in plants and animals. Synthetic miRNA sponges are usually plasmid or viral vectors which contain tandemly arrayed miRNA binding sites, separated with a small nucleotide spacer and inserted into a 3'UTR of the reporter gene driven by an RNA polymerase II promoter [40, 41]. miRNA sponges have the ability to inhibit an entire family of miRNA by using the common seed sequence, and can therefore inhibit multiple miRNAs at once. Some of the endogenous circular RNAs have been founded to function as nature miRNA sponges. For example, circRNA7 has been shown to be functions as a miRNA sponge for miRNA-7 in the mouse tissues [42]. The authors have further demonstrated that the testis-specific circRNA, sex-determining region Y (Sry), serves as a miR-138 sponge [42]. These finding suggest that circRNA functioning as a miRNA sponge to regulate miRNA expression may be a common phenomenon in human and animals.

6.3 miRNA masking

MicroRNA-masking antisense oligonucleotide technology (miR-mask) is another strategy for miRNA-based therapeutics. In contrast to miRNA sponges, miR-masks consist of single-stranded 2'-O-methyl modified antisense oligonucleotides that are fully complementary to the expected miRNA binding site in the 3'-UTR of target mRNA [43]. A miR-mask does not directly interact with its target miRNA but binds to the binding site of that miRNA in the 3' UTR of the target mRNA by fully complementary mechanism. Therefore, the miR-mask blocks the access of its target miRNA to the binding site so as to rescue its target mRNA via blocking the action of its target miRNA. miR mask is designed to be fully complementary to the target mRNA sequence of a miRNA, which suggests that the anti-miRNA action of a miR-mask is gene-specific. The strategy of miRNA masking has been used to disrupt miRNA function and involves masking the target site on target mRNA using a modified single-stranded RNA complementary to the target

sequence [44, 45]. The miRNA-masking method, in which only specific mRNA is masked, may lead to more specific and safer therapeutic strategies.

6.4 Small molecule inhibitors

Several drugs may possess the ability to modulate miRNA expression, targeting signaling pathways in miRNA biogenesis, ultimate converging on the activation of transcription factors involved in the regulation of miRNA encoding genes. The first specific molecule founded to be effective for inhibition of miRNA is an azobenzene [46]. The authors have demonstrated that the azobenzene can inhibit miRNA-21 by inhibiting miRNA-21 precursor in live cells [46]. MicroRNA-21 is significantly overexpressed in many types of human cancers, thus miR-21 is a potential therapeutic target. Recently, Naro et al. [47] have reported that, using a luciferase-based reporter assay, a high-throughput screen of >300,000 compounds led to the discovery of a new aryl amide class of small-molecule miR-21 inhibitors. Their studies further found that four aryl amide derivatives were very potent and selective miR-21 inhibitors [47]. The small molecule miRNA inhibitors are currently limited by their relatively low potencies and issues with specificity to a particular miRNA, however, they are much easier to deliver and have the promise for development of therapeutics.

6.5 miRNA mimics

In addition to miRNA inhibition as a major miRNA therapeutic approach, miRNA replacement treatment with miRNA mimics should be another miRNA therapeutic approach in disease associated with decreased miRNAs expressions. Synthetic miRNA mimics can assume the regulatory role of natural miRNAs. In diseases such as cancer, some tumor suppression-related miRNAs are downregulated. Therefore, artificial double-stranded miRNA (miRNA mimic) has been introduced to inhibit cancer [48]. Recent studies have reported that miRNA-34 is a master regulator of tumor suppression and a well-defined miRNA tumor suppressor [49]. It acts on several cancer relevant cellular pathways, including the p53 and wnt/β-catenin pathways. Down-regulation of miR-34 expression has been found in many tumor types, including lung, liver, breast, and colon carcinoma, and treatment with miR-34 mimic has been shown to inhibit tumor growth and progression [49, 50]. Consequently, miR-34 mimic, the first miRNA replacement therapy, is headed to the clinic for treatment of cancer [49, 50]. Replacement of oncosuppressor miRNAs with their mimics provides an effective strategy against cancer.

6.6 Viral vectors

Viral vector administration and encoding of miRNAs have been used for various therapeutic purposes [51]. A range of viruses can be employed for these purposes, including lentiviruses, adenoviruses, and adenoassociated viruses (AAVs). Since these vectors do not integrate into the genome, they can be eliminated efficiently with minimal toxicity, yet show remarkable efficiency in transferring RNA-encoding vectors into the nucleus of mammalian cells, ensuring high expression of miRNA [51]. Previous studies have shown that systemic lentivirus delivery of miR-15a/16 reduces lymphocytic leukemia progression in a mouse model [52]. In a murine model of muscular dystrophy-associated chronic dilated cardiomyopathy [53], intraventricular delivery of AAV vectors containing miR-669a induces long-term miR-669a overexpression and significantly decreases hypertrophic remodeling, fibrosis, and cardiomyocyte apoptosis. Furthermore, it significantly reduces adverse remodeling and enhances systolic

fractional shortening of the left ventricle in treated dystrophic mice, without significant detrimental consequences on skeletal muscle wastage [53]. Viral vector therapies have shown the highest efficacy for delivering miRNA into cells and organs in vitro and in vivo. However, their safety and toxicity remains a controversial issue.

7. miRNA therapeutics in disease

miRNAs are abundant in many mammalian cell types and appear to target about 60% of the genes of humans and other mammals [54]. Many miRNAs are evolutionarily conserved, which implies that they have important biological functions. However, growing evidence suggests that alteration of miRNAs expressions plays a key role in the development of disease. The signature miRNAs associated with disease and their potential therapeutics in the most common diseases are discussed in the following sections.

7.1 Therapeutic potential of microRNAs in cancer

Rapidly growing evidence supports that miRNAs play key roles in the pathogenesis of cancer and many miRNAs can function either as oncogenes or tumor suppressors [55]. MiRNAs can influence the development, progression, and metastasis of cancers [29, 30]. Their functional effect may differ depending on their expression levels. They have either an oncogenic potential or tumor-suppressor effect depending on their downstream impact on target genes and thereby controlling the biologic manifestations of cancers. The activity of a lost or down-regulated tumor suppressor miRNA can be restored by using miRNA mimics [56]. To date, there are some miRNA-based trials for treatment of cancers. For examples, miR-34 is one of the tumor suppressor miRNAs and it is significantly downregulated in many kinds of cancer [57]. Therefore, a cancer therapy synthetic miR-34 (MRX34) has been developed and has entered phase I clinical trial for liver cancer and metastasis from other cancers (NCT01829971) [57]. In lung cancer, miR-27a has been reported to be a potential targeted therapy for lung cancer [58]. MicroRNA-loaded minicells (miR-16-based mimic miRNA) are designed to counteract the loss of the miR-15 and miR-16 family and are used in clinic trials for small-cell lung cancer and mesothelioma [59]. The miR-205BP/S3 is a possible promising therapeutic modality for melanoma [60]. Let-7 is well recognized as one of the important tumor suppressors. So re-expression of the tumor-suppressor let-7 is another proposed miRNA therapeutic strategy to upregulate tumor-suppressor miRNA by exogenously transfecting with pre-let-7 that led to the inhibition of growth [27]. In addition to tumor suppressor miRNAs, some of the miRNAs can be served as oncogenes and used as therapeutic targets for cancer. For example, miR-21 is significantly overexpressed in many types of human cancers, thus miR-21 is a potential therapeutic target for a certain cancer [47].

7.2 Therapeutic potential of microRNAs in liver disease

Numerous studies have demonstrated that alterations in intracellular miRNAs correlated with various liver diseases [28, 38, 61]. In the liver, MiR-122 is one of the highly abundant miRNAs that affects various genes involved in hepatic cholesterol and lipid metabolism, thereby playing a central role in maintaining liver homeostasis [61]. Intriguingly, miR-122 is essential to the stability and propagation of hepatitis C virus (HCV) [61]. The finding of the role of miR-122 in the HCV replication process is one of the best examples of the potential targeted miRNA-based therapeutic

approaches. Blocking miR-122 using antisense approaches has reduced HCV replication in animal model [61]. MiR-122 binds to two closely spaced target sites (S1 and S2) in the highly conserved 5′ untranslated region of the HCV genome, thereby forming an oligomeric miR-122–HCV complex that protects the HCV genome from nucleolytic degradation or from host innate immune responses. Recently, a LNA-miR-122, known as Miravirsen, has been introduced and demonstrated that it can decrease HCV in nonhuman primates with no side effects [62]. Furthermore, in clinical trials of Miravirsen (NCT01200420), it has shown that the use of miravirsen in patients with chronic HCV genotype 1 infection can induce dose-dependent reductions in HCV RNA levels without evidence of viral resistance [61]. This miRNA-based therapeutics might deliver promising outcomes in the setting of liver disease.

7.3 Therapeutic potential of microRNAs in heart disease

Growing evidence shows miRNAs could be a promise molecular therapeutic strategy for cardiovascular disease [63, 64]. Previous studies have demonstrated that miRNA-21 level is upregulated in activated fibroblasts of the failing heart [65]. The investigators further demonstrated in an *in vivo* study of a mouse model of pressure-overload-induced heart disease that administration of a miRNA-21 antisense construct reduces the extent of heart fibrosis and overall heart function [65]. Their findings validate miR-21 as a disease target in heart failure and establish the therapeutic efficacy of microRNA therapeutic intervention in a cardiovascular disease setting. The miR-15 family is also found to be significantly increased in cardiac diseases [66]. Knockdown of the miR-15 family with LNA-modified anti-miRNAs resulted in reduced infarct size after ischaemia-reperfusion injury [66], suggesting it could serve as a therapeutic target for the manipulation of cardiac remodeling and function in the setting of myocardial infarction.

Diastolic dysfunction is a major clinical syndrome. Gain- and loss-of-function studies in animal model have shown that genetic deletion of the cardiac-specific miR-208a prevents pathological cardiac remodeling. Furthermore, therapeutic inhibition of miR-208a by subcutaneous delivery of miR-208a antisense during hypertension-induced heart failure in rats can prevent pathological myosin switching and cardiac remodeling and improve cardiac function [67, 68]. These studies suggest that miR-208 can serve as a potent therapeutic target for the modulation of cardiac function and remodeling during heart disease progression. In addition, miRNAs also play an important role in regulation of cardiovascular angiogenesis. AntimiR-92a (MRG-110) is currently used as a Phase I clinical trial for Miragen and it could offer a potential therapeutic to accelerate the healing process and revascularization in chronic ischemic disease. MRG-110 is being developed under a license and collaboration agreement with Servier for the treatment of heart failure and other ischemic disease [www.miragen.com]. To date, there is another therapeutics miRNA (MGN-5804 which targeting miR-378) in the development phase for the treatment of cardiovascular disease.

7.4 Therapeutic potential of microRNAs in renal disease

MicroRNAs can serve as mediators and therapeutic targets in many chronic renal diseases [69].

A variety of miRNAs are specifically enriched in the renal tissue as compared with other tissues, including miR-192, miR-194, miR-204, miR-215, and miR-216 [69]. miR-192 is one of the key miRNAs which is involved in diabetic nephropathy [70]. The authors reported that, in individual biopsies, tubulointerstitial fibrosis and low estimated GFR are associated with a decrease in miR-192 expression [70].

miR-192 targets E-cadherin, resulting in fibrosis of tubular cells and development of diabetic nephropathy. These findings suggests that miR-192 mimics should potentially be used as therapeutics of diabetic nephropathy. A global expression profiling study have shown that miR-21 is one of the most highly regulated miRNAs in kidneys of mice with diabetic nephropathy [71]. It has been reported that miR-21 antagonism in vitro and in vivo in streptozotocin-induced diabetic mice decreased mesangial expansion, interstitial fibrosis, macrophage infiltration, podocyte loss, albuminuria, and fibrotic- and inflammatory gene expression, which suggests that therapeutic miR-21 silencing could ameliorate diabetic kidney disease. Indeed, in a mouse model of chronic kidney disease, treatment with antagomir against miR-21 reverses both glomerular and tubular cell damage, resulting in a decrease in renal fibrosis and prolonging the life span of the chronic kidney disease-affected mice [72].

7.5 Therapeutic potential of microRNAs in neurological disease

Some of the miRNAs are highly abundant in the nervous system, where they play key roles in developmental neurobiology. Numerous studies have shown a dysregulation of miRNAs in neurological disease [73, 74]. These alterations in miRNAs expression prior to the onset of or during the course of disease pathology raises the possibility that expressing or inhibiting specific miRNAs might ameliorate the disease process and provide an effective therapeutic strategy. For example, Alzheimer's disease (AD) is being tested for potential miRNA-based therapy [75]. It has reported that a member of the miR-15/107 superfamily, miR-16 can specifically inhibits the expression of AD biomarkers Aβ and Tau, as well as brain inflammation and oxidative stress. MicroR-16 mimics delivered into the brain of mice resulted in a reduction of AD-related genes expression in a region-dependent manner, thus supporting the potential of miR-16 as an excellent therapeutic candidate for treatment of Alzheimer's disease. Similar to the changes of miRNAs in Alzheimer's disease, numerous miRNAs in human and animal models are also reported to be dysregulated in Parkinson's disease [76]. Some of these dysregulated miRNAs have been suggested to be potential therapeutic targets for Parkinson's disease. For example, Cho et al. had suggested that overexpression of miR-205 by miR-205 mimic could provide a potential therapeutic strategy to suppress the abnormal upregulation of LRRK2 protein in Parkinson's disease [77]. In addition, it has been reported that early downregulation of miR-34b/c in Parkinson's disease triggers downstream transcriptome alterations underlying mitochondrial dysfunction and oxidative stress, which ultimately compromise cell viability [78]. Therefore, upregulation of miR-34b/c may be an applicable therapeutic strategy for Parkinson's disease.

8. Future prospects

As the miRNA field dramatically grows, a better understanding of miRNA biogenesis and path-physiologic function will help to develop miRNA-based therapies. In addition, it is well known that a specific miRNA could target multiple genes and affect different organs in the same time. Therefore, research efforts should try to maximize the benefit of target diversity and prevent off-target effects. To achieve this goal, improvement of the chemical design of miRNA antisense and mimics and developing novel delivery systems are very important to ensure that the desired miRNA concentrations are achieved in organs and the targets are specifically regulated.

The use of synthetic miRNAs holds great promise as a new class of potential therapeutic agents by silencing the gene(s) of interest. Applicable to a wide variety of human diseases such as cancer, viral infections, genetic disorders, and cardiovascular disease, the attractiveness of miRNA therapeutics is their ability to target specific genes of interest, not always possible with small molecules or protein-based drugs. When designing drugs for therapeutic use, RNA sequence must be carefully designed to avoid undesired effects and immune responses in the body. The care into making a safe and relevant delivery system for miRNA-based therapies must also balance considerations of target tissue and cell delivery, cellular uptake, and nuclease degradation of the molecule.

Although a considerable number of clinical trials involving miRNA therapeutics have been conducted over the years, not all of those miRNA therapeutics have so far moved into clinical implication. The big challenges for miRNA-based therapeutics is to identify the best miRNA candidates or miRNA targets for each type of disease. The other challenges include the optimizing the miRNA delivery vehicles that can have higher targeting specificity and stability, as well as having lower toxicities and off-target effects. Although there are still hurdles to the use of mRNA-targeting approaches for clinical applications, with the rapid expansion occurring in this field, the prospects of miRNA-based therapeutics remain promising.

9. Conclusions

Growing evidences have shown that miRNAs play a key role in biological function and cell homeostasis. If the miRNAs are dysregulated, they lead to the development of many disease phenotypes. The miRNAs have immense potential in the clinical arena because they can be detected in the blood, serum, tissues, and fine-needle aspirate specimens. In addition, the discovery of miRNAs and their expression profile in a wide variety of diseases has led investigators to understand the key role of miRNAs as biomarkers during disease progression. Furthermore, because the miRNAs are relatively small size and they can regulate the network of target genes, they are promising targets for therapeutics. The most attractive feature of miRNA-based therapy is that a single miRNA could be useful for targeting multiple genes that are deregulated in disease, which can be further investigated through systems biology and network analysis that allows designing disease-specific personalized therapy. In summary, miRNAs are poised to provide diagnostic, prognostic, and therapeutic targets for several diseases. As the field continues to grow, miRNA-based therapeutics may develop a novel class of drugs for different diseases.

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