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MicroRNAs and cardiovascular medicine

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Abstract

MicroRNAs are non-coding RNA sequences that act as regulators of gene expression. They are aberrantly expressed in many pathological conditions. Cardiovascular diseases are among the leading causes of morbidity and mortality in the general population. Various knock-in and knockdown approaches have shown abnormal signature patterns of microRNAs in cardiovascular conditions like cardiac hypertrophy, myocardial infarction, heart failure, arrhythmias and vascular proliferative diseases. Since a single microRNA targets many genes, modulating a single microRNA involved in a disease carries a possible risk of undesirable side effects. The review focuses on current understanding of microRNAs in cardiovascular conditions, the possible underlying mechanisms and various approaches of modulating microRNAs.

Keywords

MicroRNAs, gene regulation, cardiovascular diseases

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Introduction

MicroRNAs (miRNAs) are an abundant class of small non-coding entities discovered first in *Caenorhabditis elegans* when the product of *lin-4* gene was found to suppress *lin-14* gene expression.[1] They provide a ubiquitous and powerful mechanism for RNA-mediated control of gene expression.[2] A total of 7000 miRNAs are defined by now, which impact the understanding of information encoded by genomic sequences and transcribed units. About 1872 precursors and 2578 mature human miRNAs have been reported in human genome as per current version of miRBase. (http://www.mirbase.org).[3] miRNAs affect gene expression at multiple levels including pre-transcriptional, transcriptional and post-transcriptional.[4] The extent of genome regulation by miRNAs is controversial, with some reports suggesting more than 70% of the human genome being regulated by miRNAs,[5] while others report a number close to 30%.[6] Each miRNA has multiple targets inside a cell, and similarly, each gene is targeted by multiple miRNAs. Recently, miRNAs have been implicated in the pathogenesis of a spectrum of diseases and are believed to serve as important functional targets of the future.

Synthesis of miRNAs

miRNAs are synthesized as primary miRNA (pri-miRNA) with a hairpin structure from non-coding region of DNA by RNA polymerase II.[7] pri-miRNA is then acted upon by DGCR8/ Pasha and Drosha (RNAase III) to form 70-to-80-nucleotides-long precursor miRNA (pre-miRNA), which is exported out of the nucleus into the cytoplasm using Exportin. In the cytoplasm, pre-miRNA is targeted by another RNAase III, Dicer and trans-activation response (TAR) RNA binding protein (TRBP) to form miRNA. The immature miRNA then associates with Ago-proteins to form mature RNA-induced silencing complex (RISC). RISC binds to the 3′-untranslated region (3′UTR) of target messenger RNA (mRNA) by a 'seed region' composed of six to seven nucleotides. Mature RISC causes degradation of mRNA if the binding sequence shows a perfect complementarity with mRNA. It can suppress translation if there is imperfect binding between mRNA and miRNA.[8] Figure 1 shows biogenesis of miRNA.

miRNAs can be transcribed independently as single transcription units (e.g. micro RNA(miR)-143)[9] or together with genes in which they are located (e.g. miR-126).[10] Furthermore, miRNAs may also be derived directly from introns when they are known as mirtrons.[11] Proteins produced by mRNAs that

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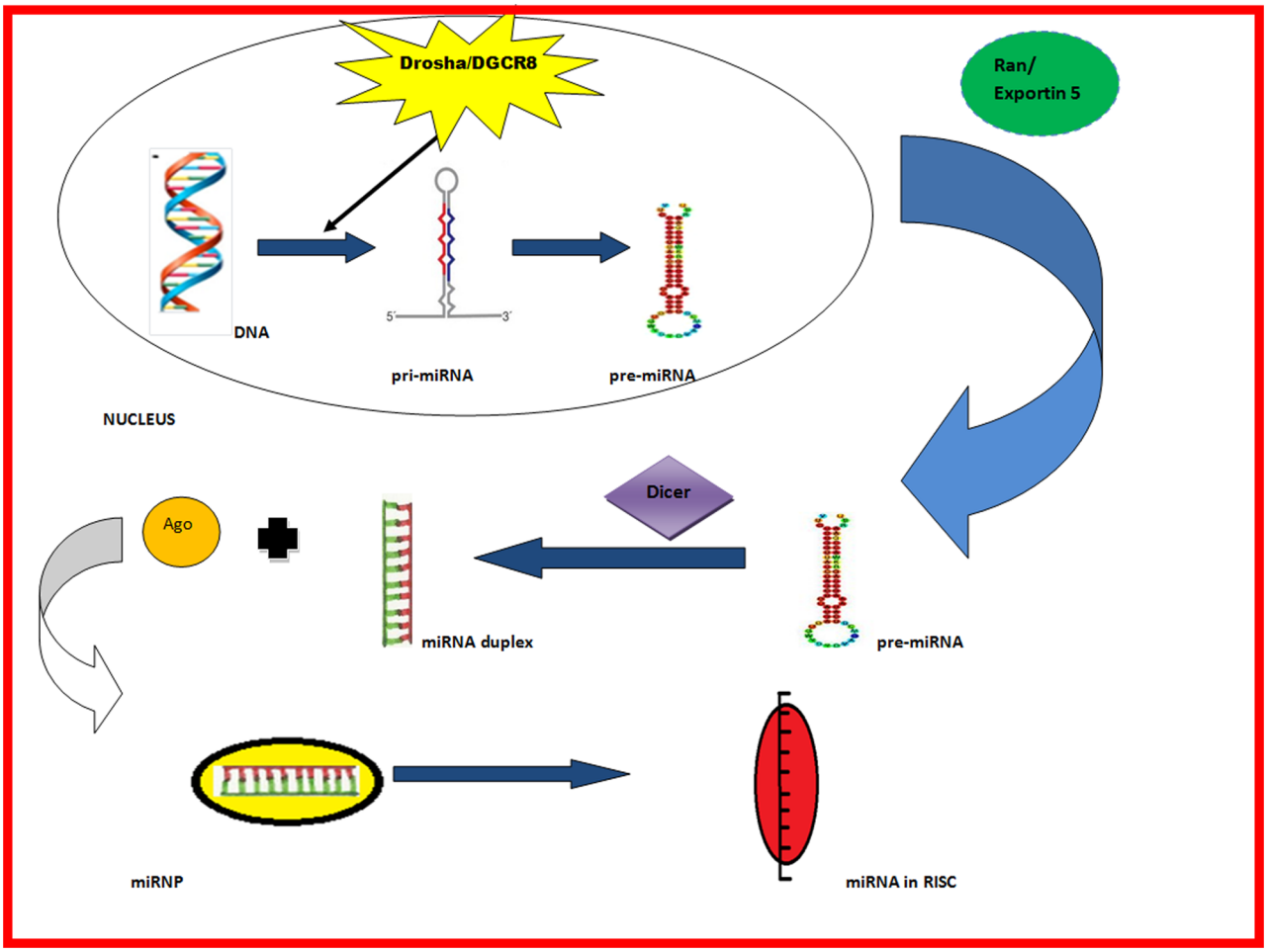

Figure 1. Biogenesis of miRNA.
miRNA: microRNA; pri-miRNA: primary miRNA; pre-miRNA: precursor miRNA; miRNP: microRNA ribonucleoprotein complex; RISC: RNA-induced silencing complex.

are targets of miRNA can also regulate the expression of many miRNAs adding complexity to miRNA-induced gene regulation.[12,13] miRNAs regulate transcription factors, which in turn regulate the expression of miRNA forming a double negative feedback. For example, p53, which is a target of multiple miRNAs, regulates miR-143.[14] Similarly, SMAD, a signal transducer of transforming growth factor–beta (TGF-β), regulates miR-21 and miR-199a.[15] Furthermore, many miRNAs are subjected to regulation by methylation patterns governed by epigenetic mechanisms, which are determined by environmental and other pathophysiological states.[16]

miRNAs and cardiac hypertrophy

Cardiac hypertrophy is a major compensatory cellular response of the heart to stressful conditions such as ischemia, injury, metabolic alterations, hypertension or genetic abnormalities. The pathological hypertrophy in fact acts as a major predictor of sudden cardiac death, heart failure and arrhythmias. It involves altered expression profile of certain miRNAs with a shift to the fetal gene program. There is a change in intracellular signaling pathways leading to increased protein synthesis and enhanced deposition of extracellular matrix proteins.[17] miRNAs upregulated in cardiac hypertrophy include miR-21, miR-23a, miR-23b, miR-24, miR-208, miR-212, miR-125, miR-129, miR-195 and miR-199, while those downregulated are miR-1, miR-133, miR-29, miR-30 and miR-150.[18–20]

One miRNA which is consistently linked with stress and functions to regulate cardiac growth is miR-21. miR-21 is involved in the pathogenesis of many cancers too and is termed as oncomiR.[21] It is highly expressed in cardiomyocytes[22] and fibroblasts.[23] miR-21 inhibition in cultured cardiac cells suppresses cardiomyocyte growth and can also block β-adrenergic receptor–mediated cardiomyocyte growth.[24] The in vivo effect of miR-21 on cardiomyocytes was shown in a study where downregulation of miR-21 was found to reduce the heart size. miR-21 targets the 'sprouty gene'—*SPRY1*—and represses its function resulting in overexpression of mitogen-activated protein kinase (MAPK/ERK) and consequently causing fibrosis. Other genes

targeted by miR-21 include programmed cell death gene (*PDCD4*) and phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*).[25] Another miRNA which is upregulated in rodent and human hearts in hypertrophic conditions is miR-195. Forced expression of miR-195 has been found to induce hypertrophic growth.[18]

miR-208 contributes to stress-induced cardiac hypertrophy by regulating triiodothyronine-dependent repression of β-myosin heavy chain (β-MHC).[26,27] It targets thyroid hormone receptor–associated protein and derepresses β-MHC resulting in hypertrophy of myocytes. miR-208 indeed works cooperatively with stress signaling to modulate gene expression. Targeting miR-208 with a locked nucleic acid (LNA)-based antagomir (antagonist of miRNA) has been recently shown to prevent functional deterioration and improve survival in patients with heart failure.[28]

miR-23 is a pro-hypertrophic miRNA, activated by nuclear factor of activated T-cells (NFAT).[29] miR-23 then targets muscle-specific ring finger protein which is thought to be cardioprotective.[29] Thus, miR-23 can be considered as a useful target in cardiac hypertrophy. More recently, miR-22 overexpression has been linked to hypertrophic pathways. miR-22 functions as an integrator of Ca^{2+} hemostasis during stress and as a regulator of protein content of myofibres.[30] miR-22 is believed to promote hypertrophy by suppressing PTEN, thereby stimulating phosphoinositide-3 kinase/Akt (PI_3K/Akt)pathway.[31] miR-22 expression–mediated hypertrophic changes have been recently shown to be modulated by the hypolipidemic drug Atorvastatin suggesting a novel role of pharmacological interventions in cardiac hypertrophy.[32]

In contrast to the above miRNAs, which have a stimulatory role in cardiac hypertrophy, downregulation of miR-133 and miR-1 manifests in cardiac hypertrophy. Significant hypertrophy was induced in mice after knockdown of miR-133 using antisense oligonucleotides, while overexpression of miR-133 by adenovirus-mediated transfer-inhibited cardiomyocyte hypertrophy. miR-133 targets RhoA and Cdc42 as a basis to suppress hypertrophy.[33] It also targets connective tissue growth factor (CTGF), and thereby inhibits extracellular matrix protein synthesis.[34] miR-1 targets hypertrophy-associated calmodulin and attenuates calcineurin–NFAT pathway involved in signaling hypertrophy.[35] miR-133 and miR-1 seem to be cardio-protective and may be targeted as a promising therapeutic strategy.

Other miRNAs which are downregulated in various models of hypertrophy and heart failure are miR-29 and miR-9. miR-29 targets elastin, fibrillin and collagen. Therefore, derepression of these proteins in 'miR-29 downregulation' opens up events leading to cardiac fibrosis. In vivo administration of cholesterol-based oligonucleotides against miR-29 has shown increased collagen expression and fibrotic changes.[36] miR-9 is downregulated in animal models of hypertrophy induced by aldosterone and isoproterenol, while nuclear factor of activated T-cells c3 (NFATc3) and myocardin appear to be overexpressed. Administration of miR-9 carries the potential to suppress myocardin expression and hence to regulate the hypertrophic program.[37]

Understanding the molecular mechanisms behind the pathological hypertrophy of the heart and finding out novel therapeutic targets is evolving as a field of immense interest.

Arrythmias and miRNAs

Normal conduction in the heart requires a proper functioning of various ion channels. Na^+ influx leads to membrane depolarization, while K^+ efflux results in repolarization. A protein Connexin 43(Cx43) is essential for conduction across gap junctions.[38,39] L-type Ca^{2+} channels determine the shape of the action potential by facilitating excitation–contraction coupling. miR-1 targets GJAI coding for Cx43[40] and KCNJ2 coding for Kir2.1.[41,42] miR-1 is increased in coronary artery disease and results in slowing up of conduction and arrhythmias. Blocking miR-1 with antisense oligonucleotides has been shown to normalize conduction and attenuate the arrhythmias seen in myocardial infarction (MI). This is complicated by the results of another study which showed increased ventricular septal defects in mice with targeted deletion of miR-1-2 owing to derepression of Irx5 transcription factor.[43] Inward rectifier potassium current–mediated repolarization is also affected by miR-133 which represses hERG (human ether-a-go-go-related gene).[44] miR-133 also targets *HCN2* gene involved in automaticity.[45] miR-1 and miR-133 may serve as targets in the management of arrhythmias.

MI and miRNAs

Oxidative stress and myocyte apoptosis are the key factors involved in the pathogenesis of ischemic heart disease. Angiogenesis and myocardial preconditioning serve as cardio-protective mechanisms in this setting. miRNAs involved in angiogenesis and ischemic preconditioning are potentially of therapeutic use in MI. Various miRNAs implicated in ischemic heart disease include miR-1, miR-133, miR-19, miR-21, miR-199 and miR-320. miR-320 has been found to be consistently overexpressed in the ischemic heart in a mouse model of ischemia reperfusion–mediated injury. It targets cardio-protective heat shock protein–20 (hsp 20) and contributes to myocyte death.[46] Furthermore, miR-21 is found to be downregulated in infarcted areas, and adenovirus-mediated expression of miR-21 leads to an attenuated rate of myocyte apoptosis by repression of *PDCD4*.[47] miR-21 may also provide cardio-protection via upregulation of hsp 70.[48]

miR-199 acts as a molecular switch when the oxygen tension in myocytes falls in short supply. It targets the protective mediators—hypoxia inducible factor–1α (HIF-1α) and sirtuins—involved in ischemic preconditioning. It can be utilized in preventing hypoxic damage to the heart.[49] miR-92a targets pro-angiogenic α-5 integrin and inhibits

angiogenesis. Antagomirs designed against miR-92a resulted in improved blood vessel growth and reduced functional deterioration of heart tissue.[50]

miR-22 which has already been discussed as a promoter of myocardial hypertrophy has been found to be protective against ischemia reperfusion–mediated injury. miR-22 targets adenosine 3′5′ cyclic monophosphate response element binding protein (CREB)-binding protein (CBP) and functions via anti-apoptotic mechanisms. Adenovirus-mediated transfer of miR-22 in animals has shown a reduction in infarct size, cardiomyocyte apoptosis and decline in creatine kinase levels.[51]

Contrastingly, a miRNA which plays a detrimental role in cardiac remodeling is miR-34. It has been seen to be elevated under conditions of pressure overload and silencing of miR-34 and, with suitable interventions, attenuates cardiac remodeling and improves systolic function in animals.[52] Apart from serving as potential therapeutic targets, miRNAs have the potential to be used as biomarkers of infarction, being present as microparticles in the circulation.[53,54]

Metabolic syndrome, cholesterol regulation and miRNAs

miR-33 is involved in cholesterol metabolism. miR-33b is co-expressed with SREBP1, and miR-33a is co-expressed with SREBP2.[55] miR-33 regulates ABCA1 and reverse cholesterol transport. Antagomirs against miR-33b have shown increase in high-density lipoprotein (HDL) levels and reduction in the size of plaques in mice deficient in low-density lipoprotein (LDL) receptors.[56,57] On the other hand, overexpression of miR-33b is linked with decreased functioning of ABCA1 and hence reduction in HDL levels. Thus, downregulation of miR-33 may be protective in atherosclerosis.

Vascular disease and miRNAs

Vascular proliferation

Vascular injury is a fundamental event in the pathogenesis of coronary artery disease, atherosclerosis and restenosis post-angioplasty. Multiple miRNAs are overexpressed in vascular diseases. miR-21 upregulation is linked with increased proliferation of vascular smooth muscle cells, fibroblasts and endothelial cells. *PTEN* and *PDCD4* are believed to be the normal targets of miR-21. Knockdown of miR-21 has been shown to inhibit vascular injury, and therefore, miR-21 can be targeted for modulating the progress in vascular disease.[58] Furthermore, miR-221 and miR-222 are also upregulated in injured carotid arteries. P27 (Kip1) and P57 (Kip2) are the major targets of miR-221 and miR-222. miR-221 and miR-222 downregulation may serve as a useful strategy in vascular proliferative disease.[59]

Inflammation

Inflammation and endothelial dysfunction are major determinants of initiation and progression of coronary artery disease. Various factors contributing to endothelial dysfunction include increased LDL, raised blood glucose, obesity, smoking and reactive oxygen species.[60] Normal endothelium does not express any adhesion molecules, but once activated, there is an increased expression of P and E selectins and vascular cell adhesion molecule–1 (VCAM-1), resulting in enhanced recruitment of leucocytes. Some miRNAs are present in endothelial cell and regulate the inflammatory status. miR-126 expressed in endothelial cell inhibits the activation of VCAM-1. Downregulation of miR-126 is linked to increased tumor necrosis factor–α (TNF-α)–mediated VCAM-1 expression.[61] miR-126 is in fact the first miRNA found to be associated with vascular inflammation. It is regulated by transcription factors Ets-1 and Ets-2.[62] Ets-1 can be induced by multiple stimuli like angiotensin II (Ang II)[63] and TNF-α.[64] miR-126 supplementation has been shown to provide atheroprotection which is thought to be because of CXCL12-mediated recruitment of progenitor cells.[65] Ang II is known to provoke vascular injury and cardiac remodeling acting via angiotensin 1 receptors (AT1R). miR-155 targets and inhibits AT1R expression as demonstrated in in vivo studies.[66] miR-155 further downregulates Ang II-mediated expression of VCAM-1 and monocyte chemotactic protein (MCP-1).[67] miR-155 seems to be protective in endothelial dysfunction and inflammation induced by Ang II.

Apart from the Ang II pathway, another pathway of note in inflammation-mediated vascular injury is nuclear factor kappa-beta (NF-κβ) signaling. Various inflammatory stimuli—LDL, lipopolysaccharides and cytokines—appear to induce the expression of inflammatory genes through NF-κβ. This results in upregulation of P-selectins, intercellular adhesion molecule–1 (ICAM-1) and VCAM-1.[68] miR-10a is protective against the NF-κβ pathway as downregulation of miR-10a is linked with increased expression of interleukin-6 (IL-6), IL-8 and VCAM-1.[69] Another miRNA believed to interfere with NF-κβ functioning is miR-181-b. miR-181-b targets importin-a3, a protein required for nuclear translocation of NF-κβ. miR-181-b levels are reduced in mice exposed to inflammatory stimuli and in patients with sepsis. miR-181-b replacement seems to protect against NF-κβ-mediated vascular injury.[70]

Angiogenesis

Angiogenesis is a major determinant of cardiac repair following MI. Vascularization depends on growth factors like vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF).[71] Various aspects of blood vessel growth are modulated by miRNAs. miR-126 promotes vascularization by targeting Spred-1, which is an inhibitor of MAPK involved in downstream signaling of VEGF.[72] Upregulation of miR-126

Table 1. Involvement of various microRNAs (miRNAs) in cardiovascular disease.

Condition	miRNAs involved
Cardiac hypertrophy	miR-21,[24] miR-22,[31] miR-23,[29] miR-1,[35] miR-29[36]
Arrythmias	miR-1,[43] miR-133[44]
Myocardial infarction	miR-320,[46] miR-22,[51] miR-21[47]
Vascular disease	miR-21,[58] miR-222,[59] miR-126,[61] miR-155[67]
Angiogenesis	miR-126,[72] miR-92[50]
Cholesterol metabolism	miR-33[56,57]
Endothelial dysfunction and inflammation	miR-126,[61,62] miR-10,[82] miR-155[66]

therefore frees VEGF signaling from being suppressed by Spred-1. miR-126 is also involved in migration of hematopoietic stem cells to the sites of cardiac injury.[73] Other miRNAs that influence blood supply include miRNA-221 and miRNA-222. miR-221 and miR-222 inhibit angiogenesis by targeting c-kit and by decreasing endothelial nitric oxide synthase (eNOS) expression.[74,75]

Heart failure and miRNAs

Majority of studies relating miRNAs and heart disease have been conducted on heart failure. Heart failure is a situation when cardiac output is unable to meet the demands of tissues. Since hypertrophy, arrhythmias and vascular disease discussed above can lead to heart failure, it would not be wrong to say that miRNAs implicated in these pathological conditions may also be involved in heart failure. Notably, the miRNAs upregulated include miR-21, miR-129 and miR-210, while those downregulated include miR-30 and miR-182.[35] As in hypertrophy, there is a shift toward fetal gene programming in heart failure too. Various miRNAs are expressed in a similar way in adult failing heart as in the fetal heart.[24] This change toward fetal gene expression may contribute to pathological features seen in heart failure. Understanding the miRNA profile expressed in failing heart may give us an opportunity to validate the targets selectively.

Preeclampsia and miRNAs

Preeclampsia acts as a risk factor for cardiovascular disease in women.[76] It also increases the risk that a child born to a mother with the condition would suffer a stroke in adulthood.[77] Results of four surveys conducted recently on miRNA expression in preeclamptic mothers show a total of 67 human miRNAs to be differently regulated.[78] These include miR-181a, miR-195, miR-584, miR-155, miR-222 and miR-210. miR-210 is induced in response to hypoxia.[79] Recently, some miRNAs have been detected in the plasma of preeclamptic mothers. These include miR-144, miR-181a and miR-130a.[80] A rich source of these miRNAs is the placenta.[81] Detection of miRNA molecular markers at an early stage may aid in diagnosing as well as prove useful in understanding the pathogenesis of preeclampsia.

Endothelial dysfunction and miRNAs

As has been discussed, endothelial dysfunction has a pivotal role in atherosclerosis and coronary heart disease. The involvement of miR-126 and miR-155 in the same has been highlighted.[61–66] miR-34 and miR-217 bring about endothelial senescence by suppressing sirtuin-1 (SIRT1), which is normally required for endothelial proliferation.[82] miR-10a inhibits endothelial inflammation by suppressing NF-κβ signaling pathway.[82]

Folic acid in high dose has been shown to improve endothelial function in various studies.[83,84] The mechanisms of folate action which have been proposed include increasing the bioavailability of tetrahydrobiopterin (BH_4), enhancing the coupling of BH_4 with eNOS, favoring the generation of active forms of BH_4 from inactive BH_2 and assuring the direct activation of eNOS independent of BH_4.[85] miRNAs may be intricately linked to the functioning of folate. Both are associated with aberrant phenotypes ranging from neural tube defects to tumor genesis. Misexpression of various miRNAs has been demonstrated in neural tube defects resulting from folate deficiency.[86] Folate deficiency state is also linked with many cancers,[87] and recently, altered expression of miRNAs has been shown under conditions of cellular stress in vitro.[88] Taking this into consideration, the improvement in endothelial function after folate supplementation in diabetes and coronary artery disease may also be linked to the modulation of miRNAs. Adding to this, obesity-linked hormones such as leptin are also subject to transcriptional regulation.[89] Table 1 shows the involvement of various miRNAs in heart disease.

miRNA-based therapies

Antagomirs

Antagomirs are a sequence of antisense oligonucleotides designed to act against the targeted miRNA involved in disease pathology. They may act either by competing with miRNA and inhibiting the RISC function or may interfere with the processing of primary miRNA.[90] Suppression of miRNA results in derepression of protein by inhibiting the degradation of concerned mRNA or preventing the translational suppression by miRNA. As discussed above, antagomirs against miR-208 and miR-21 have given useful results.[25,28] To increase the binding affinity of antagomirs to

their target miRNA, certain modifications like 2′-O-methyl and 2′-O-methoxy ethyl are introduced to the oligonucleotides. Further addition of phosphorothioate groups leads to the formation of tiny LNAs, which adds to the biostability and increases delivery to the tissues as well. Tiny LNAs may be well suited for inhibition of disease-specific miRNAs.[91] However, there are some shortcomings of this antisense technique. Being polyanionic, they are not suitable for oral use. Only routes used currently are intravenous and subcutaneous.[90] In addition, there are problems because of poor cellular uptake and degradation by nucleases in plasma. Further concern is of toxicity. Toxicities may arise because of the chemical nature of antagomirs as in case of phosphorothioate oligonucleotides, which can activate the innate immunity[92] and complement cascade.[93] Toxicity may also arise because of hybridization with and inactivation of unintended target RNA resulting in off-target side effects. These effects can be minimized by stringent selection of sequences.[90]

miRNA mimics

These are synthetic miRNAs which are useful in miRNA-deficient conditions.[94] Using a suitable viral vector, they can be continually expressed to circumvent the problem of miRNA downregulation in underlying disease. Short double-stranded RNA sequences are used for loading into RISC. Problem of off-target side effects is unlikely in this approach since only miRNA of interest with sequence same as that of natural miRNA is delivered. As discussed above, miR-1, miR-133 and miR-29 upregulation may be a useful strategy in the management of cardiac hypertrophy and heart failure.[33–36] The field of miRNA mimics is still in the period of basic research.

Several other approaches recently studied include miRNA sponges, miRNA erasers and miRNA occupiers. miRNA sponges consist of a series of oligonucleotides in the 3′UTR of a reporter gene, which have a perfect or imperfect binding site for the target miRNA. Thus, all members of a miRNA family are inhibited by miRNA sponges.[95] miRNA erasers are similar to miRNA sponges except that they are composed of only two copies of antisense sequence with a perfect binding to target miRNA. miRNA occupiers rather bind directly to 3′UTR of mRNA and inhibit the binding of miRNA to mRNA. Occupiers, therefore, carry the advantage of minimizing the off-target effects.[94,96] In vivo efficacies of these approaches are yet to be discovered.

Conclusion

miRNAs represent important entities which serve as diagnostic and prognostic markers in a variety of diseases as well as bear therapeutic interest. The role of miRNAs in cardiovascular and other conditions is being deciphered with ongoing research. Various miRNAs have been implicated in cardiovascular diseases, and targeting these miRNAs has already given useful results in experimental studies. The area needs more investigation related to the underlying mechanisms as more and more miRNAs are discovered. In days to come, technologic advances may facilitate pharmacodynamic understanding and pharmacokinetic maneuvering to make medical implications a sound reality. Clinical trials in humans employing the use of antagomirs have already begun, and the field of cardiovascular disease is hopefully the next to be targeted.

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