Targeting microRNAs in heart failure

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A B S T R A C T

MicroRNAs play pivotal roles in cardiac disease, and their therapeutic modulation raises exciting and unique opportunities, as well as challenges in the path toward clinical development and implementation. In this review, we provide a detailed overview of recent studies highlighting the important role of microRNAs in heart failure (HF) and the potential use of microRNA-based technology for diagnosis, prevention, and treatment of HF. We will focus on the strategies presently used for microRNA-based therapy by discussing their use and drawbacks, as well as the challenges and future directions for their development in the context of human HF.

Key Words: MicroRNA, Therapeutics, Heart failure.

Introduction

Heart failure (HF) is one of the major worldwide causes of death and disability. HF is a complex and progressive disease composed of several clinical syndromes, which result from the inability of the heart to provide adequate amount of blood to the organism and maintain its metabolic requirements. Moreover, HF can develop from several pathological conditions including myocardial infarction (MI), pressure overload (aortic stenosis and hypertension), inflammatory heart muscle disease (myocarditis), and volume overload (valvular regurgitation) [1]. Within these different etiologies, prolonged stress stimulates a ventricular remodeling process involving diverse molecular and cellular events such as genetic alterations, hypertrophic growth, fibrosis, apoptosis, and endothelial dysfunction with subsequent weakened cardiac structure and impaired contractile function [2].

MicroRNAs constitute a growing class of non-coding small RNAs that act as molecular switches of gene expression and are known to regulate complex cardiac signaling and transcriptional circuits during cardiac development and disease. The global significance of microRNAs during cardiac development was elucidated by the generation of cardiac-specific Dicer knockout mice. Since Dicer is an RNAse III endonuclease responsible for cleavage of the precursor microRNA into an active mature microRNA, depletion of Dicer leads to disruption of the global regulation of microRNA expression and subsequent alterations in target gene expression levels. Not surprisingly, Dicer ablation resulted in embryonic lethality due to double outlet right ventricular and ventricular septum defects [3]. Whereas, in the adult heart, conditional Dicer depletion resulted in adverse cardiac remodeling manifested by cardiac hypertrophy and fibrosis, upregulation of fetal cardiac gene expression, and cardiac dysfunction suggesting a global requirement of microRNAs to maintain homeostasis in the adult myocardium [4].

Besides exhibiting developmental stage- and tissue-specific expression patterns, microRNAs also regulate distinct cellular processes such as proliferation, differentiation, cell metabolism, apoptosis, and angiogenesis. Presently, advances in...
microRNA-based technology allow researchers to modulate the cellular levels of specific microRNAs in order to ameliorate cardiac remodeling and ultimately improve or design microRNA-based therapeutic tools. In the present review, we provide an overview of recent studies highlighting the important role of microRNAs in HF and the potential use of microRNA-based technology for diagnosis, prevention, and treatment of HF.

**microRNAs in heart failure**

The myocardial tissue is composed of different cell types including heart muscle cells, endothelial cells, smooth muscle cells, and fibroblasts, each of which contribute to the distinct structural, mechanical, biochemical, and electrical properties of the heart (Fig.). In response to cardiac injury or stress, cellular alterations such as interstitial fibrosis, angiogenesis, cellular hypertrophy, and inflammation occur, which can lead to the onset of cardiac disease and/or progression to HF and may also determine the severity of clinical outcomes. As crucial regulators of pathological cardiac remodeling, microRNAs constitute attractive therapeutic targets, and several tools have been developed to specifically and efficiently modulate microRNA levels in vivo and to directly target the different cellular processes associated with cardiac disease (Fig.).

**Fibrosis**

Cardiac fibroblasts contribute to adverse cardiac remodeling in response to stress or injury via secretion of matrix metalloproteinases and collagen leading to extracellular matrix modulation and interstitial fibrosis formation. Fibrosis is an important part of the healing process and when excessive, it hampers contractility and increases the risk for arrhythmias. Therefore, blocking or reversing the formation of fibrosis may constitute an important therapeutic avenue for the treatment of HF, and a number of microRNAs have previously been identified to critically affect fibrosis regulation.

**microRNA-21:** miR-21 is highly expressed in cardiac fibroblasts compared to other cardiac cell types and is upregulated in failing human myocardium, as well as in myocardium from murine models of HF [5]. There are, however, controversial findings concerning the importance of miR-21 in HF. miR-21 regulates fibroblast proliferation and survival by inhibiting Sprout homolog 1 (Spry1) and subsequently activating ERK-MAP kinase signaling. Inhibition of miR-21 by cholesterol-conjugated antagomirs in a pressure-overload-induced cardiac disease model reduced ERK-MAP kinase activity, decreased interstitial fibrosis, and improved cardiac function. Remarkably, inhibition of miR-21 3 weeks after aortic banding, as a model of established cardiac hypertrophy, could still attenuate cardiac fibrosis and dysfunction [5].

In contrast, others demonstrated that miR-21 inhibition by genetic deletion or systemic administration of locked nucleic acid-modified (LNA) antimiR oligonucleotides does not reduce pathological myocardial remodeling nor prevents cardiac dysfunction in different mouse models of HF [6]. These findings suggest that caution is needed when interpreting studies using antisense approaches to elucidate the function of individual microRNAs in vivo. Moreover, following acute MI, the expression levels of miR-21 in the infarcted areas were reduced, and overexpression of miR-21 in this setting even decreased myocardial infarct size [7]. These contradictory results raise questions regarding not only the discrepancy between data obtained from antisense and genetic deletion techniques but also from, maybe more important, the therapeutic potential of miR-21 in HF. Although several, mainly technical, possibilities to justify the contradictory findings were suggested by the different authors, it is clear that future studies are required to clarify the biology of miR-21 and its role in HF, preferably including cell-type-specific genetic deletion strategies for miR-21.

**microRNA-29:** miR-29 family members are selectively expressed in cardiac fibroblasts and target mRNAs that promote extracellular matrix deposition [8]. All different family members are downregulated in areas adjacent to the infarct, thereby derepressing their targets and resulting in increased cardiac fibrosis [9]. In a recent study [10] where a set of microRNAs was measured in the plasma of patients with hypertrophic cardiomyopathy to identify which

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*Fig. – microRNAs regulate different molecular processes during heart failure. MicroRNA exert their regulatory roles in the different cardiac cell types (cardiomyocytes, endothelial cells, fibroblasts, and inflammatory cells) being able to induce (red boxes) or repress (blue boxes) different pathophysiological processes that are associated with cardiac pathological remodeling in heart failure (cardiac hypertrophy, inflammation, angiogenesis, and fibrosis).*
Angiogenesis

Angiogenesis, the complex process of blood vessel formation, involves synergistic effects of distinct growth factors that can either be physiological or pathological. Pathological cardiac hypertrophy correlates with reduced capillary density, subsequent myocardial ischemia, and eventually HF. Ameliorated blood flow, revascularization, and myocardial function by means of therapeutically induced angiogenesis may promote new repairing mechanisms and myocardium survival and is therefore, a promising therapy for cardiovascular disease.

Studies aimed to better understand the angiogenic mechanisms in the heart have revealed a subset of microRNAs with the potential to regulate pro- or anti-angiogenic factors and proper endothelial cell function [17]. While a wealth of discoveries regarding the action of angiogenic microRNAs in cancer identified signaling pathways used to promote or inhibit angiogenesis, their participation and mode of action during vascular remodeling induced by cardiac pathological insults remain to be clarified. Understanding those mechanisms in various cardiac angiogenic settings could introduce new microRNA-based therapies, such as mimics and antagonists, to manage and/or cure HF.

microRNA-17–92: Gain- or loss-of-function studies targeting individual members of the miR-17 ~ 92 cluster revealed miR-17, miR-18, miR-19, and miR-20 as the ones with anti-angiogenic function, while both in vitro and in vivo assays revealed miR-17/20 inhibition to exert the most potent effect on neo-vascularization [18]. Furthermore, miR-17 inhibition in rat and mouse models of pulmonary hypertension beneficially affects lung and heart remodeling [19] by resulting in lower number of muscularized vessels, increased pulmonary artery acceleration time, decreased wall thickness of pulmonary vessels, and subsequent improved cardiac outcome. miR-92a, mostly upregulated under cardiac ischemia, inhibits angiogenesis by targeting a pro-angiogenic factor, integrin α5 (ITGA5) and treatment of infarcted mice hearts with a specific antagonist improved cardiac function due to enhanced angiogenesis [20]. A similar therapeutic approach but administrating a locked nucleic acid-modified antisense (LNA)-miR-92a to a porcine model of ischemia reperfusion also resulted in decreased infarct area, increased capillary formation and consequently, cardiac function recovery [21].

microRNA-126: This endothelial cell-enriched microRNA is crucial for maintenance of vascular integrity, endothelial cell proliferation, migration, and sprouting during embryonic development as well as in cardiac response to injury [22]. As such, 40% of miR-126 null-mice display embryonic lethality, while 50% of the surviving animals died 1 week after MI due to vascular deficiency. Furthermore, circulating miR-126 levels were decreased in time, in acute MI patients, suggesting its potential utility as a novel biomarker for clinic diagnosis of acute MI [23].

microRNA-24: This endothelial cell-enriched and pro-apoptotic microRNA is strictly elevated in the peri-infarct zone endothelium, following MI [24,25]. In vitro overexpression of miR-24 impairs endothelial cell angiogenic functions including proliferation, sprouting, and tube formation, whereas its inhibition promotes angiogenesis [24,25]. Although these effects are attributed to inhibition of the endothelium-enriched transcription factor GATA2, the p21-activated kinase PAK4 and their respective downstream targets [24], miR-24 is also known to target e-NOS, a pro-angiogenic factor [25]. In vivo inhibition of miR-24 resulted in preserved cardiac function, decreased infarct size, and enhanced vascularization after MI, as endothelial cell apoptosis was inhibited [24,25].

microRNA-210: Unlike the previously mentioned microRNAs, miR-210 directly exerts its pro-angiogenic function via the cardiomyocytes by inducing the release of angiogenic factors such as leptin, interleukin-1-a, and tumor necrosis factor-alpha (TNF-α) [26]. Overexpression of miR-210 in a mouse model of MI enhanced capillary formation, reduced apoptosis, and infarct size, and consequently improved cardiac function [26]. As diagnostic tool, patients with improved plasma brain natriuretic peptide (BNP) profiles are classified in a subgroup of patients with low plasma miR-210 levels, suggesting that plasma miR-210 levels may reflect a mismatch between cardiac pump function and oxygen demand in the peripheral tissues, and therefore be a biomarker for chronic HF in addition to plasma BNP concentrations [27].
microRNA-146a: This microRNA is abundantly expressed in the heart and is upregulated during development of HF. In mice with cardiomyocyte-restricted genetic deficiency for the Dicer protein, miR-146a was found to be upregulated at juvenile and adult ages, suggesting a non-cardiomyocyte origin of miR-146a [4]. A recent study identified miR-146a as a key player in cardiomyocyte response during peripartum cardiomyopathy (PPCM), with increased levels being triggered by the activation of endothelial cells and their subsequent exosome-mediated miR-146a release [28]. Targeting of miR-146a by LNA and antagomir strategies in a mouse model of PPCM prevented the development of the disease by reducing cardiac fibrosis, increasing capillary density, and improving cardiac function [28]. In contrast, overexpression of miR-146a in a mouse model of ischemia reperfusion conferred cardiac protection by indirect decrease of NF-κB activity [29].

microRNA-34: The miR-34 family consists of three members: miR-34a, miR-34b, and miR-34c, all of them displaying increased expression levels in response to cardiac stress and in the ageing heart [30]. In vivo ablation of miR-34a reduced age-associated cardiomyocyte death, reduced fibrosis, and improved myocardial function in a mouse model of MI. The observed effects were attributed to angiogenesis induction in the border zone of the infarcted area and reduced DNA damage in cardiomyocytes [30]. In a study where all miR-34 family members were inhibited and a common seed region was targeted, greater beneficial cardiac outcomes were observed in mouse models of MI and pressure overload, compared to inhibition of miR-34a alone [31]. The later study suggests a greater therapeutic potential by targeting whole microRNA families rather than single members.

microRNA-26a: miR-26a is an anti-angiogenic microRNA with a dynamic expression pattern in response to ischemia/reperfusion. Its expression is increased just 1 h after 45 min of ischemia-reperfusion-induced myocardial injury, whereas it was decreased 24 h later [32]. Furthermore, therapeutic inhibition of miR-26a by LNA technology in mice after acute injury induced by left arterial descending artery banding (LAD) resulted in protective cardiac effects with decreased infarct size and improved cardiac function [32]. This outcome was attributed to improved angiogenesis observed within 2 days following LAD [32]. Moreover, miR-26a seems to act as a previously unrecognized crucial regulator of pathological angiogenesis by inhibiting both the expression and phosphorylation of SMAD1 and subsequently downregulating its pro-angiogenic downstream target Id1 [32].

Hypertrophy and metabolic balance

Cardiac hypertrophy is the thickening of the ventricular walls in response to chronic cardiac stress. Intrinsic changes including re-expression of fetal genes, alterations in excitation–contraction coupling, and changes in energetic and metabolic balance induce cardiomyocyte size growth. Left ventricular hypertrophy (LVH) is the most potent predictor of adverse cardiovascular outcomes in the cardiovascular disease (CVD) population, and it is an independent risk factor for coronary heart disease, sudden death, HF, and stroke. Hence, targeting hypertrophy may significantly reduce adverse clinical endpoints and aid in the treatment of HF. Studies from the past decade have demonstrated that microRNAs are atypically expressed in hypertrophic hearts, and gain- and loss-of-function studies using appropriate disease models have revealed distinct roles for specific microRNAs in pathological cardiac hypertrophy.

microRNA-25: The role and therapeutic potential of miR-25 in HF is supported by recent but somewhat controversial findings. Cardiac expression of miR-25 decreases during the development of cardiac hypertrophy and HF in rodents, while its inhibition with an antagomir approach exacerbates the pathological remodeling response and accelerates progression to HF [33]. The observed effects were ascribed to increased levels of a basic helix-loop-helix (bHLH) transcription factor, HAND2, which besides being a central player during right ventricle development, is also activated in the stressed, adult heart as part of the embryonic gene program, a hallmark of HF [33]. Paradoxically, others reported increased levels of miR-25 in rodent and human HF, which by directly targeting SERCA2a cause diminished Ca2+ uptake and impaired cell contractility [34]. The divergences between these studies likely relate to different antisense chemistries, mode of delivery, or degree of silencing achieved. Although other studies could confirm miR-25 downregulation in HF [35,36] such conflicting reports probably reflect our poor understanding not only of the complexity of microRNA biology, regulation, and function but also of the variations between different animal models of disease.

microRNA-199b: miR-199b is a very solid example that microRNAs can incite the cardiac hypertrophic program. Cardiac expression levels of miR-199b are increased in LVH and HF, and its overexpression strongly promotes cardiac hypertrophic growth. miR-199b is directly regulated by the calcineurin/NFAT pathway [37] and is able to regulate its own signaling strength by promoting translocation of NFAT to the cytoplasm and indirectly inactivate its own regulator, Dyrk1a. Antagomir-mediated knockdown of miR-199b in a mouse model of cardiac pressure overload, prevented development of HF and, more interesting, reversed later stage cardiac failure phenotypes and dysfunction, bringing miR-199b forward as a very promising therapeutic target for the treatment of hypertrophic heart disease and HF.

microRNA-214: The miR-199a/214 cluster is encoded by a large non-coding RNA, DNM3os, which is located in the opposite strand of the DNM3 gene. Among this cluster, miR-214 plays a regulatory role in the metabolic switch from mitochondrial fatty acid oxidation towards glucose metabolism in the heart in response to hemodynamic stress [38]. Silencing miR-214 in a mouse model of cardiac pressure overload normalized mitochondrial fatty acid oxidation, attenuated cardiac pathological remodeling, and preserved cardiac function due to the derepression of its target gene PPARG [38].

microRNA-1: This anti-hypertrophic cardiomyocyte-enriched microRNA is suppressed in different murine models of HF and negatively controls the calcium signaling members calmodulin and myocyte enhancer factor 2 A (MEF2A) [39]. MiR-1 can effect Ca2+ extrusion from cardiomyocytes via its direct targets Na+-Ca2+ exchanger 1 (NCX1) and AnxA5. Because AnxA5 binds to NCX1 and controls its activity, once increased during HF...
AnxA5 impairs NCX1 function leading to reduced Ca\(^{2+}\) extrusion [40,41]. This regulatory loop is controlled by the serum response factor (SRF) transcription factor and balances the translation of NCX1 and AnxA5 proteins [40]. The AKT/FoxO3a pathway can also exert a regulatory role on miR-1 during HF [41,42]. This happens either through insulin-like growth factor 1 (IGF-1), a direct target of miR-1 that induces phosphorylation of AKT which, in turn, inactivates the FoxO3a transcription factor causing miR-1 downregulation in HF [42], or through increased Ca\(^{2+}\) levels in HF causing calcium/calmodulin-dependent protein kinase (CaMKK)-dependent activation of AKT, inactivation of FoxO3a, and subsequent decline in miR-1 levels [42]. Conversely, induced expression of miR-1 by adenoviral gene therapy or cardiotoxic adeno-associated 9 vector (AAV9) not only rescued the hypertrophic phenotype induced by isoproterenol administration in mice [39] or by chronic pressure overload in rats, respectively, but also attenuated disease progress. The observed effects were long lasting suggesting long-term therapeutic effectiveness of miR-1 overexpression in pathological cardiac remodeling [43]. Moreover, an inverse correlation between the expression of miR-1 and circulating levels of heart-type fatty acid binding protein 3 (FABP3), responsible for fatty acid uptake in cardiomyocytes, was described, suggesting that FABP3 levels can be useful in determining miR-1 expression levels in patients with heart or metabolic disease [44].

microRNA-133: Despite some controversial findings about miR-133 and its correlation with cardiac hypertrophy, miR-133 is an established muscle-enriched microRNA that is highly expressed in the healthy heart. This microRNA is clustered with miR-1 and both are encoded from two genomic loci (miR-1-1/133a-2 on mouse chromosome 2 and miR-1-2/miR-133a-1 on mouse chromosome 18). Genetic deletion of both copies of this cluster leads to increased mortality of neonatal mice as a result of ventricular-septal defects (VSDs) associated with enhanced proliferation of neonatal cardiomyocytes and abrupt expression of smooth muscle genes in the heart, mainly the direct target genes cyclin D and SRF [11]. Although deletion of a single genomic loci results in vital mice with normal cardiac morphology and contractility [45], these single mutants develop long QT durations at low heart rates pointing to the importance of miR1/miR-133a cluster during cardiac repolarization [45]. Such phenotype could be abrogated after inhibiting β-adrenergic signaling and L-type calcium channels by pharmaceutical interventions suggesting miR-1/miR-133a cluster to be a regulator of cardiac repolarization via adrenergic signaling [45]. Moreover, inhibition of miR-133 expression by a specific antagonist spontaneously induces cardiac hypertrophy and impairs cardiac function by direct targeting of RhoA and Cdc42, both being part of the Rho subfamily of small GTP-binding proteins, and NELF-A/Whsc2, a negative regulator of RNA polymerase II [46].

Conversely, gain of function studies showed that inducing cardiac-specific-miR-133 expression decreases cardiomyocyte apoptosis and collagen deposition and promoted cardiac function following chronic pressure overload. The underlying mechanism involves the direct inhibition of multiple components of the β1AR signaling pathway including β1AR itself and its downstream effectors adenylyl cyclase type VI (ACVI) and cAMP-dependent protein kinase catalytic subunit beta (PKA Cbeta), a key modulator of the β1AR-mediated accumulation of cAMP [47]. In another study, a similar approach to induce miR-133 expression in cardiac tissue also resulted in less fibrosis, less apoptosis, and improved diastolic function after pressure overload but did not affect cardiac hypertrophic growth [48] despite the long QT intervals demonstrated in the ECG. Whether overexpression of miR-133 can diminish cardiac dysfunction in the failing heart remains disputable. Nevertheless, it is clear that this microRNA has a multidisciplinary role during pathological cardiac remodeling by acting on different cellular processes. In agreement, a recent report shows miR-133a to be attenuated and contribute to cardiac hypertrophy in diabetic hearts [49], while overexpression of miR-133a attenuates cardiac fibrosis in diabetics [50]. These effects are related to control of DNA methylation by miR-133 via direct regulation of DNA methyl transferases in diabetic cardiomyocytes [51]. The role of miR-133 in regulating cardiac hypertrophy, fibrosis, epigenetic modification, and β-AR signaling points miR-133 as a promising therapeutic target in managing HF.

**microRNA-208:** The microRNA-208 family is composed of miR-208a and miR-208b, encoded by intronic regions in the α-MHC and β-MHC genes, respectively. They show a similar expression pattern in rodents as their host genes with miR-208a being expressed in the adult heart while miR-208b is enriched in embryonic hearts. Gain- and loss-of-function studies in rodents showed that miR-208a is required for the expression of β-MHC in stressed hearts, and therefore directly implicated in the isoform switch from α-MHC to β-MHC that characterizes pathologic hypertrophy and HF. In fact, inhibition of miR-208a levels in Dahl hypertensive rats, a hypertension-induced model of HF, not only prevents pathologic myosin switching and cardiac remodeling but also improves cardiac function and survival [52]. miR-208 was recently identified circulating outside of cells, in body fluids such as blood, saliva, and urine. Whether miR-208 is released from damaged cardiomyocytes into the bloodstream remains unknown, but the fact that this microRNA is enriched in the heart and detected in peripheral blood makes it potentially useful for the diagnosis and treatment of HF.

**microRNA-23:** The two isomers, miR-23a and miR-23b, are part of the miR-23a/27a/24-2 cluster [53] and are both upregulated under conditions of cardiac stress. miR-23a is involved in cardiac hypertrophy as a downstream target of the calcineurin/NFAT pathway and targets the anti hypertrophic protein, muscle ring-finger protein 1 (MURF1) [53]. Therapeutic studies in rodents using antagonist to silence miR-23a showed attenuation of cardiac hypertrophic growth in different models of HF [53,54] with the underlying mechanism involving direct targeting of the forkhead box O3 gene (FOXO3A), a transcription factor involved in the regulation of cardiac hypertrophy [54]. Moreover, miR-23a, similar to miR-199b, is regulated by the calcineurin/NFAT signaling pathway, and it will be interesting to know how both microRNAs integrate to mediate calcineurin/NFAT signaling during cardiac hypertrophy and HF.

**microRNA-378:** This anti-hypertrophic microRNA was identified in a functional high-throughput screen for morphological changes in neonatal rat cardiomyocytes after transfection with
Inflammation

Inflammation is the process of immune cell influx to the side of injury or infection. Increasing evidence point to the importance and the therapeutic potential of inflammatory pathways in the treatment of HF. Although several microRNAs have been identified to regulate the immune system and inflammation in the context of other pathological conditions (as extensively reviewed in [57]), very few microRNAs have been suggested as main regulators of the chronic inflammatory processes that accompany HF and so far only miR-155 has been extensively studied in this context.

miRNA-155: Two different studies demonstrated the involvement of miR-155 in cardiac hypertrophy via diverse mechanisms. Recently, miR-155 was shown to be enriched in cardiomyocytes and whole-body loss of miR-155 to be protective against cardiac injury induced by pressure overload [58], partly due to repression of junnonji AT-rich interactive domain 2 (jarid2) [58]. On the other hand, the cardioprotective effect of miR-155 silencing has been associated to its decreased expression levels in macrophages rather than in cardiomyocytes [59]. Silencing of miR-155 in leukocytes markedly reduced cardiac inflammation, hypertrophy, and cardiac dysfunction following pressure overload in mice. Moreover, in vivo cardiomyocyte-specific modulation of miR-155 did not affect cardiac remodeling. In macrophages, miR-155 is responsible for the repression of suppressor cytokine signaling 1 gene (Socs1), which inhibits paracrine hypertrophic stimulation [59]. The cardioprotective effect of miR-155 silencing supports the causative significance of inflammatory signals in hypertrophic cardiac disease, positioning miR-155 forward as a potential therapeutic target for cardiac hypertrophy.

Advantages and disadvantages of microRNA therapeutics

Intensive research in recent years aimed at targeting differential microRNA expression as a novel therapeutic approach. In vivo modulation of microRNAs with antisense oligonucleotides as microRNA inhibitors or with modified microRNA mimics such as plasmid or lentiviral vectors carrying microRNA sequences to increase the expression of microRNAs was proven successful in the aforementioned preclinical studies (Tables 1 and 2), but to date no clinical trials were initiated yet for cardiovascular diseases.

Two companies are currently developing inhibitors of miR-122 for the treatment of Hepatitis C virus (HCV). While Regulus Therapeutics developed a GalNAC-conjugated anti-miR-122 that recently entered a phase I study on healthy volunteers (http://www.regulusrx.com), Santaris Pharma developed another miR-122 targeting drug, miravirsen, for which phase I and phase II trials have been completed [60,61]. Such achievements confirm that pharmacological inhibition of microRNA expression and activity can be achieved and is a feasible therapeutic strategy in patients. Nevertheless, several challenges still remain to implement the clinical use of microRNA-targeting drugs. Although microRNA-based therapeutics can provide stable and sustained effects in various HF models, issues related to delivery, dosage, and specificity still remain to be addressed in the clinical use of these therapeutic tools. For instance, cell-type or tissue-specific delivery of microRNA-based therapeutics to prevent unintended off-target effects is an important issue in developing an efficient microRNA-based therapy. To overcome this issue, there are ongoing studies with ultrasound-mediated microrbuble technique to provide organ-specific delivery of miR antimiRs/mimics [62,63]. Importantly, this technique has been shown to cause no functional or histological damage in the swine hearts [62]. Additionally, to obtain efficient in vivo delivery and tissue distribution, several synthetic vehicles including cationic liposomes, polymers, inorganic (gold) nanoparticles, dendrimers, and micelle have been generated and are being investigated (extensively reviewed in [64,65]).

Another important aspect is the dosage and administration frequency of microRNA-based drugs. Anti-miRs have often been administered at high concentrations in order to obtain prolonged stable knockdown, but further assessment is necessary regarding the effect of long-term inhibition of microRNAs in vivo in order to develop effective microRNA therapeutics.

Additionally, while decreased microRNA expression levels after antimiR treatment is often assessed as a measure of effectiveness, question remains whether this is the most reliable evaluation to determine microRNA inhibition. For example, binding of an antimiR to its target microRNA may interfere with detection but, depending on the chemistry,
Table 1 – Overview of preclinical studies applying microRNA-based therapeutics to inhibit specific microRNAs in relevant models in heart failure.

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>miRNAs</th>
<th>Doses</th>
<th>Delivery</th>
<th>Target</th>
<th>Model</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antagomir</td>
<td>miR-199b</td>
<td>80 mg/kg/bw</td>
<td>IP</td>
<td>Dyrk1a</td>
<td>TAC (6 wk)</td>
<td>Decreased fibrosis and hypertrophy and improved cardiac function</td>
<td>Da Costa Martins et al. [37]</td>
</tr>
<tr>
<td></td>
<td>miR-21</td>
<td>80 mg/kg/bw</td>
<td>Implanted jugular vein catheter</td>
<td>Spry1</td>
<td>TAC (3 or 6 wk)</td>
<td>Decreased fibrosis and improved cardiac function</td>
<td>Thum et al. [5]</td>
</tr>
<tr>
<td></td>
<td>miR-29b</td>
<td>80 mg/kg/bw</td>
<td>IP</td>
<td>IGF-1 and LIF</td>
<td>TAC (2 wk)</td>
<td>No effect on cardiac function and excess amount of perivascular fibrosis</td>
<td>van Rooij et al. [9]</td>
</tr>
<tr>
<td></td>
<td>miR-17</td>
<td>8 mg/kg/bw</td>
<td>IV</td>
<td>p21</td>
<td>Hypoxia (10% O2, 28 days) or monocrotaline (36 days)-induced pulmonary hypertension (PH)</td>
<td>Decreased right ventricular systolic pressure and total pulmonary vascular resistance index, increased pulmonary artery acceleration time, normalized cardiac output, and decreased pulmonary vascular remodeling</td>
<td>Pullamsetti et al. [19]</td>
</tr>
<tr>
<td>miR-92a</td>
<td>8 mg/kg/bw</td>
<td>IV</td>
<td>ITGA5</td>
<td>MI (2 wk)</td>
<td>Improved cardiac function, enhanced capillary density and decreased apoptosis</td>
<td>Bonauer et al. [20]</td>
<td></td>
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<tr>
<td>miR-24</td>
<td>5 mg/kg/bw</td>
<td>RO</td>
<td>PAK4 and GATA2</td>
<td>MI (2 wk)</td>
<td>Increased capillary density and improved cardiac function</td>
<td>Fiedler et al. [24]</td>
<td></td>
</tr>
<tr>
<td>miR-24</td>
<td>80 mg/kg/bw</td>
<td>IV</td>
<td>N/A</td>
<td>TAC (25 wk)</td>
<td>No effect on hypertrophy, improved cardiac function, protected E-C coupling</td>
<td>Li et al. [73]</td>
<td></td>
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<tr>
<td>miR-146a</td>
<td>8 mg/kg/bw</td>
<td>IV</td>
<td>NRAS</td>
<td>Cardiomyocyte-restricted stat3 KO model for peripartum cardiomyopathy</td>
<td>Attenuated cardiac dysfunction and reduced fibrosis</td>
<td>Halkein et al. [28]</td>
<td></td>
</tr>
<tr>
<td>miR-34a</td>
<td>8 mg/kg/bw</td>
<td>IV</td>
<td>PNUTS</td>
<td>MI (2 wk)</td>
<td>Improved cardiac function, increased capillary density, and reduced apoptosis and fibrosis</td>
<td>Boon et al. [30]</td>
<td></td>
</tr>
<tr>
<td>miR-25</td>
<td>80 mg/kg/bw</td>
<td>IP</td>
<td>Hand2</td>
<td>TAC (4 wk)</td>
<td>Enhanced cardiac dysfunction and increased hypertrophy and fibrosis</td>
<td>Dirkx et al. [33]</td>
<td></td>
</tr>
<tr>
<td>miR-23a</td>
<td>25 mg/kg/bw</td>
<td>Implanted osmotic minipumps</td>
<td>MuRF1</td>
<td>Isoproterenol infusion (1 wk)</td>
<td>Improved cardiac function and reduced hypertrophy</td>
<td>Lin et al. [53]</td>
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<tr>
<td>miR-132</td>
<td>80 mg/kg/bw</td>
<td>RO</td>
<td>Fox03</td>
<td>TAC (3 wk)</td>
<td>Decreased hypertrophy and fibrosis and preserved cardiac function</td>
<td>Ucar et al. [56]</td>
<td></td>
</tr>
<tr>
<td>miR-199a–214</td>
<td>20 mg/kg/bw</td>
<td>IP</td>
<td>PPARd</td>
<td>TAC (6 wk)</td>
<td>Decreased hypertrophy and fibrosis and preserved cardiac function</td>
<td>El Azzouzi et al. [38]</td>
<td></td>
</tr>
<tr>
<td>LNA</td>
<td>miR-92a</td>
<td>0.03 mg/kg/bw</td>
<td>Catheter-based antegrade or retrograde local delivery to the heart</td>
<td>Pig ischemia/reperfusion model</td>
<td>Improved cardiac function, increased capillary density and decreased inflammation</td>
<td>Hinkel et al. [21]</td>
<td></td>
</tr>
<tr>
<td>miR-146a</td>
<td>20 mg/kg</td>
<td>IV</td>
<td>NRAS</td>
<td>Cardiomyocyte-restricted stat3 KO (model for peripartum cardiomyopathy)</td>
<td>Improved cardiac function, reduced fibrosis, and enhanced capillary density</td>
<td>Halkein et al. [28]</td>
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</table>
Antimirs are able to inhibit microRNAs without inducing their degradation [66]. The derepression levels of microRNA targets as a secondary endpoint can also be a measure of antimir efficacy. The strength of microRNA therapeutics may be explained by the fact that unlike conventional drugs (one drug and one target), microRNAs have multiple targets involved in various cellular processes. Targeting one microRNA may lead to alterations in several cellular pathways and while this may result in undesired side effects, so far these were not reported. To date, preclinical studies have demonstrated long lasting and reversible effects of microRNA-based therapeutics without adverse side effects or histopathological changes in the experimental animals. Their new mechanism of action, their ability to function as master regulators of the genome, and the lack of adverse events in healthy cells or tissue make microRNAs promising therapeutic targets for current and future technology and product development.

While generation of adjuvant carrier or delivery systems that increase stability, prevent renal clearance, and enhance cellular uptake by target tissues is scientifically and technically challenging, it will ultimately be clinically rewarding. Next to the therapeutic value, microRNAs are emerging as potential diagnostic and prognostic biomarkers in cardiovascular diseases including atherosclerosis, myocardial infarction, heart failure, and hypertension [67]. Secretion of microRNAs into apoptotic bodies, micro vesicles, exosomes, and in association with RNA binding proteins not only protects them from degradation but also enables their detection in the circulation [68–71]. However, whether these microRNAs are involved in the disease process or simply serve as biomarkers remain elusive, and further studies including large patients cohorts are needed.

**Future directions and conclusion**

Along with understanding the importance of microRNAs in various cellular functions and pathophysiologic conditions, they are emerging potential therapeutic targets in cardiac diseases. Despite the need for better understanding of microRNA function, tissue- and cell-type-specific delivery of microRNA-based therapeutics and safety to avoid off-target effects, the first clinical trials (phase I and II) using antimiR-122 against chronic hepatitis have been successful [72]. Besides antimiR technology, strategies to increase microRNA expression at specific sites are also being developed. In fact, MRNA therapeutics (http://www.mirnaRx.com) recently initiated a new phase I clinical trial to induce expression of miR-34 in primary and metastatic liver cancer. miR-34 is a naturally occurring microRNA tumor suppressor that is lost or downregulated in tumors of patients with a variety of cancers. Administration of the developed miR-34 mimic (MRX34), encapsulated using liposomal formulation induced tumor regression, enhanced the survival of mice carrying hepatocellular carcinomas, and inhibited the growth of other non-hepatic tumors.

Despite the fact that only one decade has passed since the identification of the first human microRNA, microRNAs have emerged as promising targets for therapeutic interventions in treating many types of pathologies including
<table>
<thead>
<tr>
<th>Inducers</th>
<th>miRNAs</th>
<th>Doses</th>
<th>Delivery</th>
<th>Target</th>
<th>Model</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>miR-101a</td>
<td>$1 \times 10^9$ plaque-forming units</td>
<td>Injection to left ventricular cavity</td>
<td>N/A</td>
<td>MI (4 wk)</td>
<td>Decreased fibrosis and improved cardiac function</td>
<td>Pan et al. [16]</td>
</tr>
<tr>
<td></td>
<td>miR-1</td>
<td>$2 \times 10^7$ infectious units</td>
<td>Intramyocardial injection</td>
<td>calm1 and calm2</td>
<td>Isoproterenol infusion (2 wk)</td>
<td>No hypertrophy</td>
<td>Ikeda et al. [39]</td>
</tr>
<tr>
<td></td>
<td>miR-133</td>
<td>N/A</td>
<td>Transcoronary delivery</td>
<td>Rhoa, Cdc42, and Whsc2</td>
<td>Akt transgenic mice (hypertrophy)</td>
<td>Decreased hypertrophy</td>
<td>Care et al. [46]</td>
</tr>
<tr>
<td>microRNA precursors</td>
<td>miR-210</td>
<td>25 µg</td>
<td>Intramyocardial injection</td>
<td>Efna3 and Ptp1b</td>
<td>MI (4 and 8 wk)</td>
<td>Increased angiogenesis, decreased apoptosis, and improved cardiac function</td>
<td>Hu et al. [26]</td>
</tr>
<tr>
<td>(minicircles)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lentivirus</td>
<td>miR-146a</td>
<td>$1 \times 10^8$ plaque-forming units</td>
<td>Micro-catheter in common carotid artery</td>
<td>N/A</td>
<td>Ischemia/reperfusion</td>
<td>Reduced infarct size, improved cardiac function, reduced apoptosis, and reduced inflammation</td>
<td>Wang et al. [29]</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>miR-1</td>
<td>$5 \times 10^{12}$ viral genome</td>
<td>Intravenous injection</td>
<td>Fbln2</td>
<td>Ascending aortic banding (AAB) (2 and 9 wk)</td>
<td>Improved cardiac function, decreased hypertrophy, and fibrosis</td>
<td>Karakikes et al. [43]</td>
</tr>
<tr>
<td>(AAV) serotype 9</td>
<td>miR-378</td>
<td>$1 \times 10^{12}$ viral genome</td>
<td>IV</td>
<td>Grb2, Igf1r, Ksr1, and Mapk1</td>
<td>TAC (3 wk)</td>
<td>Improved cardiac function, reduced hypertrophy, and fibrosis</td>
<td>Ganesan et al. [55]</td>
</tr>
<tr>
<td>Mimics</td>
<td>miR-26a</td>
<td>1 nmol/mouse</td>
<td>Tail-vein injection</td>
<td>SMAD1</td>
<td>Exercise (nocturnal running, 9 days)</td>
<td>Decreased physiological angiogenesis induced by exercise</td>
<td>Icli et al. [32]</td>
</tr>
</tbody>
</table>
cardiovascular diseases. The next decade should focus on increasing knowledge and improving technology to solidify microRNA biology and establish microRNA-based therapies as the most effective therapeutic approaches for different human diseases, including HF.

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