Trends in Cardiovascular Medicine ▮ (2015) ▮▮▮–▮▮▮

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/tcm

Targeting microRNAs in heart failure

*Burcu Duygu, Leon J. de Windt, and Paula A. da Costa Martins**

Department of Cardiology, CARIM School for Cardiovascular Diseases, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands

ABSTRACT

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

The authors have indicated there are no conflicts of interest.

L.D.W. was further supported by Grant 311549 from the European Research Council (ERC), Belgium. P.D.C.M. was supported by a Leducq Career Development Award, the Dutch Heart Foundation, Netherlands, Grant NHS2010B261, and a NWO MEERVOUD Grant.

* *Corresponding author*. Tel.: +31 433 882 949.

E-mail address: p.dacostamartins@maastrichtuniversity.nl (P.A. da Costa Martins).

http://dx.doi.org/10.1016/j.tcm.2015.05.008
1050-1738/

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.).

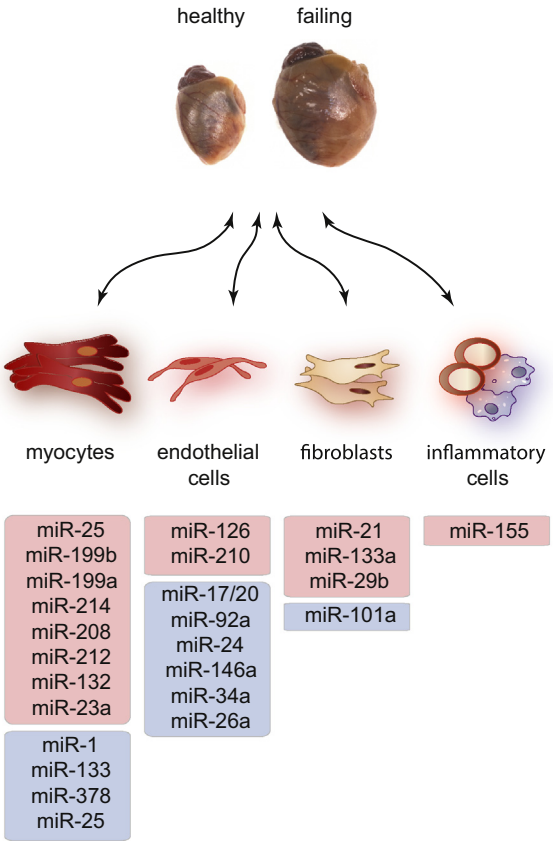

Fig – microRNAs regulate different molecular processes during heart failure. MicroRNA exert their regulatory roles in the different cardiac cell types (cardiomyocytes, endothelial cells, fibroblasts, and inflammatory cells) being able to induce (red boxes) or repress (blue boxes) different pathophysiological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

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 controversial 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

microRNAs can be regarded as biomarkers of the disease, only miR-29a levels were found to correlate with cardiac fibrosis, along with a set of microRNAs related to cardiac hypertrophy. This finding may help to correctly diagnose patients who are at risk of developing cardiac fibrotic remodeling.

microRNA-133: miR-133 is one of the most abundant microRNAs in the heart. The importance of miR-133 in cardiac fibrosis has been highlighted in several reports that miR-133a-1 and miR-133a-2 knockout mice develop a severe myocardial fibrosis response accompanied by increased cardiomyocyte apoptosis and HF [11], which could be explained by the fact that miR-133 directly repressed connective tissue growth factor (CTGF), an important secreted protein during the process of fibrosis [12]. *In vivo* and *in vitro* mechanistic studies showed that increased expression of miR-133 has cardiac protective effects not only by direct targeting the TGF-β1 receptor [13], but it can also act together with miR-29b to upregulate collagen 1A1 and exacerbate the levels of fibrosis in angiotensin II-induced hypertension [14]. Interestingly, a recent study using human foreskin fibroblasts revealed that miR-133a, in concert with several transcription factors, contributes to reprogramming of fibroblasts into cardiomyocytes [15].

microRNA-101a: This fibroblast-enriched microRNA is involved in cardiac fibroblast proliferation and inhibits FBJ osteosarcoma oncogene (FOS), known to activate specific profibrotic pathways, such as those involving miR-21. While miR-101a is downregulated in hypertrophic and post-infarcted hearts [16], its overexpression in infarcted rat hearts revealed positive effects on cardiac function mostly caused by reduced fibrotic tissue formation. Interfering with miR-101a expression may present therapeutic value even though unresolved issues regarding its mechanism of action still remain.

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 antagomirs, 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 neovascularization [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 antagomir 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 reduced 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 exert 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 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 Ca^{2+} 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 PPARβ [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 Ca^{2+} extrusion from cardiomyocytes via its direct targets Na^{+}–Ca^{2+} 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 cardiotropic 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 antagomir 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 decreased 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 adenyl 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-HMC 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 antagomir 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

a library of microRNA precursor molecules [55]. MiR-378 exerts its anti-hypertrophic function by targeting four different components of the MAPK pathway: MAPK1 (also called extracellular-regulated kinase 2), kinase suppressor of ras 1 (KSR1), growth factor receptor-bound protein 2 (GRB2), and IGF1R. Thus, miR-378 sets a good example of the ability of microRNAs to target multiple hits in a single pathway, which also augments their therapeutic value. Furthermore, *in vivo* restoration of miR-378 expression levels in a mouse model of chronic pressure overload resulted in attenuation of pathological remodeling and cardiac dysfunction [55], which could be an effective therapeutic strategy in myocardial disease.

microRNA-212/132: The miR-212/132 microRNA family is upregulated in both murine and human failing hearts [56]. Not only hypertrophic stimuli cause increased expression levels of miR-212 and miR-132 in cardiomyocytes, but also expression of both is necessary and sufficient to induce their hypertrophic growth. Genetic-targeted deletion of miR-212 and miR-132 conferred protection from pressure-overload-induced HF, while their overexpression caused pathological hypertrophy, HF, and death. Curiously, pharmacological approaches to inhibit miR-132 alone were sufficient to preserve cardiac function and attenuate hypertrophy and fibrosis in mice subjected to pressure overload [56]. These therapeutic effects are attributed to increased expression levels of FOXO3A, a miR-132 target gene, and subsequent blunted calcineurin/NFAT signaling. Although decreased expression levels of miR-212/132 inhibit starvation-induced autophagy in cardiomyocytes [56], the study does not correlate this anti-autophagic function to the therapeutic effects of antagomir-mediated miR-132 knockdown.

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.

microRNA-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 jumonji 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 microbubble 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. AntimiRs 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.

Inhibitors	miRNAs	Doses	Delivery	Target	Model	Outcome	References
Antagomir	miR-199b	80 mg/kg/bw	IP	Dyrk1a	TAC (6 wk)	Decreased fibrosis and hypertrophy and improved cardiac function	Da Costa Martins et al. [37]
	miR-21	80 mg/kg/bw	Implanted jugular vein catheter	Spry1	TAC (3 or 6 wk)	Decreased fibrosis and improved cardiac function	Thum et al. [5]
	miR-29b	80 mg/kg/bw	IP	IGF-1 and LIF	TAC (2 wk)	No effect on cardiac function and excess amount of perivascular fibrosis	van Rooij et al. [9]
	miR-17	8 mg/kg/bw	IV	p21	Hypoxia (10% O_2, 28 days) or monocrotaline (36 days)-induced pulmonary hypertension (PH)	Decreased right ventricular systolic pressure and total pulmonary vascular resistance index, increased pulmonary artery acceleration time, normalized cardiac output, and decreased pulmonary vascular remodeling	Pullamsetti et al. [19]
	miR-92a	8 mg/kg/bw	IV	ITGA5	MI (2 wk)	Improved cardiac function, enhanced capillary density and decreased apoptosis	Bonauer et al. [20]
	miR-24	5 mg/kg/bw	RO	PAK4 and GATA2	MI (2 wk)	Increased capillary density and improved cardiac function	Fiedler et al. [24]
	miR-24	80 mg/kg/bw	IV	N/A	TAC (25 wk)	No effect on hypertrophy, improved cardiac function, protected E-C coupling	Li et al. [73]
	miR-146a	8 mg/kg/bw	IV	NRAS	Cardiomyocyte-restricted stat3 KO model for peripartum cardiomyopathy	Attenuated cardiac dysfunction and reduced fibrosis	Halkein et al. [28]
	miR-34a	8 mg/kg/bw	IV	PNUTS	MI (2 wk)	Improved cardiac function, increased capillary density, and reduced apoptosis and fibrosis	Boon et al. [30]
	miR-25	80 mg/kg/bw	IP	Hand2	TAC (4 wk)	Enhanced cardiac dysfunction and increased hypertrophy and fibrosis	Dirkx et al. [33]
	miR-23a	25 mg/kg/bw	Implanted osmotic minipumps	MuRF1	Isoproterenol infusion (1 wk)	Improved cardiac function and reduced hypertrophy	Lin et al. [53]
	miR-132	80 mg/kg/bw	RO	Fox03	TAC (3 wk)	Decreased hypertrophy and fibrosis and preserved cardiac function	Ucar et al. [56]
	miR-199a~214	20 mg/kg/bw	IP	PPARd	TAC (6 wk)	Decreased hypertrophy and fibrosis and preserved cardiac function	El Azzouzi et al. [38]
LNA	miR-92a	0.03 mg/kg/bw	Catheter-based antegrade or retrograde local delivery to the heart		Pig ischemia/reperfusion model	Improved cardiac function, increased capillary density and decreased inflammation	Hinkel et al. [21]
	miR-146a	20 mg/kg	IV	NRAS	Cardiomyocyte-restricted stat3 KO (model for peripartum cardiomyopathy)	Improved cardiac function, reduced fibrosis, and enhance capillary density	Halkein et al. [28]

Table 1 (continued)

Inhibitors	miRNAs	Doses	Delivery	Target	Model	Outcome	References
	miR-34 family	25 mg/kg/bw	SC	Vinculin, Sema4b, Pofut1, and Bcl6	Tac (11 wk) or MI (8 wk)	Improved cardiac function, reduced inflammation and fibrosis and attenuated hypertrophy	Bernardo et al. [31]
	miR-208a	33 mg/kg	IV	N/A	Dahl salt-sensitive rats/ high-salt diet (hypertension)	Increased survival, increased body weight, reduced fibrosis, and attenuated hypertrophy	Montgomery et al. [52]
	miR-155	10 mg/kg/bw	IV	Socs1	Angiotensin II infusion (4 wk)	Improved cardiac function, decreased heart weight, and reduced inflammation	Heymans et al. [59]
	miR-21	25 mg/kg/bw	IV	Pdcd4	TAC (3 wk) or angiotensin II infusion (14 days)	No effect on cardiac remodeling or function	Patrick et al. [6]
	miR-26a	24 mg/kg/bw	Tail-vein injection	SMAD1	Acute MI	Reduced infarct size, induction of angiogenesis, improved LV ejection fraction, and decreased apoptosis	Icli et al. [32]
AntimiR	miR-25	300 μg	IV	Serca2a	TAC (5.5 mo)	Improved cardiac function, reduced fibrosis and hypertrophy and halted established heart failure	Wahlquist et al. [34]
Adenovirus-mediated-miR decoy	miR-24	N/A	Local	e-NOS	MI (2 wk)	Increased capillary density, improved cardiac function, and reduced infarct size	Meloni et al. [25]

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, MIRNA 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 2 – Overview of preclinical studies applying microRNA-based therapeutics to induce specific microRNAs in relevant models in heart failure.

Inducers	miRNAs	Doses	Delivery	Target	Model	Outcome	References
Adenovirus	miR-101a	1×10^9 plaque-forming units	Injection to left ventricular cavity	N/A	MI (4 wk)	Decreased fibrosis and improved cardiac function	Pan et al. [16]
	miR-1	2×10^9 infectious units	Intramyocardial injection	calm1 and calm2	Isoproterenol infusion (2 wk)	No hypertrophy	Ikeda et al. [39]
	miR-133	N/A	Transcoronary delivery	Rhoa, Cdc42, and Whsc2	Akt transgenic mice (hypertrophy)	Decreased hypertrophy	Care et al. [46]
microRNA precursors (minicircles)	miR-210	25 μg	Intramyocardial injection	Efna3 and Ptp1b	MI (4 and 8 wk)	Increased angiogenesis, decreased apoptosis, and improved cardiac function	Hu et al. [26]
Lentivirus	miR-146a	1×10^8 plaque-forming units	Micro-catheter in common carotid artery	N/A	Ischemia/ reperfusion	Reduced infarct size, improved cardiac function, reduced apoptosis, and reduced inflammation	Wang et al. [29]
Adeno-associated virus (AAV) serotype 9	miR-1	5×10^{11} viral genome	Intravenous injection	Fbln2	Ascending aortic banding (AAB) (2 and 9 wk)	Improved cardiac function, decreased hypertrophy, and fibrosis	Karakikes et al. [43]
	miR-378	1×10^{12} viral genome	IV	Grb2, Igf1r, Ksr1, and Mapk1	TAC (3 wk)	improved cardiac function, reduced hypertrophy, and fibrosis	Ganesan et al. [55]
Mimics	miR-26a	1 nmol/mouse	Tail-vein injection	SMAD1	Exercise (nocturnal running, 9 days)	Decreased physiological angiogenesis induced by exercise	Icli et al. [32]

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.

Acknowledgments

L.D.W. acknowledges support from the Netherlands Cardio-Vascular Research Initiative: the Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organization for Health Research and Development (ZonMW), and the Royal Netherlands Academy of Sciences. L.D.W. was further supported by grant 311549 from the European Research Council (ERC). P.D.C.M. was supported by a Fondation Leducq Career Development Award (no.11 CDA02), the Dutch Heart Foundation grant NHS2010B261 and a NWO MEERVOUD grant (2012/01688/ALW).

REFERENCES

[1] Johnson FL. Pathophysiology and etiology of heart failure. Cardiol Clin 2014;32:9–19 [vii].
[2] Kehat I, Molkentin DJ. Molecular pathways underlying cardiac remodeling during pathophysiological stimulation. Circulation 2010;122:2727–35.
[3] Saxena A, Tabin CJ. miRNA-processing enzyme Dicer is necessary for cardiac outflow tract alignment and chamber septation. Proc Natl Acad Sci U S A 2010;107:87–91.
[4] da Costa Martins PA, Bourajjaj M, Gladka M, Kortland M, van Oort RJ, Pinto YM, et al. Conditional dicer gene deletion in the postnatal myocardium provokes spontaneous cardiac remodeling. Circulation 2008;118:1567–76.
[5] Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature 2008;456:980–4.
[6] Patrick DM, Montgomery RL, Qi X, Obad S, Kauppinen S, Hill JA, et al. Stress-dependent cardiac remodeling occurs in the absence of microRNA-21 in mice. J Clin Invest 2010;120:3912–6.
[7] Dong S, Cheng Y, Yang J, Li J, Liu X, Wang X, et al. MicroRNA expression signature and the role of microRNA-21 in the early phase of acute myocardial infarction. J Biol Chem 2009;284:29514–25.
[8] Abonnenc M, Nabeebaccus AA, Mayr U, Barallobre-Barreiro J, Dong X, Cuello F, et al. Extracellular matrix secretion by cardiac fibroblasts: role of microRNA-29b and microRNA-30c. Circ Res 2013;113:1138–47.
[9] van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci U S A 2008;105:13027–32.
[10] Roncarati R, Viviani Anselmi C, Losi MA, Papa L, Cavarretta E, Da Costa Martins P, et al. Circulating miR-29a, among other up-regulated microRNAs, is the only biomarker for both hypertrophy and fibrosis in patients with hypertrophic cardiomyopathy. J Am Coll Cardiol 2014;63:920–7.
[11] Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R, et al. microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. Genes Dev 2008;22:3242–54.
[12] Duisters RF, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I, et al. miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling. Circ Res 2009;104:170–8 [6p following 178].
[13] Shan H, Zhang Y, Lu Y, Zhang Y, Pan Z, Cai B, et al. Downregulation of miR-133 and miR-590 contributes to nicotine-induced atrial remodelling in canines. Cardiovasc Res 2009;83:465–72.
[14] Castoldi G, Di Gioia CR, Bombardi C, Catalucci D, Corradi B, Gualazzi MG, et al. miR-133a regulates collagen 1A1: potential role of miR-133a in myocardial fibrosis in angiotensin II-dependent hypertension. J Cell Physiol 2012;227:850–6.
[15] Nam YJ, Song K, Luo X, Daniel E, Lambeth K, West K, et al. Reprogramming of human fibroblasts toward a cardiac fate. Proc Natl Acad Sci U S A 2013;110:5588–93.
[16] Pan Z, Sun X, Shan H, Wang N, Wang J, Ren J, et al. MicroRNA-101 inhibited postinfarct cardiac fibrosis and improved left ventricular compliance via the FBJ osteosarcoma oncogene/transforming growth factor-β1 pathway. Circulation 2012;126:840–50.
[17] Wang S, Olson EN. AngiomiRs—key regulators of angiogenesis. Curr Opin Genet Dev 2009;19:205–11.
[18] Doebele C, Bonauer A, Fischer A, Scholz A, Reiss Y, Urbich C, et al. Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. Blood 2010;115:4944–50.
[19] Pullamsetti SS, Doebele C, Fischer A, Savai R, Kojonazarov B, Dahal BK, et al. Inhibition of microRNA-17 improves lung and heart function in experimental pulmonary hypertension. Am J Respir Crit Care Med 2012;185:409–19.
[20] Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A, et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science 2009;324:1710–3.
[21] Hinkel R, Penzkofer D, Zuhlke S, Fischer A, Husada W, Xu QF, et al. Inhibition of microRNA-92a protects against ischemia/reperfusion injury in a large-animal model. Circulation 2013;128:1066–75.
[22] Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 2008;15:261–71.
[23] Long G, Wang F, Duan Q, Chen F, Yang S, Gong W, et al. Human circulating microRNA-1 and microRNA-126 as potential novel indicators for acute myocardial infarction. Int J Biol Sci 2012;8:811–8.
[24] Fiedler J, Jazbutyte V, Kirchmaier BC, Gupta SK, Lorenzen J, Hartmann D, et al. MicroRNA-24 regulates vascularity after myocardial infarction. Circulation 2011;124:720–30.
[25] Meloni M, Marchetti M, Garner K, Littlejohns B, Sala-Newby G, Xenophontos N, et al. Local inhibition of microRNA-24 improves reparative angiogenesis and left ventricle remodeling and function in mice with myocardial infarction. Mol Ther 2013;21:1390–402.
[26] Hu S, Huang M, Li Z, Jia F, Ghosh Z, Lijkwan MA, et al. MicroRNA-210 as a novel therapy for treatment of ischemic heart disease. Circulation 2010;122(suppl 11):S124–31.
[27] Endo K, Naito Y, Ji X, Nakanishi M, Noguchi T, Goto Y, et al. MicroRNA 210 as a biomarker for congestive heart failure. Biol Pharm Bull 2013;36:48–54.
[28] Halkein J, Tabruyn SP, Ricke-Hoch M, Haghikia A, Nguyen NQ, Scherr M, et al. MicroRNA-146a is a therapeutic target and biomarker for peripartum cardiomyopathy. J Clin Invest 2013;123:2143–54.
[29] Wang X, Ha T, Liu L, Zou J, Zhang X, Kalbfleisch J, et al. Increased expression of microRNA-146a decreases myocardial ischaemia/reperfusion injury. Cardiovasc Res 2013;97:432–442.

[30] Boon RA, Iekushi K, Lechner S, Seeger T, Fischer A, Heydt S, et al. MicroRNA-34a regulates cardiac ageing and function. Nature 2013;495:107–10.

[31] Bernardo BC, Gao XM, Winbanks CE, Boey EJ, Tham YK, Kiriazis H, et al. Therapeutic inhibition of the miR-34 family attenuates pathological cardiac remodeling and improves heart function. Proc Natl Acad Sci U S A 2012;109:17615–17620.

[32] Icli B, Wara AK, Moslehi J, Sun X, Plovie E, Cahill M, et al. MicroRNA-26a regulates pathological and physiological angiogenesis by targeting BMP/SMAD1 signaling. Circ Res 2013;113:1231–41.

[33] Dirkx E, Gladka MM, Philippen LE, Armand AS, Kinet V, Leptidis S, et al. Nfat and miR-25 cooperate to reactivate the transcription factor Hand2 in heart failure. Nat Cell Biol 2013;15:1282–93.

[34] Wahlquist C, Jeong D, Rojas-Munoz A, Kho C, Lee A, Mitsuyama S, et al. Inhibition of miR-25 improves cardiac contractility in the failing heart. Nature 2014;508:531–5.

[35] Thum T, Galuppo P, Wolf C, Fiedler J, Kneitz S, van Laake LW, et al. MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. Circulation 2007;116:258–67.

[36] Varga ZV, Kupai K, Szucs G, Gaspar R, Paloczi J, Farago N, et al. MicroRNA-25-dependent up-regulation of NADPH oxidase 4 (NOX4) mediates hypercholesterolemia-induced oxidative/nitrative stress and subsequent dysfunction in the heart. J Mol Cell Cardiol 2013;62:111–21.

[37] da Costa Martins PA, Salic K, Gladka MM, Armand AS, Leptidis S, el Azzouzi H, et al. MicroRNA-199b targets the nuclear kinase Dyrk1a in an auto-amplification loop promoting calcineurin/NFAT signalling. Nat Cell Biol 2010;12:1220–7.

[38] El Azzouzi H, Leptidis S, Dirkx E, Hoeks J, van Bree B, Brand K, et al. The hypoxia-inducible microRNA cluster miR-199a approximately 214 targets myocardial PPARdelta and impairs mitochondrial fatty acid oxidation. Cell Metab 2013;18:341–54.

[39] Ikeda S, He A, Kong SW, Lu J, Bejar R, Bodyak N, et al. MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes. Mol Cell Biol 2009;29:2193–204.

[40] Tritsch E, Mallat Y, Lefebvre F, Diguet N, Escoubet B, Blanc J, et al. An SRF/miR-1 axis regulates NCX1 and annexin A5 protein levels in the normal and failing heart. Cardiovasc Res 2013;98:372–80.

[41] Kumarswamy R, Lyon AR, Volkmann I, Mills AM, Bretthauer J, Pahuja A, et al. SERCA2a gene therapy restores microRNA-1 expression in heart failure via an Akt/FoxO3A-dependent pathway. Eur Heart J 2012;33:1067–75.

[42] Elia L, Contu R, Quintavalle M, Varrone F, Chimenti C, Russo MA, et al. Reciprocal regulation of microRNA-1 and insulin-like growth factor-1 signal transduction cascade in cardiac and skeletal muscle in physiological and pathological conditions. Circulation 2009;120:2377–85.

[43] Karakikes I, Chaanine AH, Kang S, Mukete BN, Jeong D, Zhang S, et al. Therapeutic cardiac-targeted delivery of miR-1 reverses pressure overload-induced cardiac hypertrophy and attenuates pathological remodeling. J Am Heart Assoc 2013;2:e000078.

[44] Varrone F, Gargano B, Carullo P, Di Silvestre D, De Palma A, Grasso L, et al. The circulating level of FABP3 is an indirect biomarker of microRNA-1. J Am Coll Cardiol 2013;61:88–95.

[45] Besser J, Malan D, Wystub K, Bachmann A, Wietelmann A, Sasse P, et al. MiRNA-1/133a clusters regulate adrenergic control of cardiac repolarization. PLoS One 2014;9:e113449.

[46] Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, et al. MicroRNA-133 controls cardiac hypertrophy. Nat Med 2007;13:613–8.

[47] Castaldi A, Zaglia T, Di Mauro V, Carullo P, Viggiani G, Borile G, et al. MicroRNA-133 modulates the β1-adrenergic receptor transduction cascade. Circ Res 2014;115:273–83.

[48] Matkovich SJ, Wang W, Tu Y, Eschenbacher WH, Dorn LE, Condorelli G, et al. MicroRNA-133a protects against myocardial fibrosis and modulates electrical repolarization without affecting hypertrophy in pressure-overloaded adult hearts. Circ Res 2010;106:166–75.

[49] Feng B, Chen S, George B, Feng Q, Chakrabarti S. miR133a regulates cardiomyocyte hypertrophy in diabetes. Diabetes Metab Res Rev 2010;26:40–9.

[50] Chen S, Puthanveetil P, Feng B, Matkovich SJ, Dorn GW 2nd, Chakrabarti S. Cardiac miR-133a overexpression prevents early cardiac fibrosis in diabetes. J Cell Mol Med 2014;18:415–21.

[51] Chavali V, Tyagi SC, Mishra PK. MicroRNA-133a regulates DNA methylation in diabetic cardiomyocytes. Biochem Biophys Res Commun 2012;425:668–72.

[52] Montgomery RL, Hullinger TG, Semus HM, Dickinson BA, Seto AG, Lynch JM, et al. Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. Circulation 2011;124:1537–47.

[53] Lin Z, Murtaza I, Wang K, Jiao J, Gao J, Li PF. miR-23a functions downstream of NFATc3 to regulate cardiac hypertrophy. Proc Natl Acad Sci U S A 2009;106:12103–8.

[54] Wang K, Lin ZQ, Long B, Li JH, Zhou J, Li PF. Cardiac hypertrophy is positively regulated by MicroRNA miR-23a. J Biol Chem 2012;287:589–99.

[55] Ganesan J, Ramanujam D, Sassi Y, Ahles A, Jentzsch C, Werfel S, et al. MiR-378 controls cardiac hypertrophy by combined repression of mitogen-activated protein kinase pathway factors. Circulation 2013;127:2097–106.

[56] Ucar A, Gupta SK, Fiedler J, Erikci E, Kardasinski M, Batkai S, et al. The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. Nat Commun 2012;3:1078.

[57] Raisch J, Darfeuille-Michaud A, Nguyen HT. Role of microRNAs in the immune system, inflammation and cancer. World J Gastroenterol 2013;19:2985–96.

[58] Seok HY, Chen J, Kataoka M, Huang ZP, Ding J, Yan J, et al. Loss of MicroRNA-155 protects the heart from pathological cardiac hypertrophy. Circ Res 2014;114:1585–95.

[59] Heymans S, Corsten MF, Verhesen W, Carai P, van Leeuwen RE, Custers K, et al. Macrophage microRNA-155 promotes cardiac hypertrophy and failure. Circulation 2013;128:1420–32.

[60] Gebert LF, Rebhan MA, Crivelli SE, Denzler R, Stoffel M, Hall J. Miravirsen (SPC3649) can inhibit the biogenesis of miR-122. Nucleic Acids Res 2014;42:609–21.

[61] Sanchez-Nino MD, Ortiz A. HCV infection and miravirsen. N Engl J Med 2013;369:877–8.

[62] Liu Y, Li L, Su Q, Liu T, Ma Z, Yang H. Ultrasound-targeted microbubble destruction enhances gene expression of microRNA-21 in swine heart via intracoronary delivery. Echocardiography 2015;00:1–10.

[63] Gill SL, O'Neill H, McCoy RJ, Logeswaran S, O'Brien F, Stanton A, et al. Enhanced delivery of microRNA mimics to cardiomyocytes using ultrasound responsive microbubbles reverses hypertrophy in an in-vitro model. Technol Health Care 2014;22:37–51.

[64] Wang H, Jiang Y, Peng H, Chen Y, Zhu P, Huang Y. Recent progress in microRNA delivery for cancer therapy by non-viral synthetic vectors. Adv Drug Deliv Rev 2015;81:142–160.

[65] Zhang Y, Wang Z, Gemeinhart RA. Progress in microRNA delivery. J Control Release 2013;172:962–74.

[66] Davis S, Propp S, Freier SM, Jones LE, Serra MJ, Kinberger G, et al. Potent inhibition of microRNA in vivo without degradation. Nucleic Acids Res 2009;37:70–7.

[67] Kinet V, Halkein J, Dirkx E, Windt LJ. Cardiovascular extracellular microRNAs: emerging diagnostic markers and mechanisms of cell-to-cell RNA communication. Front Genet 2013;4:214.
[68] Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;9:654–9.
[69] Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, et al. Detection of microRNA expression in human peripheral blood microvesicles. PLoS One 2008;3:e3694.
[70] Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. Sci Signal 2009;2:ra81.
[71] Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A 2011;108:5003–8.
[72] Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, et al. Treatment of HCV infection by targeting microRNA. N Engl J Med 2013;368:1685–94.
[73] Li RC, Tao J, Guo YB, Wu HD, Liu RF, Bai Y, et al. In vivo suppression of microRNA-24 prevents the transition toward decompensated hypertrophy in aortic-constricted mice. Circulation research 2013;112:601–5.