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The therapeutic potential of microRNAs in heart and vascular diseases

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Abstract

The potential use of microRNAs (miRNAs) to design novel therapeutic approaches in cardiovascular diseases (CVD) has emerged as a promising strategy. miRNAs are small, noncoding RNAs that regulate gene expression epigenetically. Increasing evidence suggests that miRNAs operate as regulatory networks that target functionally related genes in physiological and pathological conditions. This property offers a unique opportunity to devise functional interventions that can overcome any redundancy mechanisms in disease. Additionally, miRNAs typically exert mild effects under baseline conditions, but have a more pronounced response under stress suggesting that off-target effects on uninjured tissue will be limited. Attractive as it may seem, miRNA biology can also be a serious concern for the safety of the potential applications, as miRNAs tend to function in a context-dependent manner and participate in both positive and negative feedback loops. Besides their role as mediators of disease, the presence of miRNAs in biological fluids has now been well documented. These circulating miRNA signatures may offer a great diagnostic and prognostic tool as disease biomarkers. Here we will review the recent developments in miRNA research with a particular emphasis on secreted miRNAs as targets for novel therapeutic approaches to cardiovascular diseases. ■ *Heart Metab*. 2014;65:21-25

Keywords: cardiovascular disease; microRNA; therapeutic application

The role of microRNAs (miRNAs) in disease pathologies is ever increasing, and substantial evidence has accumulated for their role in cardiovascular diseases, raising possibilities for therapeutic intervention. miRNAs, 18-25 nucleotide single-stranded noncoding RNAs, are responsible for the negative epigenetic regulation of gene expression through complementary base pairing to their target messenger RNAs (mRNA). This miRNA-mRNA complementarity occurs in a 6-8 nucleotide "seed" region at the 5' miRNA end, although evidence exists for recognition sites outside the seed region too.[1] The function of miRNAs is to either prevent translation or promote transcript degradation. A single miRNA can target many effectors of a biological process, while cotargeting networks of miRNAs that regulate the expression of a specific component of a signaling pathway have also been reported, indicating miRNA redundancy and extremely high complexity of miRNA regulatory networks.[1] Specific miRNAs have been implicated in a number of cardiovascular events that could lead to heart failure, including cardiac hypertrophy, myocardial infarction, cardiac fibrosis, and arrhythmias.

Abbreviations

ABCA1: adenosine triphosphate–binding cassette transporter A1; **EC:** endothelial cell; **HCM:** hypertrophic cardiomyopathy; **HCV:** hepatitis C virus; **HDL:** high-density lipoprotein; **hsTNT:** high-sensitivity troponin T; **miRNA:** microRNA; **SMC:** smooth muscle cell; **STEMI:** ST-segment–elevation myocardial infarction; **VLDL:** very-low-density lipoprotein

In the vasculature, tissue remodeling in atherosclerosis and aortic aneurysms was shown to be orchestrated by miRNAs that tightly regulate different aspects of the disease. Here we will discuss the therapeutic potential of miRNAs in cardiovascular diseases.

MicroRNA targets in the heart

Since the first report of embryonic lethality in mice with cardiac deletion of Dicer,[2] multiple studies have explored the role of miRNAs in heart development and function under physiological conditions and in disease. Several miRNAs highly expressed in the heart have been discovered, and remarkable findings for their mechanisms of function have been uncovered.[1] miRNA-based therapeutic applications for the heart have provided promising results in mouse models.[3] These studies are now slowly progressing to large animal models *(Table I)*.[4-7] Systemic delivery of miR-15 inhibitors in pigs led to no overt toxicity and a relatively equal distribution of the inhibitors across the heart. This was accompanied by a dose-dependent miRNA inhibition in the cardiac tissue.[6] Both subcutaneous and intravenous delivery resulted in comparable levels of inhibition. In a preclinical pig model, local inhibition of miR-92a significantly improved cardiac function after ischemia/reperfusion injury.[7] These results highlight the feasibility of local delivery of miRNA reagents and provide a proof of concept for the clinical use of such therapeutic approaches. In most instances, though, designing novel therapeutic applications will have to take into consideration the complex regulatory mechanisms that often operate with opposing effects in different cell types in the same tissue and thus adapt more targeted approaches. A typical example is miR-24, which was recently shown to induce apoptosis in endothelial cells following cardiac ischemia,[8] while suppressing apoptosis through repression of Bim in cardiomyocytes.[9]

MicroRNA targets in the vasculature

miRNAs have been shown to link alterations in hemodynamic forces in the vessel wall, inflammation, hyperlipidemia, and vascular remodeling. Atherosclerosis is a major underlying cause of cardiovascular disease, and distinct miRNA expression profiles were associated with its progression. Most of the studies were conducted in mice using established models of atherosclerosis apolipoprotein E–deficient mice (ApoE-/-), low-density lipoprotein receptor knockout mice (LDLR-/-), but several miRNA transgenic mice were also gen-

Species	Target miRNA	Disease model	Intervention	Delivery route	Outcome	Reference
Nonhuman primates	miR-122	None	LNA-122	Systemic (intravenous)	Decrease in plasma cholesterol	Elmen et al,[4] 2008
Nonhuman primates	miR-33	High carbohydrate, moderate cholesterol diet	AntimiR33	Systemic (subcutaneous)	Increase in plasma HDL Lower VLDL	Rayner et al,[5] 2011
Pigs	miR-15	Myocardial infarction	LNA-15b	Systemic (intravenous)	Silencing of miR-15 family expression	Hullinger et al,[6] 2012
Pigs	miR-92a	Subcutaneous ischemia/ reperfusion	LNA-92a	Systemic (intravenous) Local (catheter)	Reduced infarct size Improved cardiac function	Hinkel et al,[7] 2013

Table I *miRNA-based therapeutic applications in large animal models.*
Abbreviations: *anti-miR33, 2'-fluoro/methoxyethyl (2'-F/MOE)-modified, phosphorothioate-backbone-modified, antisense miR33; LNA, locked nucleic acid modified antisense; HDL, high-density lipoprotein; miRNA, microRNA; VLDL, very-low-density lipoprotein.*

erated to elucidate potential effects on atherosclerosis lesion progression.[10] Screening of the miRNA pool in human atherosclerotic plaques also demonstrated differential miRNA expression (miR-21, miR-34a, miR-92a, miR-146a/b, miR-210, miR-322-5p).[11-13] The role of miRNAs in cholesterol homeostasis and its impact on atherosclerosis regression is one of the best studied mechanisms. miR-33 was shown to modulate expression of genes involved in cholesterol efflux. Silencing miR-33 in vivo in mice increased hepatic expression of *ABCA1* (adenosine triphosphate–binding cassette transporter 1) and plasma high-density lipoprotein (HDL) levels. This reverse cholesterol transport led to atherosclerosis regression.[14] More importantly, promising findings were obtained from inhibition of miR-33 in nonhuman primates, African green monkeys. In line with observations in mice, a sustained increase in plasma levels of HDL was detected while very-low-density lipoprotein (VLDL) levels were reduced without any evidence of adverse effects,[5] suggesting that targeting miR-33 could be an attractive strategy to combat atherosclerosis.

Vascular remodeling in abdominal aortic aneurysms (AAA) is also mediated by miRNAs. miR-29b emerged as a key regulator of extracellular matrix deposition in several models of aortic dilatation and AAA in mice following systemic injection of antimiRs.[15,16] Interestingly, in mice, the increased miR-29b expression in the aged aorta renders it susceptible to aneurysm formation.[15] In humans, differential expression of miR-29b was detected in the ascending aorta in Marfan syndrome, a connective tissue disorder that can lead to the development of aortic root aneurysms.[17] Local instead of systemic administration of inhibitors for miR-29 will safeguard against off-target fibrotic effects in organs such as the liver and kidneys, which receive extremely high doses of the compounds.

Clinical studies

Despite the encouraging data from animal models of injury, more preclinical studies are required before miRNA therapeutics can enter any clinical trials. There are several issues that need to be addressed. Application of antisense oligonucleotides that act as inhibitors of a miRNA may be toxic for the liver and kidneys, which will clear most of these compounds from the circulation. These reagents will have to be chemically modified to increase their resistance to nucleases and thus their stability, enhance their cellular uptake, and reduce their renal clearance. Their specificity and route of delivery may be additional causes of concern.[18] Nevertheless, miravirsen, an inhibitor against miR-122, was successfully used for phase 1 and 2 trials against hepatitis C virus (HCV). No significant renal toxicity was observed, the compound was biostable, and displayed extremely high target affinity. A prolonged dose-response reduction in the viral RNA was detected, indicating that miR-122 inhibition could be a safe and effective strategy to combat HCV infection.[19] This is the most advanced miRNA therapeutic application so far. Of note, targeting miR-122 has benefited from its unique pattern of expression. miR-122 is highly expressed in the liver and therefore can be effectively targeted even when very low concentrations of the reagents are applied.

MicroRNAs as biomarkers of disease

Circulating miRNAs represent an attractive tool for the development of noninvasive diagnostic tests, and distinct signatures have been proposed to correlate with various cardiovascular diseases.[20] Encouraging results were also obtained for their value as potential prognostic markers in CVD. A signature of three miRNAs (miR-126, miR-223, and miR-197) was reported to have a predictive value for incident myocardial infarction (MI) in a prospective population-based cohort (n=820), and addition of these miRNAs to the Framingham Risk Score for hard coronary heart disease led to a significant improvement in risk classification that actually exceeded the impact of alternative biomarkers such as C-reactive protein.[21] Furthermore, in an independent cohort of patients who received percutaneous coronary intervention (PCI) and dual antiplatelet therapy (n=491), circulating miR-126 was significantly associated with major adverse cardiovascular events within 1 year.[22] miRNA signatures with a potential value as early biomarkers of acute coronary syndrome (miR-1, miR-499, and miR-21, n=332) that added diagnostic value to high-sensitivity troponin T (hsTnT) have also been proposed.[23] In a patient cohort of hypertrophic cardiomyopathy (HCM) patients and their controls (HCM, n=82), a circulating signature of three miRNAs (miR-27a, miR-29a, and miR-199a-5p) was reported to correlate with left ventricular mass. Importantly, miR-29a also associated with myocardial fibrosis, hence providing the first po-

tential biomarker for myocardial remodeling in HCM.[24] In the vasculature, circulating miR-126 correlated with subclinical and manifest peripheral artery disease in a prospective cohort (n=820), suggesting a direct link between miRNAs and vascular homeostasis.[25]

Interestingly, circulating miRNA signatures do not always have an additive value to known biomarkers of disease. In a cohort of acute coronary syndrome (n=444), cardiac-enriched miRNAs showed no independent association with the outcome once adjusted for TnT expression.[26] In a similar manner, in a cohort of ST-segment–elevation myocardial infarction (STEMI, n=216) despite the association of circulating miR-133a with myocardial salvage, larger infarcts, and more pronounced reperfusion injury, no independent prognostic value for adverse cardiovascular events was detected.[27] In a separate study, upregulation of miR-208b and miR-499 in the circulation of STEMI patients (n=397) correlated well with creatine kinase, but failed to provide a significant reclassification index following adjustment for hsTnT.[28]

While some recently identified confounding factors[29] in such studies will have to be taken into consideration and advanced statistical methods employed to address the high dimensional and collinear data obtain from profiling of the extracellular miRNA pools,[20] large multicenter independent studies are urgently needed to clarify the robustness of the circulating miRNA signatures and their potential as biomarkers of cardiovascular diseases *(Figure 1)*.

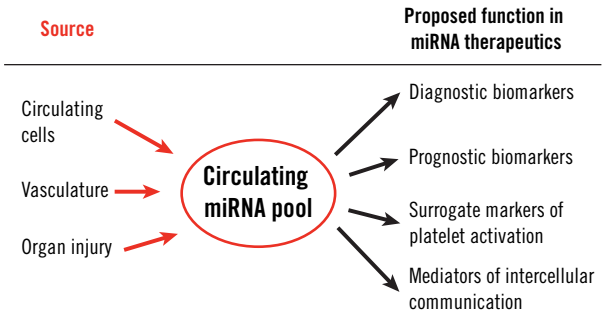

Fig. 1 *Circulating microRNAs (miRNAs) and potential therapeutic applications in cardiovascular disease.*

MicroRNAs as mediators of intercellular communication

The discovery that membrane vesicles contain functional miRNA pools sparked enthusiasm for their potential role in intercellular communication. These actively secreted vesicles may act as miRNA carriers and mediate paracrine effects. In the vasculature, regulation of vascular smooth muscle cell (SMC) contractility and proliferation by endothelial cell (EC)–secreted miRNAs was demonstrated. Vesicle-mediated transfer of miR-143/145 from ECs to SMCs was shown to confer atheroprotection. Shear stress stimulated EC secretion of vesicles enriched in miR-143/145 that could effectively target SMC gene expression and prevent neointima formation in vivo.[30] In a different study, laminar flow–regulated transfer of miR-126 by ECs to SMCs was demonstrated. This transfer had a significant effect on SMC gene expression and function toward an atherogenic phenotype.[31] Besides this local effect on the vessel wall, transfer of miRNAs from circulating cells to the vessel wall has also been explored. Monocyte-derived microvesicles enriched in miR-150 could enhance EC migration via a c-Myb–mediated mechanism both in vitro and in vivo.[32] Likewise, miR-223–derived platelet microparticles induced apoptosis in the recipient ECs in response to glycation end products via targeting the expression of insulin like growth factor 1 receptor (IGF-1R).[33] HDL complexes were recently proposed as carriers of miR-223 to ECs. Intriguingly, miR-223, which is not normally expressed in these cells, induced downregulation of intercellular adhesion molecule 1 (ICAM-1) expression, thus conferring an anti-inflammatory phenotype to the ECs.[34] Although these studies are extremely interesting, our understanding of the molecular mechanisms involved is still poor. While the functionality of the miRNA pools in vesicles/HDL complexes has been clearly demonstrated, evidence for a specific role of the delivered miRNAs in the recipient cell is still sparse. Of note, identifying an exogenous miRNA that has the potential to perform a function does not necessarily imply that this function is indeed exerted. Delineating the mechanisms of delivery will be vital in exploiting microvesicles as a new delivery route for therapeutic agents.

Conclusions

miRNA-based therapeutics is still in its infancy. Evidence from the studies performed mostly in preclinical models is extremely promising, but further work is required to determine the safety and efficacy of such therapeutic approaches. Designing protocols for local administration rather than systemic delivery of miRNA

reagents would greatly facilitate a targeted manipulation and limit potential side effects. Nevertheless, miRNA therapeutics as a novel approach to combat cardiovascular diseases is worth exploring. ■

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