INTRODUCTION

MicroRNAs (miRNAs) are short, noncoding RNAs (21-23 nucleotides) that modify gene expression by downregulating genes at the post-transcriptional level during normal function of the cells and also in various developmental or disease processes (1). These RNAs bind to their targets in the 3’ untranslated region (UTR) of messenger RNAs (mRNAs) to inhibit translation or evoke degradation of the mRNA. In rare cases, miRNAs can also target 5’ UTR or coding regions (2). MiRNAs are ubiquitously expressed, but differ in tissue expression patterns (3).

MiRNAs originate from transcripts that are either coexpressed with the host gene transcript or located in intergenic regions of the genome (1,4). Initially, the early transcript, called primary (pri)-miRNA, is processed within the cellular nucleus by the ribonuclease III enzyme Drosha and the double-stranded RNA-binding protein DGCR8 into a so-called precursor (pre)-miRNA (a short hairpin RNA molecule) and thereafter exported to the cytosol by exportin-5 (1,5,6). Within the cytosol, there is a digestion of pre-miRNA into small mature RNA molecules by the endonuclease Dicer, followed by binding to the RNA-induced silencing complex (RISC) complex (4).

![Diagram of miRNA biogenesis and function](https://via.placeholder.com/150)

Fig. 1. MiRNA biogenesis and function. miRNA genes are usually transcribed by RNA polymerase II to form a pri-miRNA precursor termed a pre-miRNA, that forms a hairpin-shaped loop structure, called pre-miRNA, which is cleaved by an enzyme called Drosha. The pre-miRNA is exported from the nucleus into the cytoplasm, where it is further cleaved by the RISC. The RISC, composed of Dicer and RNA, cleaves the pre-miRNA into a mature miRNA molecule. The mature miRNA then interacts with the RNA-induced silencing complex (RISC) to cleave or inhibit translation of target mRNAs.

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There are three major important points on the usefulness of miRNAs:

1. miRNA research has identified a new layer of mechanistic complexity in genome research and has opened a new angle on how to identify new and potentially important mechanisms of cellular functions.

2. miRNAs emerged as drug-able targets, which, within a couple of years, made their way into clinical phase 1 and 2 trials and have an enormous future therapeutic potential. Research has started using small animal and large animal models of diseases.

3. miRNAs have been shown to be stable in plasma and other body fluids and have been shown to be potentially interesting biomarkers of diseases, especially for diagnostic and prognostic evaluations (8).

MicroRNA targets in the heart

MiRNA-based therapeutic applications for the heart have provided promising results in mouse models (8). These studies are now slowly progressing to large animal models (Table I)(9,10,11,12). Systemic delivery of miR-15 inhibitors in pigs led to no overt toxicity and a relatively equal distribution of the inhibitors across the heart. In a preclinical pig model, local inhibition of miR-92a significantly improved cardiac function after ischemia/reperfusion injury (12).

The 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. A typical example is miR-24, which was recently shown to induce apoptosis in endothelial cells following cardiac ischemia (13), while suppressing apoptosis through repression of Bim in cardiomyocytes (14).

<table>
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<tr>
<th>Species</th>
<th>Target miRNA</th>
<th>Disease model</th>
<th>Intervention</th>
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<tr>
<td>Nonhuman primates</td>
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<td>None</td>
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<td>miR-33</td>
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<td>Pigs</td>
<td>miR-92a</td>
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<td>Systemic (intravenous)</td>
<td>Reduced infarct size, Improved cardiac function</td>
<td>Hinkel et al, 7, 2013</td>
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Table I: miRNA-based therapeutic applications in large animal models.

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 (15).

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)(16,17,18). 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 (19).

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 (19), suggesting that targeting miR-33 could be an attractive strategy to combat atherosclerosis (15).

**MicroRNA as potencially biomarker for coronary artery disease**

Levels of circulating miRNAs are being studied extensively as a novel class of biomarker for various diseases (20). MiRNA in clinical plasma samples is stable with respect to freezing and rethawing (21) and appears to be protected from endogenous RNase activity (22). MiRNA has been found to circulate in the plasma in microvesicles, platelets and peripheral blood mononuclear cells (PBMCs) (23) and in healthy human subjects, it has been determined that over 250 miRNAs can be detected in the circulation (24).

In patients with coronary artery disease (CAD), reduced levels of miR-126 are observed, whereas cardiac muscle-enriched miRNAs (miR-133a, miR-208a) tended to be higher in patients with CAD (20). In patients with acute myocardial infarction (AMI), the muscle-enriched miRNA miR-1 was significantly upregulated in the circulation compared with non-AMI controls. A recent study found circulating long noncoding RNAs to be of diagnostic and predictive value in post-MI and heart failure patients (25).

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 (26). 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) (27).

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 (28). 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 (29). 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 potential biomarker for myocardial remodeling in HCM (30).

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 (31). 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 (32).

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 2)(15).
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.

REFERENCES


