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REVIEW TOPIC OF THE WEEK

MicroRNAs in Cardiovascular Disease

Temo Barwari, MD, Abhishek Joshi, BA, BMBCh, Manuel Mayr, MD, PhD

ABSTRACT

Micro-ribonucleic acids (miRNAs) are in the spotlight as post-transcriptional regulators of gene expression. More than 1,000 miRNAs are encoded in the human genome. In this review, we provide an introduction to miRNA biology and research methodology, and highlight advances in cardiovascular research to date. This includes the potential of miRNAs as therapeutic targets in cardiac and vascular disease, and their use as novel biomarkers. Although some miRNA therapies are already undergoing clinical evaluation, we stress the importance of integrating current knowledge of miRNA biology into a systemic context. Discovery studies focus on miRNA effects within one specific organ, whereas the expression of most miRNAs is not restricted to a single tissue. Because most miRNA-based therapies act systemically, this may preclude widespread clinical use. The development of more targeted interventions will bolster well-informed clinical applications, increasing the chances of success and minimizing the risk of setbacks for miRNA-based therapeutics. (J Am Coll Cardiol 2016;68:2577–84) © 2016 by the American College of Cardiology Foundation.

Only 1% of the human genome codes for genes that function in protein synthesis (1). The remaining 99% of deoxyribonucleic acid (DNA) was initially considered to be junk. It is now recognized that the majority of the genome may have biochemical functions, representing regulatory, noncoding ribonucleic acid (RNA). Several subcategories of noncoding RNAs exist, in particular, long noncoding RNAs and small noncoding RNAs. Among the latter, microRNAs (miRNAs/miRs) have thus far attracted most attention since their discovery in *Caenorhabditis elegans* (2). MiRNAs affect the production of proteins by interacting with transcribed messenger RNAs (mRNAs), thus silencing the expression of genes. Here, we aim to provide an overview of miRNA biology for clinicians, discussing their therapeutic and diagnostic potential, as well as their limitations.

BASIC BIOLOGY OF miRNAs

MiRNAs are short (~22 nucleotides), noncoding RNA molecules. They exert their function via the seed region, a sequence of 6 to 8 nucleotides that binds to messenger ribonucleic acid (mRNA), the so-called miRNA targets (3). MiRNA synthesis and silencing have both been extensively reviewed recently (4,5). The key biological concepts are summarized in the **Central Illustration**. Initially, a precursor transcript is produced and then forms double-stranded RNA. Later, the miRNA duplex undergoes unwinding, whereby only a single strand, the so-called guide strand, which is usually the functional unit, is loaded in the RNA-induced silencing complex (RISC). The other strand or passenger strand is often degraded, but may also function as a mature miRNA (6). In the RISC, the miRNA binds to its target mRNA, preventing its translation into a protein. Single miRNAs suppress more than 1 gene, and miRNAs with similar seed regions may suppress a similar, but nonidentical, set of genes, and to differing degrees. Gene suppression is usually partial, rather than total, and a single gene can have binding sites for multiple miRNAs. This organizational complexity, illustrated by a high false-positive rate of target prediction

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From the King's British Heart Foundation Centre, King's College London, London, United Kingdom. Dr. Barwari is an Interdisciplinary PhD student funded by the British Heart Foundation (BHF). Dr. Joshi has been awarded a BHF Clinical Research Training Fellowship. Dr. Mayr is a BHF Senior Research Fellow (FS/13/2/29892) and supported by the Fondation Leducq (MIRVAD; 13 CVD 02) and the NIHR Biomedical Research Center based at Guy's and St. Thomas' National Health Service Foundation Trust and King's College London, in partnership with King's College Hospital. King's College London and Prof. Mayr hold patents on microRNA biomarkers. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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ABBREVIATIONS AND ACRONYMS

anti-miRs = inhibitors of miRNAs

CVD = cardiovascular disease

ECM = extracellular matrix

MHC = myosin heavy chain

MI = myocardial infarction

miRNA/miR = micro-ribonucleic acid

mRNA = messenger ribonucleic acid

RISC = ribonucleic acid-induced silencing complex

SMC = smooth muscle cell

algorithms (7), presents challenges in both understanding the functions of miRNAs and manipulating their effects.

MEASUREMENT OF miRNAs

miRNAs are relatively stable, and can be reliably measured in tissues, as well as in biofluids (8). Several techniques have been developed to identify and quantify miRNAs. Benefits and disadvantages of different techniques have been summarized previously elsewhere (8). Here, we briefly discuss the most commonly used methods.

Real-time quantitative polymerase chain reaction has been the cornerstone for miRNA quantification and remains the most reliable technique for quantitative comparison of miRNA expression levels. This technique uses predefined primers to amplify and measure individual miRNAs in a sample. Microarrays use hybridization of miRNAs to specific primers, trading less accurate quantification for higher throughput and lower cost, and measuring hundreds of miRNAs in parallel. Because both of these techniques rely on predefined primer sequences, they are not able to discover previously uncharacterized miRNAs.

RNA sequencing techniques provide "hypothesis-free" identification of RNA species, allowing the discovery of new miRNAs and quantitative analysis of a comprehensive miRNA transcriptome. The use of computational solutions to resolve reads into miRNAs suffers from the risk of reporting putative sequences that do not have real-world correlates (9).

Without added spike-ins and standard curves, all techniques rely on relative rather than absolute quantification, meaning that differences in miRNAs are presented as a "fold change" between paired samples, and not as an absolute unit, requiring information on the context of abundance. Experimental work must show downstream effects of miRNA changes as readout for miRNA function, specifically by comparing the profiles of multiple miRNAs with differential expression of target proteins. Ideally, the miRNA/mRNA duplexes in the RISC are analyzed to prove direct interactions.

THERAPEUTIC MANIPULATION OF miRNAs

The central action of miRNAs is to suppress protein expression through binding and silencing specific target mRNAs, which, in turn, reduces protein synthesis. Therefore, miRNAs offer a tantalizing mechanism for manipulating protein synthesis; in most cases, overexpression of a miRNA will suppress its direct targets, whereas inhibiting an endogenous miRNA will de-repress their expression.

Unmodified RNA strands are degraded upon administration; thus, miRNA therapeutics require either efficient methods of cell type-specific delivery or modifications that enhance stability but preserve miRNA function. For now, clinical studies with miRNA therapeutics mainly use inhibitors of miRNAs (anti-miRs). Anti-miRs are synthetic single strands of RNA, consisting of complementary nucleotides to an endogenous miRNA. Various structural modifications have been designed to increase their half-life in the circulation, bypass degradation in tissues and enhance intracellular delivery (10). Cardiotropic adeno-associated viruses achieve efficient cardiomyocyte-specific miRNA delivery (11). The translational potential of adeno-associated virus-mediated oligonucleotide delivery has been reviewed elsewhere (12). Currently, overexpressing a miRNA is generally considered less safe than inhibiting an endogenous miRNA.

Miravirsen is an anti-miR targeting miR-122 for treatment of hepatitis C (13), which has completed a multicenter phase 2a trial (14) and is currently in a phase 2b trial. The choice of miR-122 as the first therapeutic target highlights the challenges when targeting cardiovascular disease (CVD). First, miR-122 shows exquisite tissue specificity, whereas most miRNAs identified as treatment targets for CVD are ubiquitously expressed, raising concerns for off-target effects. Second, anti-miRs predominantly accumulate in the liver and kidneys, circumventing the need for tissue-specific targeting (13). The latter is further illustrated by the evaluation of anti-miR-21 as a therapy for Alport nephropathy (15). These ongoing clinical trials will provide more insight into the practical use of miRNA therapeutics.

Targeting the heart or vasculature with systemic anti-miRs would require significantly higher dosing, and efficiency may be low. Animal models have shown nephrotoxicity at higher doses of some anti-miRs, although the clinical trial of Miravirsen did not find evidence for renal injury in humans (14). The human immune system has evolved to detect viral RNA. Toll-like receptors recognize both single- and double-stranded RNA (16). High doses of synthetic oligonucleotides may elicit an immune response that could compromise efficacy and safety. Thus, cardiovascular applications will require solutions for local or cell-type-specific delivery, and clinically detectable, reliable readouts to monitor successful target engagement.