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Review article

Identification of cardiovascular microRNA targetomes

J. Fiedler [a], S.K. Gupta [a], T. Thum [a,b,*]

[a] Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Germany
[b] Centre for Clinical and Basic Research, IRCCS San Raffaele, Rome, Italy

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ABSTRACT

MicroRNAs (miRNAs) are strong post-transcriptional regulators targeting multiple targets. Endogenously transcribed, miRNAs specifically bind to complementary sequences of mRNAs and repress their expression thus govern control of cellular signaling pathways. An altered miRNA expression is causally related to cardiovascular disease. Identification of miRNA-dependent pathways is therefore an important aim to develop new therapeutic approaches. To understand miRNA function in various cardiovascular cells, the identification of individual miRNA target genes is of utmost importance. Indeed, the biological function of a miRNA is dependent on the availability of potential targets in a cell. We here summarize and discuss current challenging approaches to identify miRNA targetomes which will help to understand miRNA function in cardiac homeostasis and disease.

Contents

* Corresponding author at: Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany. Tel.: +49 511 532 5273; fax: +49 511 532 5274.
E-mail address: thum.thomas@mh-hannover.de (T. Thum).

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1. Introduction

1.1. MicroRNAs (miRNAs)—biogenesis and biological function

Since discovered in the early nineties, microRNAs (miRNAs) have been shown to play major roles in different physiological settings by regulating gene expression post-transcriptionally [1–3]. Of note, miRNAs regulate 30–50% of the genome and thus are potent mediators of cellular signaling [4]. Several details about the regulation of miRNAs and their biogenesis especially in the context of cardiovascular biology have recently been reviewed [5] and therefore are not a main topic of this review article. Briefly, miRNAs are either transcribed from miRNA genes or spliced from host gene transcripts (overview on biogenesis reviewed in [6]). Once formed as so called primary (pri)-miRNAs in the nucleus, the Drosha complex cleaves the RNA to a stem–loop precursor structure (precursor (pre)-miRNA) of about 60–70 nucleotides in length. Cytosolic translocation mediated by exportin proteins triggers further processing at the Dicer complex. Here, the miRNA heteroduplex is unwinded to its biological active single-stranded format. The second strand (designated as miRNA*) is often not selected and subsequently degraded. RNA-binding proteins, e.g. proteins of the Ago family then recruit the mature miRNA to sites of RISC (RNA induced silencing complex). An antisense mechanism then mediates miRNA-dependent recognition in RISC to target mRNAs. Most commonly, miRNA responsive elements are found in 3′-UTR (untranslated region) of the mRNA and base pairing determines stringency of miRNA:mRNA interaction. In line, the strength of miRNA binding to the mRNA is crucially mediated by nucleotides 2 to 8 (the seed) and surrounding bases [7]. However, recently the central dogma of miRNA binding to 3′-UTR region has been challenged, as reports demonstrated additional miRNA binding to coding regions or even 5′-UTRs [8]. However, the main characteristics of a single miRNA or even miRNA families sharing the identical seed sequence, are their ability to simultaneously target many mRNAs which are accessible in a specific cellular context. It is crucial to decipher miRNA target genes in a single cell by different bioinformatic or experimental approaches to substantially identify intracellular signaling pathways especially in terms of cardiac disease. The still growing number of newly identified miRNAs emphasizes their cellular impact, calls for sequence annotation and a summary of characteristics in open-access databases (see below).

1.2. Cardiovascular disease is associated to miRNA deregulation

MiRNAs are crucial in the control of cardiovascular signal transduction in the heart. Indeed, knockdown of the miRNA biogenesis related enzyme Dicer in cardiomyocytes led to cardiac remodeling, enhanced fibrosis and finally heart failure [9,10]. This key study of Costa Martins and colleagues emphasized the orchestrating role of cardiac miRNA expression to sustain and balance cardiac morphology and function. Cardiac disease animal models, such as experimental myocardial infarction lead to a differential pattern of miRNA expression [11]. Noteworthy, identified miRNAs deregulated in animal models of heart diseases such as miR-21 or miR-208a are also upregulated in human heart failure [11]. When comparing miRNA expression profiles in fetal and failing human hearts, many fetal miRNAs became re-expressed during heart failure demonstrating a potential role for miRNAs to be involved in general fetal gene reprogramming during heart failure [12]. *In vitro* studies further confirmed that modulating miRNA expression can trigger molecular and phenotypic changes in different cardiovascular cells. For instance, modulating miR-133 or miR-208a in cardiomyocytes had a strong impact on hypertrophic response of cardiomyocytes [13,14]. Modulation of miRNA biogenesis by Dicer or Drosha knockdown in endothelial cells interfered with the angiogenic response [15]. Manipulation of miR-21 or miR-29 expression in fibroblasts induced changes in fibroblast proliferation or collagen expression implicating a potential therapeutic interest [16,17]. Using genetic animal techniques, miRNA gain- and loss-of function studies also revealed an essential role for many miRNAs in cardiac homeostasis and disease [18,19]. Taken together, these important findings have also been transferred to relevant therapeutic approaches based on antagomir or antimiR-mediated knockdown of miRNA expression in murine models of cardiac disease [17,20–22]. Of note, miRNA modulators were also translated into bigger animal studies such as in a primates' model of hepatitis C infection [23] and currently miRNA-based therapeutics also have entered the clinics based on ongoing phase I and II studies.

2. Identification of miRNA target genes (the miRNA targetome or RISCome)

In the field of miRNA research, identifying miRNA target genes is one of the most important but unfortunately one of the toughest goals requiring different approaches (excellently reviewed in [24]). Detailed knowledge of downstream effectors of miRNAs is important to estimate their individual functional relevance in healthy conditions but especially during disease. This information is especially needed when miRNA expression is modulated by miRNA-therapeutics such as antagomirs or anti-miRs. Here, new tools were recently developed to investigate miRNA target genes on a more global level (miRNA targetome or the RISCome analysis) in cardiac cells [25]. Different technical approaches to uncover miRNA targets are now available and will be presented and discussed in the following sections (see Fig. 1).

2.1. Bioinformatics, proteomics and other molecular biology-related approaches

2.1.1. Bioinformatics

Computational analysis of potential miRNA target genes relies mainly on open-access databases quite easily to use for the scientific community. Several working groups have developed software algorithms as target prediction tools which seem to be the first choice in miRNA target search. In common, the software tools screen mRNAs 3′-UTR for putative miRNA-responsive elements (MRE). As previously reported, MRE are more likely bound by miRNA when they are located either in the beginning or the end of the 3′-UTR of a gene [26]. Tandem repeats of MREs in a single 3′-UTR often enhance the possibility of miRNA binding. In contrast, secondary structure effects of RNA due to characteristic nucleotide sequence might impede miRNA binding in the center of a specific mRNA 3′-UTR. However, the underlying algorithms to predict targets vary and often the overlap of predicted

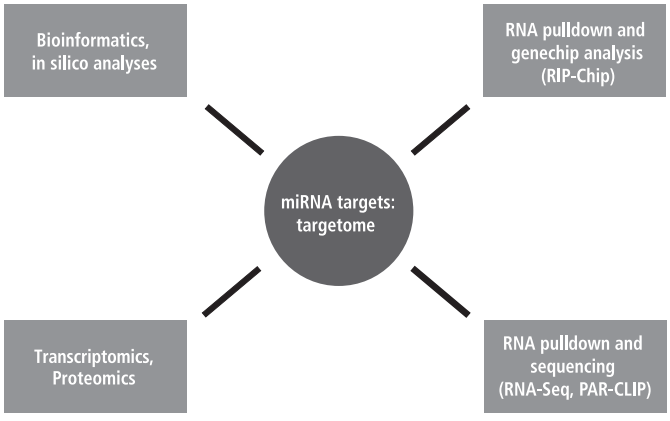

Fig. 1. Various approaches exist to delineate miRNA targets — the "targetome". Bioinformatics and *in silico* predictions, RNA pulldown and genechip analysis, transcriptomics or proteomics and RNA sequencing methods can be applied to identify miRNA targets on a cellular level.