MicroRNA-133: Biomarker and Mediator of Cardiovascular Diseases

13

J. Francisco Nistal, Ana V. Villar, Raquel García, and María A. Hurlé

Contents

J.F. Nistal (✉)
Servicio de Cirugía Cardiovascular, Hospital Universitario Marqués de Valdecilla, Universidad de Cantabria, Santander, Spain

Instituto de Investigación Valdecilla (IDIVAL), Santander, Spain
e-mail: jfnistal@gmail.com; hurlem@unican.es

A.V. Villar • R. García • M.A. Hurlé
Instituto de Investigación Valdecilla (IDIVAL), Santander, Spain

Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, Santander, Spain
e-mail: villarav@unican.es; raquel.garcia.lop@gmail.com; hurlem@unican.es

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Abstract

If we consider the molecular size of major nucleic acids and the time elapsed since we knew about their existence, microRNAs (miRs) comparatively appear as recently discovered and structurally small pieces of genetic material, but we should make no mistake to underestimate these modest but powerful molecules. Regulating no less than half of the transcriptome, their influence is a key for the fine-tuning of almost any biological process. miRs are considered elements that add precision and robustness to a myriad of physiological and pathological processes and commonly function as components of cellular networks by buffering extremes in gene expression. Their small size limits the side effects of these molecules and eases a pervasive behavior that explains their presence in different body fluids but also opens a complete new field of clinical opportunities for diagnostic, prognostic, or risk stratification applications which benefit from the possibility of bedside measurement.

From a purely investigational perspective, miRs constitute also a very useful tool. Their pleiotropic repressing effect on various, often functionally related, target mRNAs gives us clues on the molecular mechanisms underlying many pathological phenomena. On the other hand, since mRNA complementarity is dictated by a relatively short sequence (seed region) of nucleotides in the miR, computer-based prediction of miR targets is readily available. Additionally, "the control on the controllers" is tempting with the aim of modifying favorably the natural history of miR-related diseases. Several techniques to block or overexpress miRs have been launched, and, even though there are significant difficulties and drawbacks in their administration, some of them are starting to be used in human therapeutics.

miR-133 is muscle specific and one of the most abundant miRs in the heart. It plays major roles in the developing heart on cell differentiation into muscle tissue and also in later stages of cardiac morphogenesis. It is included among the handful of molecules able to cooperate for the experimental reprogrammation of fibroblasts into cardiac-like myocytes. miR-133 participates in the molecular pathology of myocardial hypertrophy, be it primary or secondary, in the transition and evolution of cardiac failure, in myocardial fibrosis and in cardiac cell apoptosis. In stable coronary artery disease and, particularly, in the acute coronary events, miR-133 levels decrease in the myocardium and increase in the circulation proportionally to the extent of the infarcted area. The value of circulating levels of miR-133 as a diagnostic and prognostic biomarker in these patients during the acute ischemic episode and after primary revascularization has already been established. Finally, research studies undertaken during the last 5 years show that dysregulation of miR-133 contributes to the pathological vascular remodeling underlying essential hypertension, vascular calcification, atherosclerosis, and aneurysmal disease.

At this time, it could be said that miRs are ready for prime time for diagnostic and prognostic purposes in the clinical arena, but more is coming on miR molecular manipulation with miR mimics and anti-miRs and even more with targeting of miR-related pathways. Meanwhile, other future uses of miRs in the

fields of regenerative medicine and tissue engineering await for further refinement before they can be clinically applicable.

Keywords

Biomarkers • MicroRNAs • miR-133 • Cardiogenesis • Cardiac remodeling • Left ventricular hypertrophy • Myocardial fibrosis • Arterial aneurysm • Coronary arterial disease • Vascular smooth muscle

Abbreviations

AAA	Abdominal aortic aneurysm
ACS	Acute coronary syndrome
AMI	Acute myocardial infarction
BAV	Bicuspid aortic valve
ceRNA	Competing endogenous RNA
CHD	Coronary heart disease
CTGF	Connective tissue growth factor
cTn	Cardiac troponin
ICA	Intracranial aneurysm
lnc-RNA	Long noncoding RNA
LV	Left ventricle
LVAD	Left ventricular assist device
MI	Myocardial infarction
miR	MicroRNA
MMP	Matrix metalloproteinase
MRE	miR recognition element
NSTEMI	Non ST-elevation myocardial infarction
qRT-PCR	Real-time polymerase chain reaction
ROC	Receiver operating characteristic
STEMI	ST-elevation myocardial infarction
TAA	Thoracic aortic aneurysm
TAC	Transverse aortic constriction
TAD	Thoracic aortic dissection
TAV	Tricuspid aortic valve
TGF-β	Transforming growth factor-β
UA	Unstable angina
UTR	Untranslated region
VSMC	Vascular smooth muscle cell

Key Facts of MicroRNAs in Cardiovascular Disease

- The World Health Organization estimates that in 2015 there will be about 20 million deaths due to cardiovascular diseases, accounting for 30 % of the global death toll. Cardiovascular disease will continue to dominate mortality trends in the near future.

- Early detection of disorders before severe cardiovascular damage takes place may benefit from the identification of new tissue-specific biomarkers that can improve the sensitivity and accuracy of the diagnostic process.
- Aside from their value for risk assessment, early diagnosis, and prognosis, biomarkers may also act as mediators of disease, what underscores their interest in the elucidation of the pathogenesis of cardiovascular disease.
- miRs are powerful regulators of many, if not all, biological processes in the cell, and their dysregulation plays important roles in cardiovascular diseases.
- More than 2,500 miRs have been identified in humans. One miR can modify the translation to protein of up to hundreds of different mRNAs, and a single mRNA can be targeted by several miRs.
- Manipulation of miR expression has emerged as a valuable tool for therapeutic intervention, and, despite their recent discovery, several miR candidates have already progressed into clinical development.
- miRs are released by cells, remain in the circulation in a stable cell-free form, and can be detected by sensitive, specific, and minimally invasive methods. These characteristics make miRs potential bedside biomarkers for cardiovascular diseases.

Definitions

Anti-miRs Synthetic single-stranded antisense oligonucleotides that hybridize by base pairing to target miRs and block their function.

Cardiac remodeling Genome expression resulting in molecular, cellular, and interstitial changes and manifested clinically as changes in size, shape, and function of the heart originated from cardiac load or injury.

MicroRNAs (miRs) Short non-coding, single-stranded RNAs involved in the posttranscriptional control of gene expression. miR binding to its mRNA targets results in the inhibition of translation to proteins.

miR-133 Muscle-specific miR that is expressed by skeletal and cardiac striated myocytes and by smooth muscle cells.

miR cluster miRs transcribed as a single unit from the same primary transcript that are regulated in a similar way.

miR family Group of miRs that have identical seed regions and share targets and functions. The occurrence of miRs belonging to distinct "seed" families within the same cluster is common.

miR recognition element (MRE) mRNA sequence in the 3′-UTR end that binds a specific miR usually by partial or complete base pairing.

miR sponges Are synthetic RNA molecules harboring complementary binding sites to the seed sequences of a given miR or a miR family.

Myomirs Group of miRs that are highly expressed in muscular tissue.

Seed region Sequence of 6–8 nucleotides at the 5′ end of miRs which binds by base pairing with complementary sequences of target mRNAs.

Transverse aortic constriction Experimental model of LV pressure overload induced by surgical banding of the mid aortic arch.

Introduction

MicroRNAs (miRs) are short (~22 nucleotide long) noncoding, evolutionary conserved, single-stranded RNAs involved in the posttranscriptional control of gene expression. miRs bind to mRNA transcripts that have complementary target sequences to produce mRNA silencing, either through the inhibition of mRNA translation or, less often, by the induction of mRNA degradation (Ha and Kim 2014). miRs were first described in the nematode *Caenorhabditis elegans* as regulatory factors that control the expression of genes involved in larval development (Lee et al. 1993). Since then, more than 2,500 mature miRs have been identified in humans, and each of them can modify the translation of up to hundreds of different mRNAs. The role of miRs as powerful regulators of many, if not all, biological processes in the cell has been widely studied, and their dysregulation plays important roles in numerous human diseases. Identification and validation of miR targets are steps of fundamental importance for the comprehensive understanding of miR roles in physiological and pathological conditions and, therefore, of their possible therapeutic applications (Dangwal and Thum 2014). miRs can be released by cells and remain in the circulation in a remarkably stable cell-free form. Detection of miRs can be sensitive, specific, and minimally invasive. Such characteristics warranted a general interest on circulating miRs as potential measurable bedside biomarkers for detecting a wide range of diseases, including those affecting the cardiovascular system, and for monitoring disease conditions and efficacy of therapeutic regimens (Creemers et al. 2012).

miR-133a is one of the most abundant miRs in the heart. The combination of experimental models of human pathologies with tools that modulate miR-133 activity has provided important insights on the role played by this miR and their targets in very prevalent cardiovascular pathologies (van Rooij and Kauppinen 2014). It contributes to cell differentiation into muscle tissue during development and in later stages of cardiac morphogenesis. It is included among the handful of molecules able to cooperate for the experimental reprogramming of fibroblasts into cardiac-like myocytes. miR-133 participates in the molecular pathology of myocardial hypertrophy, be it primary or secondary, in the transition to and evolution of cardiac failure, in myocardial fibrosis and in cardiac cell apoptosis. In stable coronary artery disease and, particularly, in the acute coronary events, miR-133 levels

decrease in the myocardium and increase in the circulation proportionally to the extent of the infarcted area. The value of circulating levels of miR-133 as a diagnostic and prognostic biomarker in these patients during the acute ischemic episode and after primary revascularization has already been established. Finally, research studies undertaken during the last 5 years show that dysregulation of miR-133 contributes to the pathological vascular remodeling underlying essential hypertension, vascular calcification, atherosclerosis, and aneurysmal disease.

MicroRNA Biogenesis, Structure, and Biology

The biogenesis of a functional miR (Fig. 1) (Ha and Kim 2014) starts in the nucleus with the transcription of primary transcripts (pri-miRs) by RNA polymerase II from intronic, exonic, intergenic, or polycistronic loci. The pri-miR has a stem-loop structure that incorporates one or more miR-encoding precursors. pri-miR is processed in the nucleus by a protein complex formed by the RNAse III DROSHA and DGCR8 (DiGeorge syndrome chromosomal region 8) to a ~70 nucleotide hairpin-shaped precursor miR (pre-miR), which contains the mature miR in either the 5′ or 3′ arm. Pre-miRs are transported to the cytoplasm, where the RNase III DICER and its cofactor TRBP (the human immunodeficiency virus transactivating response RNA-binding protein) cleave the pre-miR hairpin and separate the loop from the double-stranded stem, forming miR duplexes comprised by the passenger and the guide strands. DICER and the miR duplex integrate into a complex with argonaute proteins, which loads the guide strand to the miR-induced silencing complex (RISC). Usually, the guide strand of the miR duplex is the mature and active miR form that guides RISC to target mRNAs, while the passenger strand is degraded. However, for some miRs, the passenger strand can be also active. The most critical determinant for miR targeting of mRNAs is the "seed region," constituted by six to eight nucleotides at the 5′ end of miRs. Nucleotides at the seed region form Watson-Crick pairs with complementary sequences, called miR recognition elements (MREs), in the 3′-UTR end of the mRNA, producing either repression of translation or destabilization of the target mRNA (Ha and Kim 2014). Recent studies have shown that the functional consequences of the interaction between miR's seed regions and mRNAs are not univocal. The so-called competing endogenous RNAs (ceRNAs) are RNA transcripts [mRNAs, transcribed pseudogenes, and long noncoding RNAs (lnc-RNAs)] that, by sharing MREs, compete for binding to miRs and then regulate each other's expression. The cross talk between ceRNA-miRNA-mRNA maintains the functional balance of gene networks in the cell (Salmena et al. 2011).

MicroRNA Targets

The reliable prediction of potential miR targets is possible with computational methods. Silico predictions often use algorithms which account for interactions between the 3′-UTR sequences of mRNAs and the seed regions of miRs, the

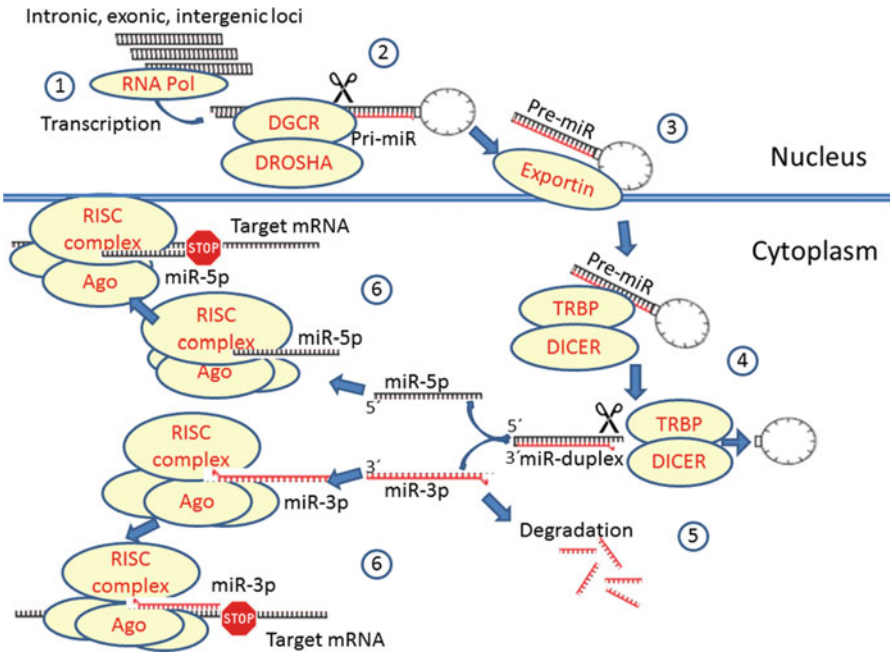

Fig. 1 Biogenesis of a functional miR. The biogenesis of a functional miR starts in the nucleus with the transcription of primary transcripts (*pri-miRs*) by RNA polymerase II from intronic, exonic, intergenic, or polycistronic loci (*1*). The pri-miR is processed in the nucleus by a complex formed by the RNAse III DROSHA and DGCR8 (DiGeorge syndrome chromosomal region 8) to a hairpin-shaped precursor miR (*pre-miR*), which contains the mature miR in either the 5′ or 3′ arm (*2*). Pre-miRs are transported by exportin to the cytoplasm (*3*), where the RNase III DICER and its cofactor TRBP (the human immunodeficiency virus transactivating response RNA-binding protein) cleave the pre-miR hairpin, forming miR duplexes of passenger and guide strands (*4*). DICER and the miR duplex integrate into a complex with argonaute proteins, which loads the guide strand to the miR-induced silencing complex (*RISC*). Usually, the passenger strand is degraded (*5*) and the guide strand is the active form that guides RISC to target mRNAs (*6*). For some miRs, the passenger strand can be also active (*6*). The interaction miR-mRNA represses the translation of targeted mRNAs to proteins

presence of multiple target sites, the thermodynamic stability of miR-mRNA hybrid, and the phylogenetic conservation. Today, many web-accessible resources organize all kinds of miR-related data and provide information regarding predicted and experimentally verified targets, biological- and disease process-related miRs, tissue expression patterns, regulatory networks and signaling pathways, etc. Representative examples are the following: HMDD; miRanda, miRBase; miR2Disease; miRSearch; miRecords; PicTar; PITA; TarBase; Targetscan; etc.

It is known that 3′-UTR sequences with perfect complementarity with the miR are not necessarily functional, while mRNA sites with imperfect pairing can be very good miR targets; therefore, bioinformatic predictions are prone to false positives. However, there are multiple exceptions regarding the requirement for miR-binding sites to be located in the 3′ UTR. Thus, all miR targets have to be validated in vitro

and in vivo since prediction programs only propose plausible candidates for validation.

Besides their intracellular functions, recent studies demonstrate that miRs can be released by cells and remain in the circulation in a remarkably stable cell-free form. miRs have been found in various body fluids, including serum, plasma, saliva, tears, urine, amniotic fluid, colostrum, breast milk, bronchial lavage, cerebrospinal fluid, peritoneal fluid, pleural fluid, and seminal fluid. miRs are easily detected and quantified in body fluids by microarray assays, next-generation sequencing, northern blot, and real-time polymerase chain reaction (qRT-PCR). Detection of miRs can be sensitive, specific, and minimally invasive. Such characteristics warranted a general interest on circulating miRs as potential bedside biomarkers for detecting a wide range of cardiovascular diseases and for monitoring disease progression and efficacy of therapeutic regimens (Wang et al. 2013a; Villar et al. 2011, 2013; Garcia et al. 2013; for a review see Creemers et al. 2012).

Circulating miRs are released from cells incorporated into extracellular vesicles (exosomes and microvesicles) or bound to RNA-binding proteins such as argonaute or lipoprotein complexes such as high-density lipoproteins (HDL), etc (Valadi et al. 2007). These types of complexings provide protection against degradation by the endogenous RNAse activity (reviewed in: Creemers et al. 2012). Identification of exosomes containing miRs, which are functional upon delivery to the recipient cells, has unveiled a new role for miRs as juxtacrine/paracrine/endocrine mediators of cell-to-cell communication, involved in the transfer of genetic information between neighboring cells or cells located at distant organs (Kuwabara et al. 2011). As an example, cell-specific inflammatory exosomes (released from platelets, endothelial cells, and monocyte or monocyte-derived cells) are enriched in a subset of miRs that are functionally involved in the pathogenesis of cardiovascular diseases and whose circulating levels have been proposed as putative biomarkers of such diseases (Hulsmans and Holvoet 2013). Exosomes also constitute a novel therapeutic strategy for delivering cargos of miRs, RNAs, or even drugs (Fleury et al. 2014).

Modulation of MicroRNA Activity

miR dysregulation is a common feature in cancer, central nervous system disorders, inflammation, cardiovascular diseases, and metabolic disorders. The manipulation of miR expression is an attractive tool for therapeutic intervention, and several miR candidates have already progressed into clinical development (van Rooij and Olson 2012). There are two approaches to developing miR-based therapeutics: miR antagonists and miR mimics (reviewed in: Dangwal and Thum 2014; van Rooij and Kauppinen 2014). miR mimics are used to restore the level of a downregulated miR during disease progression. Delivery of miR replacement therapies in vivo was accomplished using lentiviral or adenoviral constructs expressing the miR of interest, miR-liposomal formulations, miR-polyethyleneimine or miR-atelocollagen complexes, etc.

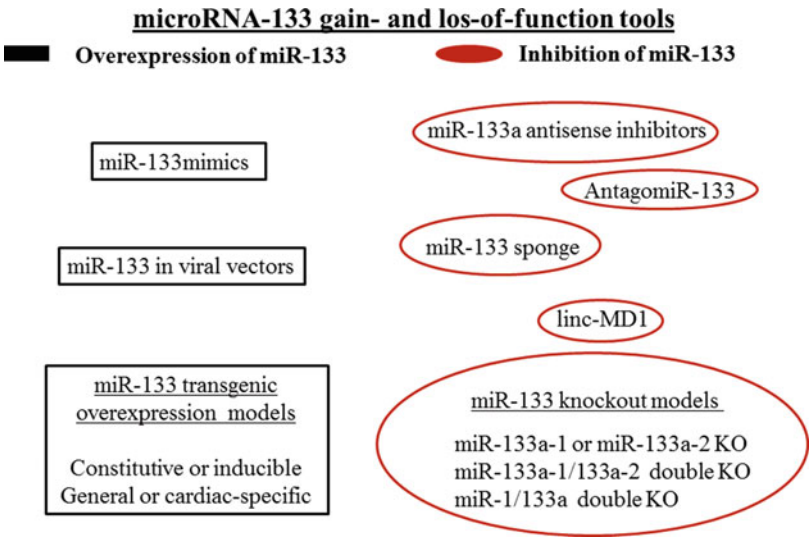

Fig. 2 MicroRNA-133 gain- and loss-of-function strategies

The objective of using miR antagonists is to inhibit endogenous miRs that are upregulated in a particular disease process. The function of mature miRs can be blocked using either anti-miRs or miR sponges. Anti-miRs are synthetic single-stranded antisense oligonucleotides that hybridize by Watson-Crick base pairing to target miRs and block their function. miR sponges are synthetic RNA molecules harboring complementary binding sites to the seed sequences of a given miR or a miR family. The miRs of interest that bind to the sponge become unavailable to bind their targets. Chemical modification strategies of the oligonucleotides, including 2′-O-methylation or locked nucleic acid (LNA) modification, have been developed to improve miR binding affinity and biostability. Cholesterol conjugation of anti-miRs (antagomiRs) improves their cellular uptake.

Modulation of miR activity has proven useful as experimental tool (Dangwal and Thum 2014; van Rooij and Kauppinen 2014). Gain- and loss-of-function approaches (Fig. 2) have been applied to ascertain processes regulated by miR-133 in the cardiovascular system, during the embryonary period and in adulthood. Classical knockout technology allowed unraveling the role of miR-133 and miR clusters in cardiac development using mice deficient for either miR-133a-1 or miR-133a-2 or both (Liu et al. 2008) and mice with targeted inactivation of miR-1/133a clusters (Wystub et al. 2013). The combination of experimental models of human disorders with miR-133 activity modulation provided insights on the role played by this miR and their targets in cardiovascular pathologies. For example, the pathogenetic role miR-133 in murine hypertrophic cardiomyopathy was demonstrated in gain- and loss-of-function studies using adenoviral vectors containing a miR-133 mimic and specific miR-133 sponges, respectively (Caré et al. 2007). Also, a modulatory role for miR-133 on myocardial fibrosis and apoptosis was demonstrated using mouse models of cardiac-specific miR-133a transgenic overexpression (Castaldi

et al. 2014). Similar approaches were used to demonstrate the participation of miR-133 in murine models of arterial wall injury and atherosclerosis (Torella et al. 2011; Gao et al. 2014).

The muscle-specific lnc-RNA, linc-MD1, constitutes an example of ceRNA which governs muscle differentiation by targeting both miR-133 and miR-135. linc-MD1 prevents miR-133 and miR-135 to regulate the expression of transcription factors that activate muscle-specific gene expression. Downregulation or overexpression of linc-MD1 correlates with retardation or anticipation of the muscle differentiation program, respectively (Cesana et al. 2011).

MicroRNA-133 Taxonomy and Targets

miR-133 was first characterized in mice and, since then, homologues have been discovered in the genomes of nonmammalian vertebrates and in many mammalian species, including the *Homo sapiens* (see miRbase). miR-133's presence in the miRNome has been firmly established in health and disease, suggesting that it plays similar functions in different organisms. There are three known miR-133 human genes: two gene loci (bicistronic) for miR-133a and one for miR-133b (miR map). The first location of a miR-133a precursor is intronic (Mib1 gene) in chromosome 18 (18q11.2), in a sequence of 88 nucleotides (GGGAGCCAAAUGCUUUGCUAGAGCUGGUAAAAUGGAACCAAAUCGACUG UCCAAUGGAUUUGGUCCCCUUCAACCAGCUGUAGCUGUGCAUUGAUGGCG CCG) called miR-133a-1 stem loop or pre-miR-133a-1. The catalytic cleavage by DICER of pre-miR-133a-1 gives rise to miR-133a-1-3p, which contains the 53–74 bp (UUUGGUCCCCUUCAACCAGCUG) from the 3′ arm and to the marginally co-expressed miR-133-1-5p, formerly called miR-133a*, which corresponds to the 5′arm 16–37 bp (AGCUGGUAAAAUGGAACCAAAU). Although miR-133-1-5p degradation after its separation from the duplex was reported, today both forms of miR-133 are considered mature functional molecules, and bioinformatic and experimental studies are unraveling their targets. A different additional precursor of miR-133a, called pre-miR-133a-2, is also intronic (putative uncharacterized protein C20orf166 gene) in chromosome 20. Its sequence (GGGAGCCAAAUGCUUUGCUAGAGCUGGUAAAAUGGAACCAAAUCGACUG UCCAAUGGAUUUGGUCCCCUUCAACCAGCUGUAGCUGUGCAUUGAUGGCG CCG) also contains the previously described 3p and 5p miR-133a sequences that, though identical, were called miR-133a-2-3p and miR-133a-2-5p (Fig. 3). The third miR-133 is miR-133b (UUUGGUCCCCUUCAACCAGCUA). Its mature form comes from the 3′arm of pre-miR-133b (CCUCAGAAGAAA GAUGCCCCCUGCUCUGGCUGGUCAAACGGAACCAAGU CCGUCUUCCUGAGAGGUUUGGUCCCCUUCAACCAGCUACAGCAGGGCUGG CAAUGCC CAGUCCUUGGAGA) which is located in chromosome 6.

The members of the miR-133 family differ depending on the species. The human family of miR-133 includes miR-133a-3p, miR-133a-5p, and miR-133b. In mice and

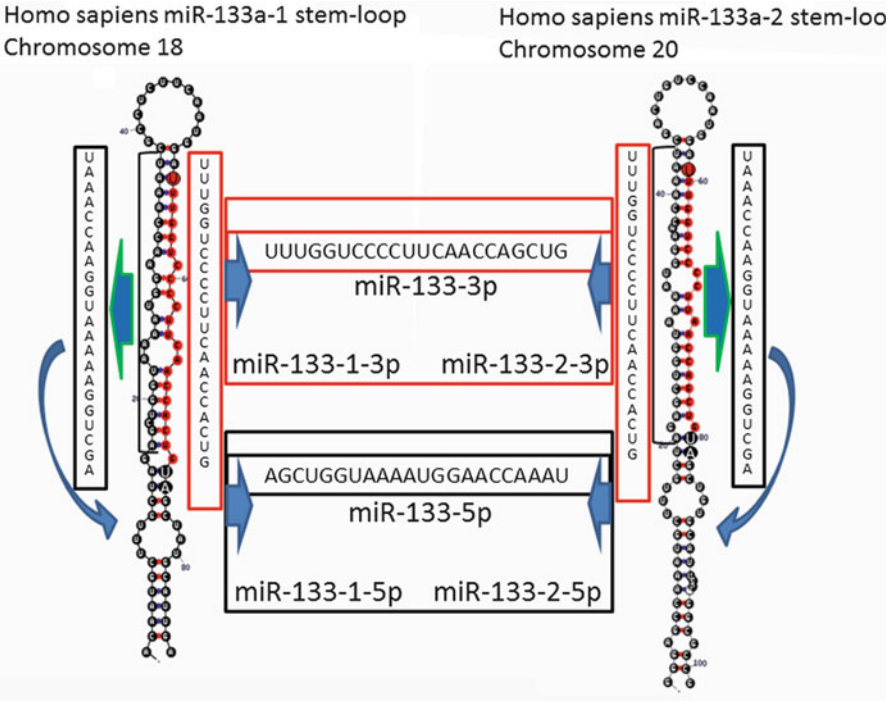

Fig. 3 The *Homo sapiens* microRNA-133a family. There are two gene loci (bicistronic) for miR-133a located in chromosome 18 (miR-133a-1) and 20 (miR-133a-2). The sequence of the precursor pre-miR-133a-1 (stem loop of the *left*) includes two mature molecules of miR-133a: the major species miR-133a-1-3p (depicted in *red*) is derived from the 3′ arm; the minority miR-133-1-5p is derived from the 5′arm (in *black*). The bicistronic precursor pre-miR-133a-2 (stem loop of the *right*) contains two miR-133a sequences that are identical to the former but, in this case, are called miR-133a-2-3p and miR-133a-2-5p (Modified from miRNA map: http://mirnamap.mbc.nctu.edu.tw)

rats, there are five different miR-133 members: miR-133a-3p, miR-133a-5p, 133b-3p, miR-133b-5p, and miR-133c. In all species, miR-133a-3p is the most abundantly expressed (see miRSearch V3.0).

The presence of multiple miRs with identical or similar mature sequences is a common and evolutionary conserved feature, indicating functional significance. Both miR-133 and miR-1 are muscle-specific miRs (myomiRs) that are found in bicistronic position in the mammalian genome and are transcribed together. miR-1 and miR-133a are even encoded by duplicated bicistronic loci (miR-1-1/miR-133a-2 and miR-1-2/miR-133a-1) with identical sequences of the mature miRs. There is another bicistronic cluster encoding the nearly identical miR-206/miR-133b (Wystub et al. 2013).

Up-to-date, the combination of computational analysis and experimental approaches revealed that miR-133a-3p has 399 predicted targets, 42 of which have

been validated experimentally. A non-comprehensive list of validated targets includes the following: collagen type I α1, fibrilin, tropomyosin, TGF-β1, TGF-βRII, MEF-2, HCN2, KCNQ1, ERG, NFATc4, RhoA, Runx2, and PITX3.

Embryogenesis, Myocardial Regeneration, and Cell Reprogramming

miR-133a is muscle specific, expressed by skeletal and cardiac striated myocytes, and participates in various physiological and pathological phenomena (Mooren et al. 2014; Thum et al. 2007; Condorelli et al. 2014). Together with miR-1 represses non-muscle genes in human and murine embryonic stem cells, favoring mesoderm formation and modulating their differentiation into muscle tissue (Ivey et al. 2008). In the heart, miR-133 is exclusively expressed by cardiomyocytes (Townley-Tilson et al. 2010) and the miR-133a-1/miR-1-2 and miR-133a-2/miR-1-1 clusters play a significant role in the maturation of embryonic cardiomyocytes to more differentiated fetal cardiomyocytes through the adjustment of the levels of myocardin (Wystub et al. 2013). These miRs are direct transcriptional targets of several muscle differentiation regulators, the most important of which is serum response factor (SRF), which points to the existence of a common set of control elements that modulate the development of cardiac and skeletal muscle (Zhao et al. 2005). miR-133a plays also an indispensable role in cardiomyocyte proliferation and ventricular septation during embryonic cardiac development. The lack of miR-133a results in ectopic activation of smooth muscle genes in the heart and aberrant cardiomyocyte proliferation, whereas its overexpression derives in a low cardiomyocyte proliferation with hypotrophic organogenesis and loss of embryonic viability (Liu et al. 2008). Overexpression of miR-133 by transgenesis in zebra fish hampers myocardial regeneration after cardiac apex avulsion, and, conversely, miR-133 depletion enhances cardiomyocyte proliferation and speeds up the regenerative healing after apical severance in this model (Yin et al. 2012). These findings led Yin et al. to suggest that cardiac regeneration in the adult zebra fish is evolutionarily related to myocardial hypertrophy in mammals and that miR-133 downregulation is a remnant shared by both remodeling phenomena.

Recent studies demonstrate that miR-133a, together with the combination of Gata-binding protein 4, Hand2, T-box5 and myocardin (Nam et al. 2013), or Gata4 plus Mef2c and Tbx5 (GMT), or GMT plus Mesp1 and myocardin (Muraoka et al. 2014), is able to promote the reprogramming of neonatal and adult mouse or human fibroblasts to cardiac-like myocytes. In addition, transplantation of cardiac progenitor cells overexpressing miR-133a improves the heart function in a rat model of myocardial infarction by increasing cardiomyocyte proliferation and vascularization and reducing cardiomyocyte apoptosis and myocardial fibrosis (Izarra et al. 2014).

Pathologic Cardiac Remodeling

There is abundant evidence of miR-133a involvement in pathological forms of cardiac plasticity. The reappearance in adulthood of the embryonic gene expression pattern, characterized by diminished expression of the miR-133 cluster, would be teleonomically sound, as programs for cardiac growth experiment a robust activation in response to the demands imposed by either the somatic development or a pathologic cardiac mechanical overload (Table 1) (Thum et al. 2007).

Cardiac miR microarray analyses in rodent models of cardiac hypertrophy show a reduction in miR-133a expression coincident with an increase in cardiac mass (van Rooij et al. 2006; Ye et al. 2013). Validation of these data with northern blotting or qRT-PCR confirms a decrease in the cardiac levels of miR-133 in exercised rats, in mice subjected to transverse aortic constriction (TAC), in several transgenic mice models of LV hypertrophy, and in human LV from patients with pathologies that promote hypertrophic growth (van Rooij et al. 2006; Carè et al. 2007). In the mouse model of TAC, miR-133 cardiac expression is reduced 1 week after surgery but recovers basal levels at 3 weeks, time at which LV mass typically plateaus with this model (Matkovich et al. 2010). These observations have been confirmed with functional in vitro and in vivo experiments of gain and loss of function. Overexpression of miR-133 with adenoviral vectors in cultured neonatal murine cardiomyocytes results in significant blunting of the hypertrophic hallmark features induced by phenylephrine or endothelin-1 treatments. Conversely, molecular sequestration of miR-133a, using adenoviral vectors carrying MREs that act as a decoy and silence the miR, results in evolution toward a hypertrophic phenotype of fetal or adult mouse cardiomyocytes in culture (Carè et al. 2007). Also, mice treated subcutaneously or transcoronary with an antagomiR targeted to miR-133a showed reactivation of the fetal gene expression profile, activation of the cardiac hypertrophic program, and significant increases in LV wall thicknesses and total cardiac mass. On the other hand, overexpression of miR-133 in Akt transgenic mice, a model of cardiac hypertrophy, resulted in volume reduction of cardiomyocytes and reduced expression of fetal genes (Carè et al. 2007).

Not all the data on the role of miR-133 in hypertrophy models are univocal though. Overexpression of this miR in transgenic mice does not palliate the increase in cardiac mass observed after TAC, which is similar to their wild-type littermates even though the transgenic animals, contrary to the wild-type, do not exhibit a postoperative reduction in miR-133 expression levels. Also, miR-133 expression is not reduced in some models of genetically induced cardiac hypertrophy (Matkovich et al. 2010).

The plasma levels of the muscle-related miRs are altered in humans in response to both acute aerobic and endurance exercise. In athletic runners, exercise induced an increase in the circulating levels of miR-133a which exhibit positive correlations with the maximal oxygen uptake, with the running speed at individual anaerobic lactate threshold, and with the cardiac interventricular septal thickness (Mooren et al. 2014). At the other end, unloading the heart in patients with advanced heart

Table 1 miR-133 in patients with cardiac disease

Pathology	Cohorts	miR-133 regulation	Other major findings	References
Aortic stenosis	Patients with moderate to severe AS ($n = 112$) and healthy controls ($n = 40$)	↓miR-133a qRT-PCR Plasma samples	miR-1 and miR-378 reduced in AS miR-378 is a predictor of LVH	Chen et al. (2014)
	Patients with isolated AS ($n = 9$) and nonhypertrophic controls ($n = 4$)	↓miR-133a in AS qRT-PCR Myocardial samples	↓miR-30c CTGF: target of miR-133a; overexpressed in AS	Duisters et al. (2009)
	AS patients ($n = 46$), surgical controls with no pressure or volume overload ($n = 23$)	↓miR-133a in patients with persistent LVH 1 year after aortic valve replacement qRT-PCR Myocardial samples	Myocardial miR-133a: predictor of postoperative LV mass loss and mass normalization one year after surgery	Villar et al. (2011)
	AS patients ($n = 74$)	↓circulating miR-133a in patients with persistent LVH 1 year after aortic valve replacement qRT-PCR Myocardial samples Plasma samples: Peripheral and coronary sinus blood	Circulating miR-133a is a predictor of postoperative LV mass loss and of mass normalization one year after surgery. The heart is a main contributor to circulating miR-133a	García et al. (2013)
Hypertrophic cardiomyopathy	HCM patients ($n = 4$) and controls ($n = 2$) Patients with mitral stenosis and atrial dilatation ($n = 3$), and controls ($n = 3$)	↓miR-133 Northern blot Myocardial samples		Carè et al. (2007)
	HCM patients ($n = 41$) and age and sex matched healthy subjects ($n = 41$)	↑miR-133a in plasma qRT-PCR Plasma samples	miRs -199a-5p, -27a, and -29a correlated with hypertrophy and miR-29a correlated also with fibrosis	Roncarati et al. (2014)

(*continued*)

Table 1 (continued)

Pathology	Cohorts	miR-133 regulation	Other major findings	References
Left ventricular hypertrophy	ICM ($n = 19$), DCM ($n = 25$), and AS ($n = 13$) patients and controls ($n = 10$)	miR-133a: no change miR high-throughput bead-based platform qRT-PCR Myocardial samples	↑miR-214 in all pathological groups ↓miR-19a and -19b in DCM and AS Distinct miR profiles in patients with different diagnosis	Ikeda et al. (2007)
Heart failure	LVs with DCM ($n = 6$), normal adult LVs ($n = 4$) and fetal hearts ($n = 6$)	miR-133: no change miR and mRNA microarrays qRT-PCR Myocardial samples	Striking similarity of miR and mRNA expression profiles in HF and fetal hearts as compared with adult healthy hearts	Thum et al. (2007)
LV mechanical circulatory support	Patients with cardiomyopathy ($n = 17$) supported with LVAD	During LVAD: ↓miR-133a in DCM and ↑miR-133a in ICM qRT-PCR Myocardial samples	Similar trends for miR-1 and miR-133b	Schipper et al. (2008)
	Patients with congestive HF with ($n = 10$: 4 ischemic; 6 nonischemic) or without ($n = 17$: 7 ischemic; 10 nonischemic) LVAD. Nonfailing hearts ($n = 11$)	↑miR-133 in HF versus non-HF and normalizes with LVAD miR-PCR-array Myocardial samples	Changes in miR signature more sensitive than mRNA profile in HF. Changes in miRs nearly normalized with LVAD	Matkovich et al. (2009)
	Patients with DCM ($n = 28$) supported with LVAD: 14 support dependent and 14 experienced LV recovery. Test cohort: $n = 14$; Validation cohort: $n = 14$. Control nonfailing hearts ($n = 7$)	miR-133: no change miR-PCR-array qRT-PCR Myocardial samples	Pre-support expression of miR-23a and -195 differs between hearts that recovered and hearts that did not	Ramani et al. (2011)

(*continued*)

Table 1 (continued)

Pathology	Cohorts	miR-133 regulation	Other major findings	References
	Patients with ICM ($n = 10$) and DCM ($n = 9$) supported with LVAD	miR-133: no change miR-PCR-array qRT-PCR Plasma samples Myocardial samples	↑circulating miR-483-3p with LVAD Baseline miR-1202 identified good and bad LVAD responders	Morley-Smith et al. (2014)
	Paired samples of failing LVs with ICM ($n = 8$) and DCM ($n = 8$) before and after support with LVAD. Nonfailing LVs ($n = 8$)	No specific reference to miR-133 Next-generation sequencing of RNA Myocardial samples	lncRNA profiles discriminate better different HF pathologies than mRNA or miR signatures. lncRNAs profile improves more with LVAD support than either mRNAs or miRs	Yang et al. (2014)
	Failing LVs with ICM ($n = 13$) and DCM ($n = 21$) before and after support with LVAD. Nonfailing adult LVs ($n = 8$) and fetal hearts ($n = 5$)	miR-133: no change Small RNA sequencing Myocardial samples Plasma and serum samples	↑circulating myomiRs in advanced HF and nearly normalized 3 months after LVAD support	Akat et al. (2014)

AS valvular aortic stenosis, *CTGF* connective tissue growth factor, *DCM* dilated cardiomyopathy, *HF* heart failure, *HCM* hypertrophic cardiomyopathy, *ICM* ischemic cardiomyopathy, *lncRNA* long noncoding RNA, *LVAD* left ventricular assist device, *LVH* left ventricular hypertrophy

failure under mechanical circulatory support also modifies the expression of miR-133a but in different direction, depending on the etiology of the cardiac underlying pathology. Thus, in patients supported with a LV assist device, the myocardial expression of miR-133a decreases if their underlying pathology is an idiopathic dilated cardiomyopathy or increases if it is of ischemic origin (Schipper et al. 2008). This observation, however, is at variance with the findings of other groups (Ramani et al. 2011; Morley-Smith et al. 2014) who did not find differences in miR-133a cardiac or plasma levels in heart failure patients after support with a mechanical LV assist device (Table 1).

The prognostic potential of myocardial and/or circulating miR signatures for the estimation of cardiac recoverability in patients with advanced heart failure under mechanical circulatory support is currently under active investigation. miRNomic profiling appears in this respect more promising than transcriptomic analysis. mRNA levels feature little expression changes in these patients, as compared with controls

with stable heart failure, whereas myocardial miR levels undergo generalized and profound changes both in patients with advanced heart failure and in patients treated successfully with mechanical means (Table 1) (Matkovich et al. 2009).

Reports on human cardiac pathologies which associate LV hypertrophy described downregulation of myocardial and/or circulating miR-133a often in association with miR-1 (Caré et al. 2007; Chen et al. 2014), and this phenomenon has been partially pinpointed in microarray analyses (Table 1) (Ikeda et al. 2007). In patients with pure, severe, a valvular aortic stenosis, the myocardial expression and circulating levels of miR-133 were found significantly reduced as compared with surgical controls without pressure or volume overload (Villar et al. 2011; García et al. 2013). In these patients, undergoing aortic valve replacement, both myocardial and circulating miR-133a preoperative levels were predictors of the quantitative LV mass loss and the probability of mass normalization 1 year after surgery. Interestingly, the postoperative increase in aortic valve area, as a surrogate of the reduction in pressure overload achieved by these patients, did not arise as a predictor of these variables, suggesting that in the clinical arena the mechanical stress imposed by the valve pathology acts as a trigger of the cardiac remodeling process, but there are other important players that have major roles in the regression of hypertrophy (Table 1) (Villar et al. 2011; García et al. 2013).

As to the origin of miR-133a in the circulation, there are several potential candidates: striated skeletal muscle, vascular wall, blood elements, etc. It has been shown, however, that in patients with severe aortic stenosis, there is a positive concentration gradient between the coronary outflow and the systemic circulation and a significant positive correlation between the miR levels at both spots and with the myocardial expression levels at the LV (García et al. 2013). These observations support the role of the heart as a key contributor to the miR-133a circulating levels in this context.

It is not clear whether miR-133 participates in the maladaptive cardiac remodeling that accompanies human end-stage heart failure. Indeed, transcriptional profiling of human myocardium with microarrays or next-generation sequencing have shown its lack of regulation in this scenario (Table 1) (Thum et al. 2007; Ikeda et al. 2007; Matkovich et al. 2009; Yang et al. 2014; Akat et al. 2014).

Fibrotic Remodeling of the Heart and Apoptosis

There is an ample consensus on the anti-fibrotic role of miR-133a in the cardiac stress condition. The controversy persists, though, on the precise mechanistic elements whereby fibrotic remodeling is blunted by this miR in different pathologic clinical or experimental situations. In rodent models of pressure overload and in patients with severe valvular aortic stenosis, Duisters et al. (2009) found that myocardial levels of miR-133 correlated inversely with the expression of connective tissue growth factor (CTGF), a potent inducer of extracellular matrix production acting downstream of transforming growth factor-β (TGF-β) (Table 1). Gain- and loss-of-function experiments showed that the expression levels of miR-133 correlated inversely with the levels of CTGF in cultured cardiomyocytes and with CTGF and extracellular matrix elements in fibroblasts. Luciferase reporter assays confirmed

that CTGF and the profibrogenic cytokine TGF-β1 and the TGF-β type II receptor were direct targets of miR-133 (Duisters et al. 2009; Shan et al. 2009). A discordant finding in this respect is the observation of increased mRNA CTGF levels after aortic banding in transgenic mice overexpressing miR-133a (Matkovich et al. 2010).

In a rat model of angiotensin-dependent systemic hypertension, it has been shown that gene and protein expression levels of collagen I A1 evolve with a significant inverse correlation with those of miR-133a. The bioinformatic prediction algorithm studies of putative binding sites and luciferase reporter assays confirmed collagen I A1 as a direct target of miR-133a (Castoldi et al. 2012). Other direct and indirect mechanisms of the antifibrotic role of miR-133a in cardiac stress act via inhibition of caspase-mediated cardiomyocyte apoptosis (Matkovich et al. 2010).

There is experimental in vivo evidence that, after an ischemic insult, miR-133a has a beneficial effect on the myocardial cell fate by targeting caspase-9 and the consequent reduction in apoptosis. Analyzing the participation of miRs in the salutary effects of myocardial ischemic postconditioning in a rat model of ischemia/reperfusion, it has been shown that postconditioning, as compared with simple ischemia/reperfusion, was accompanied by overexpression of miR-133a, reduction in the number of TUNEL-positive cells and in caspase-9 expression. Cardiac intramuscular pretreatment of the animals with a miR-133a mimic or an anti-miR oligonucleotide prevented or enhanced, respectively, the proapoptotic effect of ischemia/reperfusion, while blunting or increasing the expression of caspase-9. This cytoprotective effect of miR-133a was also duplicated in primary rat neonatal cardiomyocytes subjected to ischemia/reperfusion (He et al. 2011).

Recent data obtained with a novel cardiac-specific TetON-miR-133 inducible transgenic mouse model show that overexpression of miR-133 in vivo results in a significant reduction of myocardial fibrosis and cell apoptosis after TAC in these animals as compared with wild-type littermates (Castaldi et al. 2014). The authors show proof of direct targeting in the myocardium of the β_1-adrenergic signaling pathway at multiple levels (β_1-adrenergic receptor, adenylate cyclase VI, catalytic subunit β of cAMP-dependent protein kinase A, and exchange protein activated by cAMP or EPAC) by miR-133 that would explain the anti-apoptotic effect observed in their transgenic animals after aortic constriction (Castaldi et al. 2014).

Ablation of the expressions of both miR-133a-1 and miR-133a-2 in double knockout mice resulted in a high lethality, but the approximately 24 % of animals that survived to adulthood displayed extensive myocardial fibrosis and systolic dysfunction and evolved toward dilated cardiomyopathy at age 5–6 months with a high incidence of sudden death (Liu et al. 2008; Da Costa Martins and De Windt 2012).

Coronary Artery Disease

Acute coronary syndrome (ACS), which includes ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), and unstable angina (UA), is the leading cause of death worldwide. Currently, cardiac troponins

are the most commonly used biomarkers for myocardial damage in this context, but their clinical value is limited in many cases.

Several publications evidenced that a number of miRs are differentially regulated in patients with coronary heart disease (CHD) and investigated the usefulness of their circulating levels as prognostic and diagnostic biomarkers (reviewed in Fichtlscherer et al. 2011; Fiedler and Thum 2013; Condorelli et al. 2014). Herein, we summarize up-to-date research dealing with the potential value of miR-133 in this entity (Table 2).

Experimental studies using models of acute myocardial infarction (AMI) in mice showed significant downregulation of miR-133a and miR-133b in the damaged myocardium in comparison with control animals (D'Alessandra et al. 2010; Kuwabara et al. 2011). The clinical relevance of these findings was supported by studies that, using autopsy samples from AMI patients, confirmed that the expression of both miRs was subjected to downregulation in the infarcted and border zones in comparison with non-infarcted myocardium or from transplant donors (Kuwabara et al. 2011; Boštjančič et al. 2010, 2012).

Opposite in direction, circulating levels of miR-133a/b exhibited early elevations after coronary occlusion in experimental models of AMI in mice (D'Alessandra et al. 2010), rats (Wang et al. 2010), and pigs (Gidlof et al. 2011), with a time course that closely paralleled the increase in cardiac troponin I (cTnI). The specificity of these features was supported by the lack of changes in circulating miRs following skeletal muscle damage induced by acute hind-limb ischemia (D'Alessandra et al. 2010).

The time course of circulating miRs after acute myocardial ischemia has been studied after clinical transcoronary interventricular septal ablation in patients with hypertrophic obstructive cardiomyopathy (Liebetrau et al. 2013). This therapeutic maneuver reproduces an experimental myocardial infarction that makes it particularly suitable to assess with precision the initial phases of cardiac miR release to the circulation after induction of acute ischemia. An added advantage is that these patients do not have previous coronary artery disease that may alter acute miR release by a preexistent ischemic preconditioning. Plasma concentrations of both miR-1 and miR-133a rose consistently (100 % of patients) and very early (15 min) after induction of ischemia. miR-133a peaked 75 min after coronary occlusion, when its circulating level was over 50-fold the control value, and declined between the fourth and the eighth hour but still was increased (30-fold change) 24 h after induction of ischemia (Liebetrau et al. 2013). These results demonstrate a very quick and very sensitive increase in miR-133a after acute myocardial ischemia that is comparable, and perhaps confirmatory, of cTnT shifts.

In the clinical setting, elevated serum/plasma levels of miR-133a and/or miR-133b were consistently reported by a number of studies assessing the value of miR-133 as diagnosis biomarker in patients with ACS (Corsten et al. 2010; D'Alessandra et al. 2010; Wang et al. 2010; DeRosa et al. 2011; Fichtlscherer et al. 2010; Gildlöf et al. 2011; Kuwabara et al. 2011; Zile et al. 2011; Devaux et al. 2013; Wang et al. 2013b; Yao et al. 2014), as well as in patients with stable CHD (Fichtlscherer et al. 2010). Of note, the circulating levels of miR-133a/b in MI patients exhibited positive correlations with the extent of myocardial damage as

Table 2 microRNA-133 in patients with coronary arterial disease

Pathology	Cohorts	miR-133 regulation	Other major findings	References
Stable coronary disease (SCAD)	Derivation cohort: SCAD patients ($n = 36$) and healthy volunteers ($n = 17$) Validation cohort: SCAD patients ($n = 31$) and healthy volunteers ($n = 14$)	↑miR-133a (derivation cohort); no change (validation cohort) qRT-PCR Plasma	↓circulating miR-126, -17, -92a, -145, -155 ↑miR-208a	Fichtlscherer et al. (2010)
Acute coronary syndrome (ACS)	AMI patients ($n = 93$) and healthy subjects ($n = 66$)	No change miR-133a/b qRT-PCR Plasma	↑miR-1 in AMI; normalization at discharge	Ai et al. (2010)
	AMI ($n = 50$) patients and healthy trauma victims ($n = 8$)	↓miR-133a qRT-PCR Myocardium	↓miR-1 and ↑miR-208 in infarct tissue	Boštjančič et al. (2010)
	AMI patients ($n = 32$) and control subjects ($n = 36$)	↑miR-133 qRT-PCR Plasma	miR-208b and -499 correlated with cTnT and CPK	Corsten et al. (2010)
	STEMI patients ($n = 33$) and healthy subjects ($n = 7$)	↑miR-133a/b Microarray, qRT-PCR Serum	↑miR-1, -499-5p ↓miR-122 and -375 Time courses of miR-1 and miR-133a/b similar to cTnI	D'Alessandra et al. (2010)
	AMI patients ($n = 33$) and healthy subjects ($n = 30$)	↑miR-133 qRT-PCR Plasma	↑miR-208a miR-133a correlated with cTnI ROC curve: plasma miR-133a and miR-208a sensitive predictors for CAD miR-208a highest sensitivity and specificity	Wang et al. (2010)
	No CAD ($n = 7$), SCAD ($n = 31$), and ACS ($n = 19$) patients	↑miR-133a qRT-PCR Aortic and coronary sinus plasma	↑miR-133a,-208a,-126,-92a, and -155 ↑miR-133a and -499 transcoronary gradients ↓miR-126 transcoronary gradient	DeRosa et al. (2011)
	STEMI patients ($n = 26$) and healthy subjects ($n = 11$)	↑miR-133a qRT-PCR Plasma	↑ miR-1, -133a, -208b, and -499-5p within 12 h of the onset of MI symptoms miR-1 and miR-133 detectable in urine	Gildlof et al. (2011)

(*continued*)

Table 2 (continued)

Pathology	Cohorts	miR-133 regulation	Other major findings	References
	ACS patients ($n = 444$)	miR-133a STEMI > NSTEMI qRT-PCR Plasma	↑miR-133a,-208: ↑mortality risk	Widera et al. (2011)
	AMI patients ($n = 12$) and age-matched subjects ($n = 12$)	↑miR-133 qRT-PCR Plasma	Time courses of plasma miR-21, -29a, -133a, and -208 during 90 days after AMI	Zile et al. (2011)
	AMI ($n = 6$) patients who died one week after MI	↓miR-133a/b Microarray, qRT-PCR Infarct versus remote myocardium	↓ SERCA2 in infarcted tissue 43 differentially expressed miRs Bioinformatic prediction of SERCA targeting by regulated miRs	Boštjančič et al. (2012)
	STEMI patients ($n = 216$) undergoing primary angioplasty	↑miR-133a qRT-PCR Plasma	Prognostic information of major cardiovascular events within 6 months after AMI	Eitel et al. (2012)
	ACS ($n = 29$) and non-ACS ($n = 42$)	↑miR-133a qRT-PCR Plasma	↑miR-1 miR-1 and miR-133a correlated with cTn	Kuwabara et al. (2011)
	AMI patients ($n = 246$) and control subjects ($n = 127$)	Unchanged miR-133a qRT-PCR Plasma	miR-133a and -423-5p stable from baseline to 1-year follow-up	Bauters et al. (2013)
	1115 patients with chest pain (AMI = 224)	↑miR-133a in AMI patients qRT-PCR Plasma	↑miR-208b, -499, and -320a miR-208b: highest diagnostic accuracy for AMI	Devaux et al. (2013)
	AMI patients ($n = 13$), angina pectoris patients ($n = 176$), and control subjects ($n = 127$)	↑miR-133a qRT-PCR Plasma	miR-133a correlated with cTnI in AMI patients miR-133a: sensitive predictor for CAD	Wang et al. (2013b)
	TTC ($n = 36$), STEMI ($n = 27$) patients, and healthy subjects ($n = 28$)	↑miR-133a in TTC and STEMI qRT-PCR Plasma	Unique signature including miR-1, -16, -26a, and -133a differentiated TTC from AMI	Jaguszewski et al. (2014)

(*continued*)

Table 2 (continued)

Pathology	Cohorts	miR-133 regulation	Other major findings	References
	STEMI ($n = 25$), NSTEMI ($n = 51$), and non-AMI patients ($n = 110$)	↑miR-133 STEMI and NSTEMI qRT-PCR Plasma	miR-133 in STEMI > NSTEMI	Peng et al. (2014)
	Patients undergoing coronary angiography with abnormal ($n = 26$) and normal ($n = 22$) endothelial function	↑miR-133a in coronary sinus versus aorta qRT-PCR Plasma	↑transcoronary gradients of miR-92a and miR-133 in patients with CED	Widmer et al. (2014)
	CABG patients with ($n = 28$) and without ($n = 89$) perioperative MI	↑miR-133 qRT-PCR Plasma	miR-133a levels peaked earlier and correlated with cTnI	Yao et al. (2014)

CABG coronary artery bypass graft, *CED* coronary endothelial dysfunction, *cTnI* cardiac Troponin I, *cTnT* cardiac Troponin T, *MI* myocardial infarction, *ROC* receiver operating characteristic, *STEMI* ST-segment elevation MI, *TTC* Takotsubo cardiomyopathy

assessed by cTnI or cTnT and achieved an earlier concentration peak (D'Alessandra et al. 2010; Wang et al. 2010; Widera et al. 2011; Kuwabara et al. 2011; Wang et al. 2013b; Yao et al. 2014). Also, receiver operating characteristic (ROC) analysis further indicated that miR-133 might be a sensitive biomarker for ACS diagnosis (Wang et al. 2010; Kuwabara et al. 2011; Yao et al. 2014). In a recent meta-analysis of 19 articles, Cheng et al. (2014) reviewed literature dealing with the association between circulating miRs and myocardial infarction. Four of these studies, including 285 patients, dealt with miR-133a. The summary ROC curves of the meta-analysis indicated that miR-133a [sensitivity, 0.89 (95 % CI, 0.83–0.94; $P = 0.0047$); specificity, 0.87 (95 % CI, 0.79–0.92; $P = 0.026$)] may be suitable as diagnostic biomarker of MI (Cheng et al. 2014).

Increased circulating levels of miR-133a across the coronary circulation have been reported in patients with early coronary atherosclerosis (manifested by coronary microvascular endothelial dysfunction), patients with stable CHD, and cTn-positive ACS patients (Fichtlscherer et al. 2010; De Rosa et al. 2011; Widmer et al. 2014). These findings suggest a release of miR-133 from cardiomyocytes into the coronary circulation during myocardial injury as part of the first steps of the CHD and support circulating level of miR-133 as a sensitive surrogate marker for progression of atherosclerosis in the coronary tree, even in its initial phases.

A specific signature of miRs dysregulated in plasma, including miR-133a, miR-1, miR-16, and miR-26a, has been identified in Takotsubo disease, a cardiomyopathy with an acute phase clinically indistinguishable from AMI (Jaguszewski et al. 2014).

However, a previous study (Kuwabara et al. 2011) reported no specific profile in the circulating levels of miR-133a in either UA or Takotsubo cardiomyopathy.

In a series of 246 patients, Bauters et al. (2013) reported that miR-133a circulating levels remained stable from baseline to 1 year after AMI and lacked any relationship with cardiac biomarkers, including B-type natriuretic peptide, C-reactive protein, and cTnI, or with LV remodeling during the year following the acute episode.

In STEMI patients treated with percutaneous coronary intervention, higher circulating levels of miR-133a associated larger infarcts, more severe reperfusion injury, and decreased myocardial salvage, as determined by cardiac magnetic resonance. Circulating miR-133a constituted an independent positive risk factor for death or other major adverse cardiovascular events within 6 months after AMI (Widera et al. 2011; Eitel et al. 2012). However, miR-133a concentrations did not add prognostic information to troponins. In patients with angiographically documented CHD, Wang et al. (2013b) reported a significant positive correlation of circulating miR-133a levels with the severity of coronary lesions and constituted a sensitive independent predictor for coronary disease.

Peripheral Vascular Disease

There is growing evidence for a pivotal role of the plasticity and phenotypic switching of vascular smooth muscle cells (VSMCs) in vascular diseases. Adult VSMCs are normally quiescent and programmed for contraction, but they maintain remarkable plasticity and, depending on the signals present in their local environment, can acquire dedifferentiated phenotypes with increased ability to migrate, proliferate, and promote extracellular matrix production, inflammatory signals, and calcification. VSMC phenotypic switch plays a critical role in vascular repair. Dysregulation of this plasticity program contributes to the alterations of the arterial wall architecture, called remodeling, which can be found in several vascular disorders in humans, such as essential hypertension, atherosclerosis, vascular calcification, aneurysmal disease, or restenosis (Lacolley et al. 2012; Condorelli et al. 2014).

Several miRs, including miR-1, miR-21, miR-24, miR-26a, miR-29b, miR-143/145, miR-146a, miR-221/222, and miR-663, regulate dynamically VSMC differentiation and phenotypic switching in vitro, in animal experimental models and in human vascular pathologies (Davis-Dusenbery et al. 2011). miR-133a and miR-133b are expressed in VSMC at levels comparable to those of some typical vascular-enriched miRs (Albinsson et al. 2011; Torella et al. 2011).

The modulatory role of miR-133 in the phenotypic switch of cultured VSMCs has been described in recent reports (Torella et al. 2011; Liao et al. 2013; Gao et al. 2014). miR-133 appeared downregulated in proliferating VSMCs while it was upregulated when quiescence was induced in vitro. Proliferation of VSMCs was prevented by miR-133 overexpression and augmented by anti-miR-133 (Nazari-Jahantigh et al. 2012; Torella et al. 2011). Of note, the MAPK/ERK1/2 signaling pathway constitutes a key regulatory mechanism involved in miR-133 downregulation in cultured VSMCs primed for phenotypic switching. The

mechanism of the antiproliferative effect of miR-133 in vitro involves direct repression of its target, the specificity protein 1 or Sp1 (Torella et al. 2011), a transcription factor activated in VSMCs by phenotypic switch promoting stimuli, which is a known regulator of the Krüppel-like factor 4 (KLF4)/myocardin axis (Owens et al. 2004).

The antiproliferative effect of miR-133 observed in vitro was successfully translated to the experimental animal. Thus, proliferating VSMCs from rat carotid arteries subjected to angioplasty exhibited strongly reduced miR-133 levels in comparison with normal uninjured arteries (Ji et al. 2007; Torella et al. 2011). Arterial overexpression of miR-133 by adenoviral infection reduced VSMC activation and neointimal hyperplasia after the balloon injury. On the contrary, systemic administration of anti-miR-133 exacerbated VSMC hyperplasia which resulted in an increased neointimal formation, thickening of the tunica media, and restenosis (Torella et al. 2011). VSMCs from advanced atherosclerotic lesions of $ApoE^{-/-}$ mice expressed lower levels of miR-133a which associated downregulation of the insulin-like growth factor-1 receptors, and, as a result, VSMC proliferation was attenuated (Gao et al. 2014).

Contractile VSMCs may also experience a phenotypic transition into osteoblast-like cells, capable of synthesizing the proteins required for calcification, a hallmark feature of pathological arterial calcification. This entity is a complex, highly organized, and regulated process, similar to bone formation, which has been observed in cardiovascular diseases including atherosclerosis, Mönckeberg's medial calcific sclerosis, aortic valve calcification, and calciphylaxis (Demer and Tintut 2008). VSMC can transdifferentiate into osteo/chondrocytic-like cells by upregulation of transcription factors such as runt-related transcription factor 2 (Runx-2), a validated target of miR-133a. Studies in vitro have shown that β-glycerophosphate-induced osteogenic differentiation of VSMCs associated downregulation of miR-133a levels. Gain- and loss-of-function experiments, using miR-133a mimics and inhibitors, identified miR-133a as a negative regulator of osteogenic transdifferentiation of VSMCs by targeting Runx2. Accordingly, the pro-osteogenic effect of miR-133a inhibitor was abrogated in Runx2-knockdown cells, whereas overexpression of Runx2 prevented miR-133a-induced inhibition of VSMC transdifferentiation into osteoblast-like cells (Liao et al. 2013).

Several studies investigated in patients the contribution of miR-133 dysregulation to the pathological remodeling of the vascular wall in diseases such as essential hypertension and arterial aneurysms (Lacolley et al. 2012). VSMC-modulating miRs, including miR-133, have been reported to be closely related to essential hypertension in humans. Thus, in a cohort of patients with untreated essential hypertension, the expression levels of miR-133 in peripheral blood mononuclear cells correlated significantly and positively with clinical markers of the disease. Therefore, miR-133 might represent a useful biomarker of clinical status and potential therapeutic target in hypertension (Kontaraki et al. 2014).

Arterial aneurysm formation is a poorly understood, complex, multifactorial process of destructive vascular remodeling characterized by local chronic inflammation, oxidative stress, phenotypic switch of VSMC, VSMC loss, and

fragmentation of the extracellular matrix. Similar structural changes have been reported in intracranial (ICA), thoracic aortic (TAA), and abdominal aortic (AAA) aneurysms, though their etiology differs (Norman and Powell 2010). Arterial aneurysm rupture and dissection are major causes of morbimortality in the clinic, and there is still a need for accurate predicting aneurysmal growth and potential rupture risk (Norman and Powell 2010). The pathophysiological role of miRs in aneurysm formation and their value as biomarkers in the diagnosis, prognosis, and management of aneurysmal disease are a matter of intensive research (for reviews see Maegdefessel et al. 2013; Wei et al. 2013). In human patients, several studies comprehensively analyzed miR expression profiles for aneurysmal tissues of different locations. The profiles of regulated miRs may reflect the different genetic and molecular mechanisms involved in the etiology of the aneurysmal disease depending on location (Norman and Powell 2010). Most of these studies evidenced that miR-133a and miR-133b were consistently downregulated in both aortic and intracranial aneurysms (Table 3), which suggests that both miRs may be involved in some common pathways implicated in aneurysm formation.

Pahl et al. (2012), using miR microarray and qRT-PCR validation, reported downregulation of miR-133b and miR-133a expressions in AAA from patients undergoing elective open repair compared with autopsy or tissue bank samples (Pahl et al. 2012). In a miR microarray study, Kin et al. (2012) reported a trend toward lower expression levels of miR-133a in atherosclerotic AAA, although ascending thoracic aorta was used as control tissue, which is a limitation of the study (Kin et al. 2012). On the other hand, Cheuk and Cheng (2014) did not detect differences in the levels of miR-133 in VSMCs isolated of aneurysm samples from AAA patients in comparison to normal VSMCs from sex-matched organ donors.

In the thoracic aorta, the expression levels of miR-133a and miR-133b, determined by microarray and qRT-PCR assays, appeared significantly downregulated in the diseased wall from patients with thoracic aortic dissection (TAD) compared with normal thoracic aorta samples from age-matched donors. Using bioinformatic tools, the authors proposed type-V collagen (COL5A3) and laminin subunit beta-3 (LAMB3) as relevant predicted targets for these two miRs (Liao et al. 2011). Similarly, Ikonomidis' group (Jones et al. 2011) reported a significant reduction of miR-133a expression levels in aortic tissue of ascending TAAs from patients with tricuspid aortic valve (TAV) in comparison with control specimens of ascending aorta, as determined by microarray and qRT-PCR analysis. Moreover, a significant inverse relationship between the expression level of miR-133a and the aortic diameter ($r = 0.42$, $p < 0.05$, $n = 25$) was evidenced (Jones et al. 2011).

In parallel, two studies analyzing miR dysregulation in ICA tissue samples using microarray and qRT-PCR analysis reported reduced expression levels of miR-133a and miR-133b in aneurysmal samples versus the control vessels from matched patients (Jiang et al. 2013; Liu et al. 2014). Bioinformatic analyses allowed the authors the prediction of a subset of potential relevant target genes of miR-133a/miR-133b, with regulatory roles on programmed cell death, extracellular matrix organization, response to oxidative stress, TGF-β signaling pathway, endothelial and

Table 3 microRNA-133 in patients with peripheral arterial disease

Pathology	Cohorts	miR-133 regulation	Other major findings	References
Systemic hypertension	Untreated hypertensive patients ($n = 60$), healthy controls ($n = 29$)	↓miR-133 in PBMC	↓miR-143, -145 ↑miR-21,-1 miR-133 correlated directly with 24-h diastolic BP and with the dipping status	Kontaraki et al. (2014)
Abdominal aortic aneurysms (AAA)	AAA patients ($n = 41$), autopsy and biobank controls ($n = 12$)	↓miR-133a/b Microarray Infrarenal aorta	Bioinformatic prediction of target genes involved in AAA	Pahl et al. (2012)
	AAA patients ($n = 13$) and controls from aortic valve replacement ($n = 7$)	Trend ↓mir-133a Microarray AAAs: infrarenal aorta Control: ascending aorta	miRs: ↑AAA tissue, ↓plasma: endothelial (let-7f, miR-20a, -21, -27, -92a, -126, -221, and -222), inflammatory (miR-124a, -146a,- 155, and -223), and fibrosis-related (miR- 29b)	Kin et al. (2012)
Thoracic aortic dissections and aneurysms (TAA, TAD)	TAD patients ($n = 6$) and control donors ($n = 6$)	↓miR-133a/b Microarray, qRT-PCR TAA segments	↑18 miRs; ↓56 miRs Bioinformatic prediction of targets, networks and pathways	Liao et al. (2011)
	TAA ($n = 30$) and control donors or CABG patients ($n = 10$)	↓miR-133a Microarray, qRT-PCR TAA segments	miRs -1, -21,-29a, -133a, and -486 down- regulated Predicted targets: MMP-2 for miR-29a and MMP-9 for miR-133a	Jones et al. (2011)
	TAA patients with BAV ($n = 21$) or TAV ($n = 21$) and control donors/ receptors ($n = 10$)	↓miR-133a: TAA segments ↑miR-133a: Plasma Microarray, qRT-PCR	Dysregulated miR-1, -21, -29, -143, -145. Little concordance plasma versus tissue TAA associated with TAV or BAV: differential predictive models (ROC curves)	Ikonomidis et al. (2013)
Intracranial aneurysm (ICA)	Ruptured ICA ($n = 14$) and matched controls ($n = 14$)	↓mir-133a/b Microarray, qRT-PCR ICA tissue versus normal middle meningeal artery segments	↓18 miRs Bioinformatic prediction: targets, networks and pathways	Jiang et al. (2013)

(*continued*)

Table 3 (continued)

Pathology	Cohorts	miR-133 regulation	Other major findings	References
	ICA patients ($n = 6$) and matched controls ($n = 6$)	↓mir-133a/b Microarray, qRT-PCR ICA tissue versus normal superficial temporal artery segments	↓85 miRs, ↑72 miRs Bioinformatic prediction of targets, networks and pathways	Liu et al. (2014)
	ICA patients ($n = 18$) and controls ($n = 6$)	No change miR-133a/b qRT-PCR Serum	Bioinformatic prediction of targets, networks and pathways	Jin et al. (2013)
	ICA patients ($n = 40$) and healthy volunteers ($n = 20$)	No change miR-133a/b Microarray Plasma	miR-16 and miR-25: independent factors for ICA occurrence in logistic regression analysis	Li et al. (2014)

BAV bicuspid aortic valve, *PBMP* peripheral blood mononuclear cells, *ROC* receiver operating characteristic, *TAV* tricuspid aortic valve

VSMC proliferation and phenotypic switch, activation of the inflammatory response, and loss of cells in the vessel wall (Jiang et al. 2013; Liu et al. 2014).

With regard to differential profiles of miR-133 in peripheral blood which could help as biomarkers for predicting the likelihood of aneurysm occurrence and/or rupture, the results are variable depending on aneurysm location. Ikonomidis et al. (2013) detected increased circulating levels of miR-133a in plasma samples from patients with TAA associated to either TAV or bicuspid aortic valve (BAV) in comparison to control patients. Univariate logistic regression analysis indicated that circulating miR-133a expression alone was not predictive of aneurysm presence. However, multivariable analysis revealed that the combinations of miR-133a with miR-143 and MMP-8 or miR-133a with MMP-2, TIMP-2, miR-143, and miR-145 predicted with high degrees of specificity and sensitivity the presence of TAA disease overall and the presence of bicuspid aortopathy, respectively. These data support the concept that specific etiologic subtypes of ascending aortic aneurysm disease present specific biological plasma signatures and that miR-133a, together with other analytes, might help to predict the presence of either aneurysmal disease in general or particular subtypes of aneurysmal disease (Ikonomidis et al. 2013).

Contrary to the findings in patients with TAA, Li et al. (2014) reported, in a cohort of 40 ICA patients (20 unruptured and 20 ruptured) and 20 healthy volunteers, no differences in miR-133 plasma levels between patients with ICA and healthy volunteers nor between patients with ruptured or intact ICAs. Similarly, Jin et al. (2013) failed to identify changes in the expression levels of miR-133a/b in a

microarray study carried out using serum from patients with aneurysms with daughter aneurysms, aneurysm without daughter aneurysms, ruptured aneurysms, and angiography negative group.

Other Diseases or Conditions

miR-133a is involved in the interindividual response variability to the anti-vitamin K anticoagulant drugs. Vitamin K 2,3-epoxide reductase complex subunit 1 (VKORC1) is the main target of dicoumarinic anticoagulant drugs and is also a validated target of miR-133a (Pérez-Andreu et al. 2013). Further, VKORC1 is also involved in the correct vitamin K-dependent γ-carboxylation of matrix-Gla protein (MGP), a natural local inhibitor of vascular and other soft tissue calcification. Thus, an increased expression of miR-133a would result in reduced levels of VKORC1, increased susceptibility to dicoumarinics, and diminished γ-carboxylation of MGP with higher tendency to soft tissue calcification (Pérez-Andreu et al. 2013). However, the precise value of miR-133a as a biomarker of these two phenomena, namely, the individual susceptibility to dicoumarinic anticoagulation and the tendency to develop soft tissue mineralization, has not been delineated as yet and awaits further research.

Owing to its specific expression in muscle cells and its role in skeletal muscle conservation and regeneration, miR-133a has been included in the group of the so-called dystromirs. However, there is a paucity of data concerning the use of its circulating levels as a clinical marker of activity of skeletal myopathies. Plasma levels of miR-133a have been proposed as reliable indicators of experimental muscular damage in rats both by the use of toxics (Miwa et al. 2015) or in a model of Duchenne's muscular dystrophy (Roberts et al. 2012). In patients with myotonic dystrophy type 1, the most common type of muscular dystrophy in adults, circulating levels of miR-133a exhibit an excellent discriminant power for the diagnosis, reflect accurately the clinical stage of muscular impairment and correlate, inversely and significantly, with a standard muscle strength score (Perfetti et al. 2014). In children with Duchenne muscular dystrophy, miR-133a serum levels tell us about the remaining muscle mass of the patient, and there is a suggestion that might also help to monitor the therapeutic effect of dystrophin restoration treatments (Zaharieva et al. 2013).

Summary Points

- miR-133 is abundantly expressed in the heart and in vascular smooth muscle cells.
- Bioinformatic tools predicted 399 mRNA targets for miR-133a, and 42 have already been validated experimentally.
- miR-133 plays major roles in cell differentiation to cardiomyocytes during early development and in later stages of cardiac morphogenesis.

- miR-133 cooperates in experimental reprogramming of fibroblasts into cardiac-like myocytes.
- Dysregulation of miR-133 is involved in the pathological myocardial remodeling, in the transition to heart failure and its evolution.
- In LV hypertrophy induced by pressure overload, miR-133 decreases in both myocardium and plasma, and its levels predict LV reverse remodeling after overload release.
- In acute coronary events, miR-133 levels decrease in the ischemic myocardium and increase in the circulation proportionally to the extent of the infarcted area.
- The value of circulating miR-133 as a diagnostic and prognostic biomarker in patients with coronary artery disease has been established.
- Dysregulation of miR-133 contributes to the pathological vascular remodeling underlying essential hypertension, vascular calcification, atherosclerosis, and aneurysmal disease.
- Circulating miR-133a, together with other analytes, can help to predict the presence of either thoracic aneurysm in general or particular subtypes of aneurysmal disease.
- Circulating levels of miR-133 failed to predict the presence of intracranial aneurysms.

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