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miRNome in myocardial infarction: Future directions and perspective

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Abstract

MicroRNAs (miRNAs), which are small and non-coding RNAs, are genome encoded from viruses to humans. They contribute to various developmental, physiological and pathological processes in living organisms. A huge amount of research results revealed that miRNAs regulate these processes also in the heart. miRNAs may have cell-type-specific or tissue-specific expression patterns or may be expressed ubiquitously. Primary studies of miRNA involvement in hypertrophy, heart failure and myocardial infarction analyzed miRNAs that are enriched in or specific for cardiomyocytes; however, growing evidence suggest that other miRNAs, not cardiac or muscle-specific, play a significant role in cardiovascular disease. Abnormal miRNA regulation has been shown to be involved in cardiac diseases, suggesting that miRNAs might affect cardiac structure and function. In this review, we focus on miRNAs that have been found to contribute to the pathogenesis of myocardial infarction (MI) and the response post-MI and characterized as diagnostic, prognostic and therapeutic targets. The majority of these studies were performed using mouse and rat models of MI, with a focus on the identification of basic cellular and molecular pathways involved in MI and in the response post-MI. Much research has also been performed on animal and human plasma samples from MI individuals to identify miRNAs that are possible prognostic and/or diagnostic targets of MI and other MI-related diseases. A large proportion of research is focused on miRNAs as promising therapeutic targets and biomarkers of drug responses and/or stem cell treatment approaches. However, only a few studies have described miRNA expression in human heart tissue following MI.

Core tip: MicroRNAs (miRNAs) contribute to various developmental, physiological and pathological processes in the heart. Cardiac diseases show abnormal miRNA regulation. Primary studies of miRNA involvement in cardiac disease analyzed mainly miRNAs that enriched in or specific for cardiomyocytes; however, growing evidence suggests that other cell-type-specific or ubiquitously expressed miRNAs are also involved in cardiovascular disease. miRNAs were found to contribute to the pathogenesis of myocardial infarction (MI) and post-MI. The majority of studies focused on miRNAs in animal models of MI, in human and animal plasma samples of MI (prognostic and diagnostic targets), and on miRNAs as promising therapeutic targets.

INTRODUCTION

MicroRNAs (miRNAs) are endogenously expressed small non-coding RNA molecules. Genes encoding miRNAs can be found in genomes of almost all organisms, including viruses. Their prime mechanism of action is post-transcriptional repression of gene expression[1]. It is suggested that the short length (22 nt) maximizes target-gene specificity and minimizes non-specific effects. It is estimated that miRNAs regulate approximately 30% of genes within the human genome[2]. There are over 2000 miRNAs known to be encoded by human genome. All sequenced and cloned miRNAs from humans as well as from other species are included in database miRBase (v20.0, June 2013, http://www.mirbase.org)[3].

Mechanism of action of miRNAs

Biogenesis (including genes encoding miRNAs), transcription and processing are beyond the scope of this review and are described elsewhere[1,2,4,5]. As mentioned above, miRNAs prime mechanism of action is repression of gene expression. By sequence-specific binding to the 3'-untranslated region (3'-UTR) of mRNAs, miRNA affects stability of the transcripts and cause mRNA degradation, which is the main mechanism in plants and happens when complementarity between miRNA and mRNA is perfect, or cause protein synthesis repression (translational repression), which happens when base pairing between these two molecules is incomplete and is the canonical mechanism in animals[1,2,4,5]. Due to incomplete base pairing in animals and humans, each miRNA could influences translation of many different mRNAs without degrading it (*i.e.*, over 200 predicted target genes) and vice versa each mRNA may be influenced by different miRNA. It appears that the most efficient translational inhibition is provided through the multiplicity, which is the consequence of numerous target sites for the same miRNAs within 3'-UTR of the same mRNA (cooperative action of multiple identical miRNAs), and trough cooperativity, which is due to numerous target sites for the different miRNAs within 3'-UTR of the same mRNA. miRNA access to the UTRs could be on one hand restricted by proteins or mRNA secondary structures, and on the other hand these structures and protein binding may facilitate recognition of the mRNA targets[6]. Some miRNAs might also have other functions, although translational repression has been suggested to be the canonical one[2,4].

miRNA in regulating physiological functions

Different approaches in *in vitro* and *in vivo* experiments have been used to reveal function of majority of miRNA. Using mutated miRNA or its mutated complementary site within mRNA, consequently disrupting regulation of mRNA by miRNA, leads to the determination of the phenotypic consequence of this non-binding. Another possibility is use of transgenic constructs of either 3'-UTR or miRNA expressing vector and ectopic expres-sion of the either miRNA or mRNA[1,7]. Perhaps the best evidence that miRNA are playing a significant role in normal physiological functions was established, when the components of the miRNA biogenesis pathway were depleted[8]. In normal cell conditions, miRNAs can repress translation in different ways: (1) as a switching-off the targets, when protein production is reduced to inconsequential levels in a cell type, where target mRNAs should not be expressed; (2) as fine-tuning expression of target gene, when protein output can be adjusted in a way, which provides customized expression in one cell type and uniformly expressed level within another cell type; and (3) as neutralizers of target gene expression, when mRNA downregulation by miRNAs is negated through feedback processes[1]. The role of miRNAs can be combinatorial (defined as cooperativity), different in different cell types, and either specific or housekeeping[9].

Through the studies of expression profiling of normal and disease tissues it has been shown that miRNAs are expressed in spatial as well as in temporal manner. *miR-208* is a good example of expression in tissue-specific manner. Its expression can be detected specifically in the hearts, as well can be *miR-122* found primarily in the liver. As an example of cell-type-specific miRNAs are *miR-223*, which is primarily expressed in granulocytes, and *miR-1* and *miR-133*, which are believed to be myocyte-specific[10]. miRNAs are involved in a myriad of biological processes, including proliferation, apoptosis, metabolism, differentiation, epithelial-to-mesenchymal transition, regulation of insulin secretion, division of stem cells, embryonic development and pattering, fetal growth, immune system, including resistance to viral infection and vice versa viral production (in a case of HCV), *etc*[8]. miRNA activity is believed to have crucial role in regulatory role in maintaining tissue identity during embryogenesis as well as in adult life. Distinct miRNA expression profile, with completely different gene expression patterns might be observed in every cell type at each developmental stage[9-11].

Target prediction and bioinformatics

As mentioned above, miRBase is major database of all known miRNAs, which can also predict possible miRNA targets[3]. Predicting possible miRNA binding sites for specific mRNAs or potential targets for certain miRNA is usually the first step in target identification and for this purpose numerous computational methods have been developed. Main characteristics that are included in established programs are: evolutionarily conservation of the complementary 3'-UTR sequence, quality and stability of mRNA:miRNA pairing and involvement of "seed sequence". It is believed that for base pairing the most important is "seed sequence" of the miRNA (2-8 nt at the 5'-end) and its interaction with seven consecutive nucleotides in the target mRNA[12]. However, all predicted targets have to be validated *in vitro* and *in vivo* since none of these programs can independently validate the targets[7,13]. Due the facts that 3'-UTR sites with

perfect complementarity to the miRNA are not necessary functional and that mRNA sites with imperfect complementarity can themselves be very good miRNA targets, are bioinformatic analysis more prone to false positives[6]. Therefore, experimental demonstration that overexpression of the miRNA represses a luciferase reporter fused to the 3'-UTR of the predicted target and that this repression is not established by point mutations in the 3'-UTR target sequence is the gold standard for miRNA target identification[13,14]. Finally, association of mRNA:miRNA pairs with disease pathogenesis should be confirmed by expression profiling in human diseases by co-expression analyses[7,14]. Human MicroRNA Disease Database identifies all disease-related miRNAs with their tissue expression patterns[15]. Further, Tarbase lists experimentally validated miRNA targets for all organisms[12].

miRNAs AND DISEASE

Mutations, single-nucleotide polymorphisms and the epigenetics of miRNAs

There are several genetic and epigenetic abnormalities within miRNA genes that might contribute to a wide range of diseases. These abnormalities include small- and large-scale genomic alterations, as are rearrangements and chromosomal translocations, copy-number variation, nucleotide expansion, and single-nucleotide polymorphisms (SNPs) that beside protein-coding region also affect regions that code for non-coding RNAs. First, it has been shown that approximately 50% of the miRNA genes are encoded within fragile chromosomal sites or sites that are prone to cancer-associated rearrangements[10]. Second, although some SNPs are silent and cause no obvious functional consequence, other might cause disruption of binding between miRNAs and their targets, which can potentially lead to gain or loss of the function of miRNA or its target gene and consequently contribute to the disease state[16]. Variants identified in miRNA or their precursors (pri-miRNA or pre-miRNA) that beside targeting might also affect the processing and expression of miRNAs, are rarely observed. However, potential of variation in miRNA target sites is more huge[6,16]. Third, the aberrant DNA methylation of gene promoters has been shown to results in the inactivation of different genes, including miRNAs, and in parallel, miRNAs can also regulate proteins involved in DNA methylation[17].

Aberrant expression of miRNAs

Epigenetic mechanisms and genomic abnormalities frequently lead to abnormal miRNA expression profiles thus causing pathogenetic events in diseases. Numerous advances in miRNA research and numerous expressions profiling of diseased human tissue are suggesting that miRNAs are associated with various pathological conditions. miRNAs have been linked to wide range of diseases, including cancer genetic and immunological disorders, neurodegenerative and cardiovascular disorders[10].

THERAPEUTIC POTENTIAL OF miRNAs

miRNA expression patterns are dynamically regulated during various diseases and can also be used for pharmacological manipulation. Studies have demonstrated that the systemic use of antagomirs is well suited to block miRNA function in small animal models. For targeting a specific miRNA or disrupting binding between miRNA and its target mRNA the chemically modified oligonucleotides have been developed. miRNAs as small molecules of approximately 22 nt in length are more feasible delivered *in vivo*. Synthetic miRNAs can be therefore delivered systematically and may thus serve as therapeutic targets in the future[18].

Replenishing small RNAs

Underexpressed miRNA might be restored by reintroduction of the mature miRNA into the target tissue consequently restoring regulation of the miRNA target gene. miRNAs as potential therapeutic agents can be easily targeted and delivered to the appropriate tissue. Three major approaches are described below. Artificial miRNA or miRNA "mimics" enhance the expression of beneficial miRNAs. Artificial miRNAs are transient transfections of double-stranded miRNAs and possess the ability to bind to the homologous target site in various mRNAs. Another option is the introduction of a viral vector or plasmid expressing a specific miRNA from a short hairpin (sh) duplex (pre-miRNA-like shRNA*)*. A high level of shRNA might lead to effective target knockdown; however, it may also saturate the miRNA biogenesis pathway and lead to off-target effects with fatal consequences. Therefore, another possibility arises, namely miRNA scaffolds. In scaffolds of endogenous pri-miRNA or pre-mRNA, siRNA is inserted and introduced to the target tissue leading to the degradation of homologous mRNA. This approach is advantageous in terms of specificity and stability over conventional shRNA because both siRNA and shRNA may trigger a non-specific interferon response in addition to off-target effects. As an example in cardiovascular disease, overexpression of *miR-133* was used in a study of cardiac hypertrophy. By adenovirus delivery of a miRNA expression cassette, expression of *miR-133* was restored, which results in protection of experimental animals from agonist-induced cardiac hypertrophy[18].

Inhibiting small RNAs

ASOs are short single-stranded antisense oligonucleotides, which are called anti-miRNA oligonucleotides, AMOs or antagomirs when talking about inhibition of miRNA. Antagomirs have been shown to efficiently and specifically silence endogenous miRNAs in mice. Overexpressed miRNA can also be downregulated by reducing the loop region of the miRNA precursor (pre-miRNA). The loop regions of different pre-miRNAs are not conserved and might therefore limit their application. Another approach is to use miRNA sponges, which are miRNA inhibitory transgenes containing multiple tandem binding

sites for an endogenous miRNA and can inhibit several closely related miRNAs. miRNA sponges may be useful for sequestering a miRNA family with overlapping and redundant targets. miR-masks and miR-erasers have also been developed. Similarly to a miR-sponge, a miR-eraser sequesters more than one miRNA, except that there are only two copies of the antisense sequence. For masking the miRNA binding site on the target gene, miR-mask or miRNA masking antisense approach has been designed, which forms a duplex with target mRNA. They are also called antisense oligodeoxynucleotides (ODNs). Two approaches have been used in the context of studying cardiovascular diseases. A miRNA decoy using miRNA sponges was designed and used in research studying the effect of *miR-133* in the pathogenesis of cardiac hypertrophy. Another approach used ODNs entirely complementary to the miRNA target motifs in the 3'-UTR of 2 cardiac pacemaker channel genes, *HCN2* and *HCN4*[18,19].

Delivering miRNAs or its inhibitor to the target tissue

Current limitations exist in the following areas and need improvement: the efficiency of delivery of miRNA therapeutics to target tissues; systemic administration of drug; the potential inhibition of non-target genes (off-target effects); redundancy in the efficacy of different miRNAs; potential toxicity; and immunogenic responses. Modifications have improved the stability of miRNAs or blocked their inhibition (*i.e.*, nuclease resistance and pharmacokinetic properties such as half-life in serum and cellular uptake). Stabilization and facilitation of intravenous delivery of antagomirs could be improved by chemical modifications and cholesterol conjugations. However, toxicity due to chemical modifications should be taken into account. Local administration in easily accessible tissues has been used in the majority of the developed protocols; a major challenge remains for tissue- and cell-type-specific targeting. Viral and non-viral delivery systems have been developed in conjugation with homing signals for tissue- or cell-type-specific delivery, *e.g.*, linked to lipids and/or proteins, cationic liposomes, cholesterol, bacterial phage, aptamers, *etc*[18,19].

miRNA IN MYOCARDIAL INFARCTION

MicroRNA in cardiovascular diseases

miRNAs contribute to the regulation of developmental, as well as physiological and pathological processes in the heart. Loss of cardiomyocyte renewal is a hallmark of numerous cardiac diseases, which might also influence miRNA expression patterns in the diseased heart[20]. Primary studies of miRNA involvement in hypertrophy, heart failure and myocardial infarction analyzed miRNAs that are enriched in or are specific for cardiomyocytes; however, growing evidence suggest that other miRNAs, not cardiac- or muscle-specific, play a significant role in cardiovascular disease[21-24]. Using experimental animals and human samples dysregulation of specific miRNAs has been shown that are distinct than those involved in

other heart diseases as are hypertrophy and heart failure (HF). Cell lines and an animal models of different forms of myocardial ischemia, including myocardial infarction (MI), have been used to perform miRNA microarray expression profiling[20]. It has been shown that in response to limited amount of oxygen, numerous miRNAs are up- or downregulated. Many of dysregulated miRNAs are dependent on a transcription factor that plays an important role in response to low oxygen, hypoxia-inducible-factor. Further analyses showed that oxidative stress also activates other transcription factors that beside miRNA expression influence different homeostatic and physiological processes as are metabolism, angiogenesis, cell survival and oxygen delivery[25,26]. Numerous other pathways are activated in response to MI, including apoptosis and fibrosis, as well as numerous cell types such as cardiomyocytes, immune cells, fibroblasts and endothelial cells (ECs)[27]. However, the majority of studies were performed in terms of expression analysis and target gene identification, with only one publication focusing on MI, specifically, target site polymorphisms and the risk for MI.

Cardiac and muscle specific miRNAs in heart

There are five miRNAs recognized as muscle- and/or cardiac-specific or enriched, *miR-1*, *miR-133*, *miR-206*, *miR-208* and *miR-499*. For *miR-1* and *miR-133* it is believed that are muscle-specific and that regulate heart development[28]. *miR-208* has been identified as cardiac-specific and *miR-499* as cardiac-enriched.

In the current review we have focused on miRNAs involved in MI pathogenesis and their diagnostic, prognostic and therapeutic potential regarding MI. The function of various miRNAs analyzed in cell lines, animal models of MI and patients with MI are presented. Table 1 summarizes all free-circulating miRNAs in experimental model of MI and human MI, describes their suggested function and predicted targets. We further overviewed the results of free-circulating miRNAs in different bodily fluids of patients and/or an animal model with MI. Table 2 summarizes all miRNAs as potential diagnostic and/or prognostic targets in MI. Lastly, therapeutic opportunities using miRNA strategies in the context of MI are also presented. More detailed description of all these miRNAs is below.

Cell line models

miR-21: *miR-21* was upregulated after inducing injury of cardiac myocytes using H_2O_2, and H_2O_2-induced cardiac cell death and apoptosis were increased by a *miR-21* inhibitor. Programmed cell death 4 (PDCD4) has been identified as a target of *miR-21*, and activator protein 1 (AP-1) has been identified as a downstream signaling molecule of PDCD4[29]. All miRNAs with suggested role in apoptosis in MI are summarized in Figure 1.

miR-15b and *miR-106b*: After retrieving 119 MI-related miRNAs from publications, GO and pathway analyses

Table 1 miRNAs with suggested role in experimental models of myocardial infarction and in myocardial infarction in humans

miRNA	Role/function	Expression in MI	Target genes	Species	Ref.
miR-1	After heat-shock up, protective against I/R	Nd	Repressing pro-apoptotic and up-regulating anti-apoptotic genes	Mouse	[40]
	Nd	Down	Nd	Mouse	[59]
	Pro-apoptotic	Up	IGF1	Rat	[45]
	Pro-arrhythmogenic	Up	Ion channels: Cx43, Kir2.1	Rat	[46]
	Predictive	Down, up	Predictive	Human	[55,56]
miR-15b	Anti-angiogenic	Down	Suggested VEGF and Ang2	Endothelial cells	[30]
miR-21	H₂O₂ induced cell injury	Up	PDCD4	Cardiomyocytes	[29]
	After heat-shock up, protective against I/R	Nd	Repressing pro-apoptotic and up-regulating anti-apoptotic genes	Mouse	[40]
	Response to I/R	Up in cardiac fibroblasts	PTEN	Mouse	[23]
	Anti-apoptotic	Down, up	PDCD4	Rat	[47]
	Pro-arrhythmogenic	Up	Sprouty-1, collagen Ⅰ, collagen Ⅲ	Rat	[48]
miR-24	Anti-fibrotic	Down	Furin	Mouse	[35]
	Anti-angiogenic, induce endothelial cell apoptosis	Down in cardiomyocytes and fibroblasts, up in endothelial cells	GATA2, PAK4 eNOS	Mouse Mouse	[36] [37]
	After heat-shock up, protective against I/R	Nd	Repressing pro-apoptotic and up-regulating anti-apoptotic genes	Mouse	[40]
miR-29 family	Anti-fibrotic	Down	Proteins involved in fibrosis (COL1A1-2, COL3A1, FBN1, ELN)	Mouse, human	[34]
miR-29b	Anti-fibrotic	Down	Proteins involved in fibrosis (COL1A1, COL3A1, αSMA)	Rat	[52]
miR-34a	Pro-apoptotic	Up	ALDH2	Rat	[49]
miR-92a	Anti-angiogenic	Up	ITGA5	Mouse	[38]
miR-101a/b	Anti-fibrotic	Down	c-Fos	Rat	[50]
miR-106b	Anti-apoptotic	Up	p21	Cardiomyocytes	[30]
miR-133a	Nd	Down	Nd	Mouse	[59]
	Predictive	Down	Predictive	Human	[55,56]
miR-133b	Predictive	Down	Predictive	Human	[55,56]
miR-146a	Predictive: inflammation and VR	Up	Predictive	Human	[57]
miR-150	Predictive: inflammation and VR	Down	Predictive	Human	[57]
miR-155	Predictive: inflammation and VR	Down	Predictive	Human	[57]
miR-206	Pro-apoptotic	Up	IGF1	Rat	[45]
miR-208a	Nd	Down	Nd	Mouse	[59]
	Predictive	Up	Predictive	Human	[55]
miR-320	Pro-apoptotic	Down	HSP20	Mouse	[41]
miR-494	Activation of Akt pathway	Down	Pro- and anti-apoptotic proteins (PTEN, ROCK1, CaMKII; FGFR2, LIF)	Mouse	[42]
miR-499	Nd	Down	Nd	Mouse	[59]
miR-711	Involved in anti-fibrotic effect of pioglitazone	Down	SP1	Rat	[51]
miR-874	Regulated by Foxo3a in necrosis	Up	Caspase 8	Mouse	[39]

I/R: Ischemia/reperfusion; MI: Myocardial infarction; Nd: Not determinate; VR: Ventricular rupture.

for their predicted gene targets demonstrated that these dysregulated miRNAs were enriched in cardiovascular-related phenotypes. By highlighting miRNA-gene networks, overall relationships between miRNAs and gene targets were discovered, particularly in apoptosis and angiogenesis. Experimental data identified *miR-106b* as an anti-apoptotic modulator through inhibition of p21 expression and *miR-15b* as an anti-angiogenic miRNA with the possible targets vascular endothelial growth factor and Ang2 (angiopoietin 2)[30]. All miRNAs with suggested role in angiogenesis in MI are summarized in Figure 2.

To investigate the possible release of miRNAs from activated platelets, the miRNA content of platelets was screened from control patients and patients with MI. Nine miRNAs found to be differentially expressed in MI patients compared with healthy controls were screened,

and 8 of these were decreased in MI patients. Of these, *miR-22*, *miR-185*, *miR-320b* and *miR-423-5p* increased after aggregation in the supernatant of platelets and were depleted in thrombi aspirated from MI patients. Platelets from patients with MI exhibit a loss of specific miRNAs, and activated platelets shed miRNAs that can regulate EC gene expression[31].

Mouse models
Whole genome microarray analysis: Genome-wide mRNA and miRNA expression profiles were performed at three time points post-MI: 2 d, 2 wk and 2 mo. The majority of differentially expressed miRNAs were uniquely regulated at each of the time points analyzed. Bioinformatic analysis demonstrated that several genes and miRNAs in various pathways are regulated in a tem-

Table 2 miRNAs as potential diagnostic and prognostic biomarkers in myocardial infarction

miRNAs as potential biomarker	Role of biomarker	Expression in body fluid	Species and body fluid	Ref.
let-7b	Potential diagnostic value	Up	Plasma; human	[72]
let-7f	Differentiating TTC and MI	Down	Plasma; human	[79]
miR-1	Detection of AMI and AP	Up	Exosome, serum; human and mouse	[59]
	Correlation with MI size	Up	Serum; rat and human	[60]
	Differentiating AMI and AP	Up	Serum; human	[61]
	Differentiating AMI and other cardiovascular diseases	Up	Plasma; rat and human	[67]
	Similar time course to cTnI and the same trend to cTnI concentration	Up	Plasma and tissue; human and mouse	[68,70]
	Differentiating AMI and non-AMI	Up	Plasma; human	[69]
	AMI biomarkers, not superior to cTnT	Up	Plasma; human	[71]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Up	Plasma; human	[76]
	No association with 30 d mortality post-MI and diagnosis of HF	Up	Plasma; human	[81]
	Biomarker for AMI, correlated with renal elimination	Up	Plasma, urine; human, pig	[85]
	Detected in urine	Up	Urine; rat	[86]
miR-16	Differentiating TTC and MI	Down	Plasma; human	[79]
	Higher risk of impaired LV contractility	Up	Plasma; human	[83]
miR-21	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Nd	Plasma; human	[76]
	Differentiating NSTEMI and CHF	Up	Plasma; human	[78]
	Time-dependent changes 2-90 d post-MI	Down, up	Plasma; human	[80]
miR-26a	Differentiating TTC and MI	Down	Plasma; human	[79]
miR-27a	High risk of impaired LV contractility	Up	Plasma; human	[83]
miR-29a	Time-dependent changes 2-90 d post MI	Up	Plasma; human	[80]
miR-29b	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Nd	Plasma; human	[76]
miR-30a	Potential diagnostic value	Up	Plasma; human	[72]
miR-30c	Correlation with MI size	Up	Whole blood; human	[64]
miR-34a	Prognostic: correlated with LV end diastolic dimension	Up	Exosomes, serum; human	[62]
miR-101	Higher risk of impaired LV contractility	Down	Plasma; human	[83]
miR-126	The same trend to cTnI expression	Down	Plasma; human	[70]
	Positive association to the risk for MI	Nd	Plasma; human	[84]
miR-133a	Detection of AMI, AP: biomarker for cardiomyocyte death	Up	Exosome, serum; human and mouse	[59]
	AMI biomarker, correlation to cTnI	Up	Plasma and whole blood; human	[66]
	Differentiating AMI and other cardiovascular diseases	Up	Plasma; rat and human	[67]
	Similar time-course to cTnI	Up	Plasma and tissue; human and mouse	[68]
	AMI biomarkers, not superior to cTnT	Up	Plasma; human	[71]
	Differentiating AMI and AP, positive correlation to severity of coronary stenosis	Up	Plasma; human	[75]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Up	Plasma; human	[76]
	Differentiating TCC and MI	Up	Plasma; human	[79]
	Biomarker for AMI, correlated with renal elimination	Up	Plasma, urine; human, pig	[85]
miR-133b	Similar time-course to cTnI	Up	Plasma and tissue; human and mouse	[68]
miR-134	Differentiating AMI and AP	Up	Serum; human	[61]
miR-145	Correlation with MI size	Up	Whole blood; human	[64]
miR-146a	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Nd	Plasma; human	[76]
miR-150	Associated with LV remodeling	Down	Plasma; human	[82]
	Higher risk of impaired LV contractility	Down	Plasma; human	[83]
miR-155	Prognostic for cardiac death within 1 yr after MI	Up	Serum; human	[63]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Nd	Plasma; human	[76]
*miR-181c**	Novel mirna dysregulated during MI	Nd	Whole blood; human	[65]
miR-186	Differentiating AMI and AP	Up	Serum; human	[61]

miR-192	Prognostic for development of ischemic HF	Up	Exosomes, serum; human	[62]
miR-194	Prognostic: correlated with LV end diastolic dimension	Up	Exosomes, serum; human	[62]
miR-195	Potential diagnostic value	Up	Plasma; human	[72]
miR-197	Negative association to the risk for MI	Nd	Plasma; human	[84]
miR-208	Differentiating AMI and AP	Up in AP compared to AMI	Serum; human	[61]
	Differentiating AMI and other cardiovascular diseases	Up	Plasma; rat and human	[67]
	Time-dependent changes 2-90 d post MI	Up	Plasma; human	[80]
	Detected in urine	Up	Urine; rat	[86]
miR-208b	AMI biomarkers, correlation to cTnT but not superior to cTnT	Up	Plasma; human	[71,76]
	Differentiating STEMI and NSTEMI	Higher in STEMI	Plasma; human	[73]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Up	Plasma; human	[76]
	Higher risk for 30 d mortality post-MI and HF	Up	Plasma; human	[81]
	Biomarker for AMI, correlated with troponin	Up	Plasma, urine; human, pig	[85]
miR-223	Differentiating AMI and AP	Up	Serum; human	[61]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Down	Plasma; human	[76]
	Negative association to the risk for MI	Nd	Plasma; human	[84]
miR-328	AMI biomarker, correlation to cTnI	Up	Plasma and whole blood; human	[66]
miR-380*	Prognostic for cardiac death within 1 yr after MI	Up	Serum; human	[63]
miR-423-5p	Before PCI compared to after	Up	Plasma; human	[77]
miR-499	Differentiating AMI and AP	Up in AP compared to AMI	Serum; human	[61]
	Differentiating AMI and other cardiovascular diseases	Up	Plasma; rat and human	[67]
	Similar time course to cTnI	Up	Plasma and tissue; human and mouse	[68]
	AMI biomarkers, correlation to cTnT but not superior to cTnT	Up	Plasma; human	[71,76]
	Differentiating STEMI and NSTEMI	Higher in STEMI	Plasma; human	[73]
	Differentiating MI, CHF and unstable AP	Up	Plasma; human	[74]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Up and also in acute HF	Plasma; human	[76]
	Differentiating NSTEMI and CHF	Up	Plasma; human	[78]
	Higher risk for 30 d mortality post MI and HF	Up	Plasma; human	[81]
	Biomarker for AMI, correlated with renal elimination	Up	Plasma, urine; human, pig	[85]
miR-1915	Novel miRNA dysregulated during MI	Nd	Whole blood; human	[65]
11 miRNAs	Prognosis after MI	Up and down	Serum; human	[63]
20 miRNAs	Predicting AMI (96% specificity; 90% sensitivity; 93% accuracy)	Up and down	Whole blood; human	[64]
A subset of miRNAs	Dysregulated during AMI course	Nd	Whole blood; human	[65]
34 miRNAs	AMI biomarkers	20 up, 14 down	Plasma and tissue; human and mouse	[68]
19 candidate miRNAs	Prediction for risk of MI	Nd	Plasma; human	[84]

AMI: Acute myocardial infarction; AP: Angina pectoris; cTnI: Cardiac troponin I; CHF: Chronic heart failure; cTnT: Cardiac troponin T; HF: Heart failure; LV: Left ventricle; MI: Myocardial infarction; Nd: Not determine; NSTEMI: Non-ST-elevation MI; PCI: Percutaneous coronary intervention; STEMI: ST-elevation MI; TTC: Takotsubo cardiomyopathy.

poral or phenotype-specific manner[32]. In another study, a mouse MI was induced and one week after MI, a set of 29 upregulated miRNAs was found in the left ventricle originating from the Dlk1-deiodinase type 3 gene (*Dio3*) genomic imprinted region, which has been identified as a hallmark of pluripotency and proliferation. This miRNA signature was associated with an increase in expression of the *Dio3* located in this region. *Dio3* is a fetally expressed enzyme associated with cell proliferation, which was shown to be upregulated in cardiomyocytes. These data suggest that a regenerative process is initiated, but not completed, in adult cardiomyocytes after MI[33].

miR-29 and fibrosis: One of the first studies regarding MI was comparing expression profiles of miRNA from mouse border zone of the infarcted region as well as from the remote myocardium 3 and 14 d after MI. The *miR-29* family was downregulated in the region of the heart adjacent to the infarct. It has been shown that downregulation of *miR-29b* with anti-miRs induces the expression of collagen and that overexpression of *miR-29* reduces collagen. Three days after the MI, in the infarcted region, *miR-29* downregulation correlated with upregulation of collagen types I and III (COL1A1, COL1A2, COL3A1) and fibrillin, and in the remote myo-
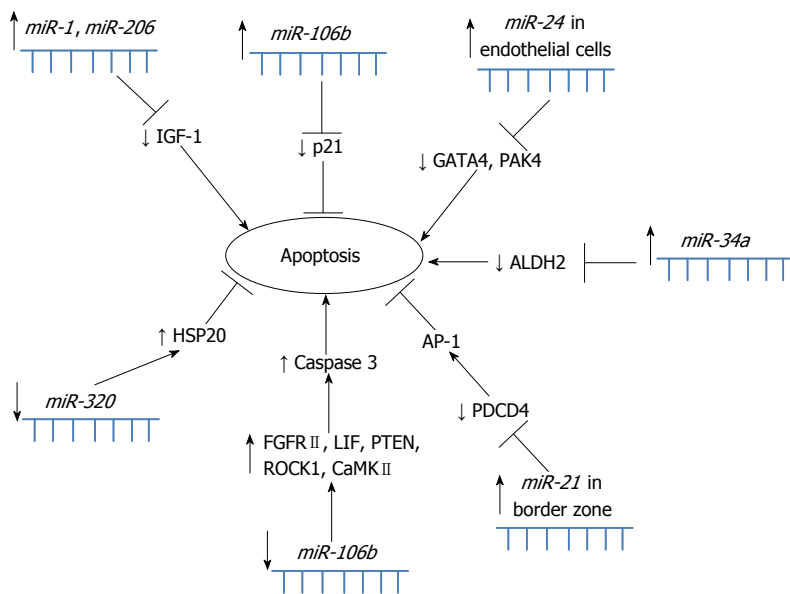
Figure 1 Schematic overview of miRNAs involved in apoptosis in myocardial infarction.
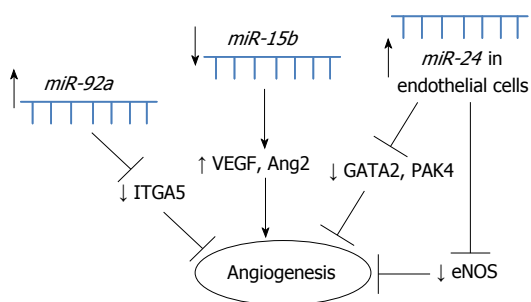

Figure 2 Schematic overview of miRNAs involved in angiogenesis in myocardial infarction.

cardium expression of elastin was increased. The *miR-29* family was thus identified as a regulator of fibrosis[34]. All miRNAs with suggested role in fibrosis in MI are summarized in Figure 3.

***miR-24*, fibrosis and angiogenesis:** The downregulation of *miR-24* in a mouse MI model was closely related to extracellular matrix remodeling. Intra-myocardial injection of *miR-24* was able to improve heart function and attenuate fibrosis in the infarct border zone. *In vitro* experiments suggested that the upregulation of *miR-24* could reduce fibrosis and decrease the differentiation and migration of cardiac fibroblasts (CFs). Transforming growth factor β (TGF-β) increased *miR-24* expression, and overexpression of *miR-24* reduced TGF-β secretion and Smad2/3 phosphorylation in CFs. Furin was found to be a potential target for *miR-24* in fibrosis and both protein and mRNA levels of furin were regulated by *miR-24* in CFs[35]. *miR-24* is markedly upregulated after cardiac ischemia and it has been also shown to be enriched in cardiac ECs. *miR-24* has been reported to induce apoptosis in ECs and abolishes endothelial capillary network formation by targeting the endothelium-
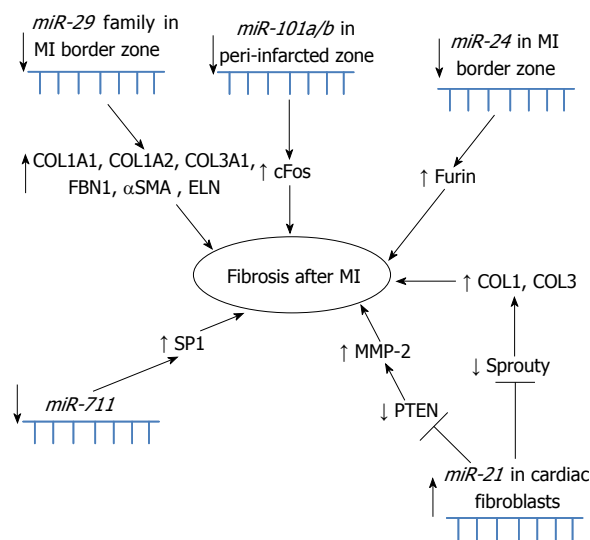

Figure 3 Schematic overview of miRNAs involved in fibrosis after myocardial infarction.

enriched transcription factor GATA2 and the p21-activated kinase PAK4. MI size in mice has been limited by blocking endothelial *miR-24*. Reduced MI size as well as preserved cardiac function and survival were probably due to prevention of endothelial apoptosis and enhancement of vascularity as a consequence of blocked *miR-24*[36]. Another mouse model showed that after a MI induction, *miR-24* expression was lower in the peri-infarct tissue and its resident cardiomyocytes and fibroblasts, while it increased in ECs. Local adenovirus-mediated *miR-24* decoy delivery increased angiogenesis and blood perfusion in the peri-infarct myocardium, reduced infarct size, induced fibroblast apoptosis and overall improved cardiac function. The *miR-24* decoy increased apoptosis in cardiomyocytes. *In vitro miR-24* inhibition enhanced EC survival and proliferation and induced cardiomyocyte and

fibroblast apoptosis. Endothelial nitric oxide synthase has been identified as a novel direct target of *miR-24* in human cultured ECs and *in vivo*[37].

***miR-92a* and angiogenesis:** *miR-92a* has been shown to control the growth of new blood vessels (angiogenesis). Systemic administration of antagomir-92a led to enhanced blood vessel growth and functional recovery of damaged tissue. Overexpression of *miR-92a* blocked angiogenesis and vessel formation. *miR-92a* was shown to be upregulated after induction of acute MI (AMI). Antagomir-92a treatment reduced the infarct size, suppressed the number of apoptotic cells and augmented the number of *in vivo* perfused vessels in the infarct border zone. Among its targets are several pro-angiogenic proteins, including integrin subunit alpha5[38].

***miR-874* and necrosis:** Another study revealed that in response to H₂O₂ treatment, *miR-874* was substantially increased. Knockdown of *miR-874* attenuated necrosis in the cellular model and also MI in the mouse model. As downstream mediator and target of *miR-874* was identified caspase-8. Caspase-8 was able to antagonize necrosis. When suppressed by *miR-874*, caspase-8 lost the ability to repress the necrotic program. Foxo3a was identified as a transcriptional repressor of *miR-874* expression. This study determined a novel myocardial necrotic regulatory model consisting of Foxo3a, *miR-874* and caspase-8[39].

***miR-1*, *miR-21*, *miR-24*, *miR-320* and ischemia-reperfusion:** Heat-shock treatment protects the heart against ischemia-reperfusion (I/R) injury. A significant induction and increase of *miR-1*, *miR-21* and *miR-24* has been observed in hearts of mice, which were subject to cytoprotective heat-shock (HS). miRNAs isolation from HS mice and injection into non-HS mice, resulted in significantly reduction of the infarct size in the heart following global I/R injury. Further analysis showed that reduction in MI size is accompanied by downregulation of expression of genes that induce apoptosis and upregulation of those that reduce apoptosis. These results showed that in the non-heat-shocked mice, miRNA function in heat-shock-like protection against I/R. Proposed mechanism of miRNAs action is through repression of pro-apoptotic genes (caspases 1, 2, 8, and 14, Bid, Bcl-10, Cidea, Ltbr, Trp53, and Fasl) and induction of anti-apoptotic genes (Bag-3 and Prdx2). Through administration of *miR-21*, it has been shown that chemically synthesized miRNA can reduce MI size, an outcome that was blocked with a *miR-21* inhibitor[40]. Another miRNA in the mouse hearts with I/R has been shown to be dysregulated, *miR-320*. *miR-320* was shown to be significantly decreased after MI and to target heat-shock protein 20. Experiments involving cardiac-specific overexpression of *miR-320* in transgenic mice resulted in increased apoptosis and infarct size in the hearts with I/R, and treatment with antagomir-320 reduced the infarct size[41].

***miR-21*, I/R and fibrosis:** Further research on I/R

models led to the identification of miRNAs with significant expression changes on days 2 and 7 post-I/R. Elevated *miR-21* levels were observed on day 2 as well as on day 7; however, *miR-21* induction in response to I/R was limited to CFs. CFs were shown to be the major cell type in the infarct zone. A marked decrease in phosphatase and tensin homolog (PTEN), a target of *miR-21*, has also been observed in the infarct zone. This decrease has been associated with increased matrix metalloproteinase-2 (MMP-2) expression, suggesting a *miR-21*-PTEN-Akt-MMP-2 pathway in CFs after MI[23].

***miR-494* and apoptosis in I/R:** A mouse model with cardiac-specific *miR-494* overexpression showed improved recovery of contractile performance during the reperfusion period. This was accompanied by a reduction of apoptosis in transgenic mice and reduced MI in I/R. Cultured adult cardiomyocytes with short-term overexpression of *miR-494* showed an inhibition of caspase-3 activity and reduced cell death after stimulated I/R. *miR-494* inhibited three pro-apoptotic (PTEN, ROCK1, CaMKⅡ) as well as two anti-apoptotic proteins (FGFR2 and LIF). *miR-494* targets both pro- and anti-apoptotic proteins and was downregulated in human infarcted hearts. Divergent targets of a miRNA may work unequally to balance a common signaling pathway and eventually affect its functional consequences[42].

Rat models

Microarray analysis: Using genome-wide expression profiling of miRNAs in an ischemic myocardium from rat, seventeen miRNAs were shown to be significantly dysregulated during the AMI progression. Expression was analyzed 2, 7 and 14 d after AMI. On day 2, four miRNAs were upregulated (*miR-31*, *miR-223*, *miR-18a* and *miR-18b*) and two were downregulated (*miR-451* and *miR-499-5p*). On day 7, four miRNAs were upregulated (*miR-31*, *miR-214*, *miR-199a-5p* and *miR-199a-3p*) and seven were downregulated (*miR-181c*, *miR-181d*, *miR-499-5p*, *miR-29b*, *miR-26b*, *miR-126* and *miR-1*). On day 14, five miRNAs were upregulated (*miR-214*, *miR-923*, *miR-711*, *miR-199a-3p* and *miR-31*). Some of these dysregulated miRNAs were related to processes included in response to low oxygen as are hypoxia, inflammation, and fibrosis[43]. In another study, propanolol was chronically administered to induce reversal of the MI. A long-term MI model in rats was established and microarray data analysis showed that long-term propranolol administration resulted in 18 of 31 dysregulated miRNAs undergoing reversed expression. *miR-1*, *miR-29b* and *miR-98* were suggested to play predominant roles in MI. Bioinformatic analysis suggested that *miR-1* regulates myocyte growth, *miR-29b* regulates fibrosis and *miR-98* regulates inflammation[44].

***miR-1* and *miR-206* and apoptosis:** The potential roles of muscle-specific *miR-1* and *miR-206* and their expression in a rat model of MI have been analyzed. Both miRNAs were significantly increased, while insulin-like growth factor 1 (IGF-1) protein levels were markedly re-

duced. Caspase-3 activity was increased in cells transfected with either *miR-1* or siRNA against IGF-1. Enhanced apoptosis could be therefore induced in cardiomyocytes with a low level of IGF-1 mediated by the post-transcriptional repression caused by *miR-1*/*miR-206*[45].

miR-1 and arrhythmia: Propranolol was shown to reduce the incidence of arrhythmias in a rat model of MI. Increased expression of *miR-1* was observed in an ischemic myocardium. Administration of propranolol reversed the upregulation of *miR-1* to near control levels, significantly diminishing the incidence of arrhythmias in the first 12 h after MI. The suggested targets for miR-1 were the cardiac ion channels Cx43 and Kir2.1[46].

miR-21, apoptosis and atrial fibrillation: The miRNA expression profiling has been performed 6 h after AMI induction in rats. Thirty-eight miRNAs were dysregulated when infarcted area has been compared to non-infarcted heart tissue and 33 in the border zone of the MI when compared to non-infarcted area. *miR-21* was significantly downregulated in the infarcted area but was upregulated in the border zone (6 and 24 h after MI). *miR-21* had a protective effect on ischemia-induced cell apoptosis by targeting PDCD4 and AP-1, which might play critical roles in the early phase of AMI. Importantly, some miRNAs in the non-infarcted area were also differentially expressed 6 h after AMI, suggesting that in addition to dysregulated miRNAs in infarcted tissue and border zone, some miRNAs dysregulated in the remote myocardium might also contribute in the pathophysiological response to AMI[47]. Another potential role of *miR-21* in the atrial fibrillation (AF) resulted from experimental HF after MI. *miR-21* was upregulated in atrial tissues following MI, along with the dysregulation of target genes sprouty-1, collagen- I, and collagen-III. Anti-*miR-21* treatment reduced atrial *miR-21* expression, decreased AF duration, and reduced atrial fibrous tissue[48].

miR-34a and apoptosis: In an experimental rat model of MI, the expression of *miR-34a* was highly increased while the expression of aldehyde dehydrogenase 2 (ALDH2) was decreased. Overexpression of *miR-34a* in neonatal rat cardiomyocytes significantly enhanced apoptosis and downregulated ALDH2, suggesting that ALDH2 is a direct target of *miR-34a*. Serum *miR-34a* levels in AMI patients and rats were significantly higher than those in controls[49].

miR-101, miR-711, miR-29b and fibrosis: Four weeks after MI induction in rats, examination of miRNAs expression in the peri-infarct area revealed down-regulation of *miR-101a/b*. In rat neonatal CFs, enforced expression of *miR-101a/b* lead to suppression of collagen production and proliferation. These effects were abrogated by co-transfection with antisense inhibitors of *miR-101a/b*. The fibroblast proto-oncogene c-Fos was suggested as a target of *miR-101a*. Anti-fibrotic action of *miR-101a* was

mimicked by silencing c-Fos using siRNA, whereas effect of *miR-101a* in cultured CFs was cancelled by enforced expression of the c-Fos. In rats with chronic MI, four weeks after overexpression of *miR-101a* using adenovirus, remarkable improvement in cardiac performance was observed as well as reduction in interstitial fibrosis and inhibition of c-Fos and TGF-β1 expression[50]. Pioglitazone was further shown to increase *miR-711* expression and significantly reduce collagen- I levels similar to CFs, and overexpression of *miR-711* suppressed collagen- I levels. Therefore, pioglitazone may upregulate *miR-711* to reduce collagen- I levels in rats with MI. The *miR-711*-transcription factor SP1-collagen- I pathway may be involved in the anti-fibrotic effects of pioglitazone[51]. Another fibrosis study has been performed showing that carvedilol protected against myocardial injury induced by AMI. In male rats, cardiac remodeling and impaired heart function were observed 4 wk after MI; the upregulation of COL1A1, COL3A1, and α-smooth muscle actin (α-SMA) mRNA was observed as well as the downregulation of *miR-29b*. COL1A1, COL3A1, and α-SMA were downregulated and *miR-29b* was upregulated by carvedilol in a dose-dependent manner in rat CFs. Enforced expression of *miR-29b* significantly suppressed COL1A1, COL3A1, and α-SMA expression[52]. An alternative strategy has also been hypothesized that overexpression of *miR-29b*, which would inhibit mRNAs that encode CF proteins involved in fibrosis, would similarly facilitate progenitor cell migration into the infarcted rat myocardium. The number of GFP-positive cells, capillary density, and heart function were significantly increased in hearts overexpressing *miR-29b*, and downregulation of *miR-29b* with anti-*miR-29b* induced interstitial fibrosis and cardiac remodeling[53].

Human MI
Microarray analysis: Our group performed genome-wide miRNA expression profiling of human MI (7 d post-MI and 4 wk post-MI) comparing fetal hearts to healthy adult hearts. A number of novel miRNAs were identified as well as some similar expression patterns between human MI and fetal hearts, suggesting involvement of cardiac gene reprogramming also in response after MI. Seven miRNAs were confirmed as dysregulated, including *miR-1*, *miR-133a/b*, *miR-150*, *miR-186*, *miR-210* and *miR-451*[54].

miR-29: Several miRNAs were shown to be dysregulated in the murine MI model, including *miR-29*. Similarly dysregulation has been observed in human MI, after obtaining border zone of the infarcted cardiac tissue from the patients that received a cardiac transplant[34].

miR-1, miR-133a/b, miR-208a: Our group further showed that *miR-1*, *miR-133a/b* and *miR-208* were differentially expressed in human MI and fetal hearts when compared to healthy adults. Time-course changes were observed in human MI, with *miR-208* upregulated across all time points and *miR-1* and *miR-133a/b* downregulated
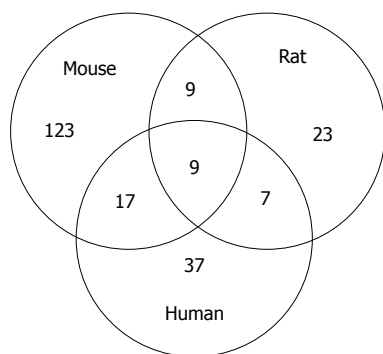
Figure 4 Venn's diagram of number of differentially expressed miRNAs in human myocardial infarction compared to mouse and rat model of myocardial infarction. Experimental data were obtained from microarray analysis performed on mouse model of MI[32,33], rat model of MI[43,44] and human MI[54,58]. MI: Myocardial infarction.

2-7 d after MI. All four miRNAs were downregulated in fetal hearts in comparison to healthy adults. We have also observed some similar patterns of miRNA expression between fetal hearts and MI[55]. The remote myocardium was also analyzed and compared to healthy adult hearts and the infarcted area. Whereas *miR-1* expression was similar in MI and healthy adults, it was upregulated in the remote myocardium. Downregulation of both *miR-133a* and *miR-133b* was observed in the infarcted tissue as well as in the remote myocardium of patients with MI when compared to healthy adult hearts[56].

miRNAs and ventricular rupture: Evidence suggests that an intense inflammatory reaction after a MI might contribute to the development of ventricular rupture (VR). In 50 patients with MI (with or without VR), we showed an altered expression of *miR-146a*, *miR-150* and *miR-155* compared to healthy adult hearts. *miR-146a* showed upregulation and *miR-150* and *miR-155* showed downregulation in patients with VR compared to those without. These miRNAs are involved in the regulation of innate immunity and the inflammatory response, providing further evidence that innate immunity resulting in an intense inflammatory reaction plays an important role in the pathogenesis of VR after a MI in humans[57].

miRNAs and SERCA2: In another study our group also showed 43 dysregulated miRNAs and decreased expression of the protein SERCA2 when infarcted tissue was compared to the corresponding remote myocardium. The prediction of miRNA binding to SERCA2 identified 213 putative miRNAs. miRNA annotation of dysregulated miRNAs revealed 18 functional and 21 disease states that are linked to the cardiovascular diseases. Half of the dysregulated miRNAs were associated with SERCA2. Free-energy binding and flanking regions were defined for 10 upregulated miRNAs (*miR-122*, *miR-320a/b/c/d*, *miR-574-3p/-5p*, *miR-199a*, *miR-140* and *miR-483*). The dysregulation of 9 miRNAs was confirmed (*miR-21*, *miR-122*, *miR-126*, *miR-1*, *miR-133*, *miR-125a/b* and *miR-98*)[58].

Comparison of the number of differentially ex-

pressed miRNAs from microarray studies performed on human MI[54,58], on mouse[32,33] and rat model of MI[43,44] is summarized in Figure 4. Only a small proportion of differentially expressed miRNAs overlaps between three different species, these are *let-7b*, *let-7f*, *miR-26b*, *miR-126-3p*, *miR-126-5p*, *miR-195*, *miR-199a-3p*, *miR-214* and *miR-451*. All these microarray analyses were performed at different time point post-MI. However, comparison has been performed including any dysregulated miRNA at any time point post-MI within species.

Circulating miRNAs
Serum and exosome miRNAs as AMI biomarkers: In patients with AMI as well as in patients with angina pectoris (AP), significant increase in serum levels was observed for *miR-1* and *miR-133a*. *miR-133a* has been recognized as a circulating marker for cardiomyocyte death, because its elevated expression is observed in patients with an injured myocardium. Using an experimental mouse model, it was further identified that significant reduction in levels of *miR-1*, *miR-133a*, *miR-208a* and *miR-499* occur in the infarcted myocardium. After stimulation of cardiomyoblasts, exosome fraction of the culture medium was obtained. The measurement of *miR-133a* was performed. Significant elevation of *miR-133a* was observed upon the detection of cell death[59]. Using an *in vitro* cardiac cell necrosis model, it was shown that cardiac *miR-1* was released into the culture media 20 min after induction, where it is stable for at least 24 h. The amount of *miR-1* released was related to the number of necrotic cardiac myocytes. Furthermore, a time-course study of serum *miR-1* in a rat model included time points at 1, 3, 6, 12, 24 h, and 3, 7, 14, 21 and 28 d after AMI. Serum *miR-1* levels were increased after AMI with a peak at 6 h, returning to the basal level 3 d after AMI, and showed a strong positive correlation with MI size. Research in humans has shown that in 31 patients with AMI, *miR-1* was significantly increased within 24 h after AMI and showed a positive correlation with serum creatine kinase-MB, suggesting its relationship to MI size also occurs in humans. At days 3 and 7, the serum levels returned to baseline[60]. In another study, serum samples were taken from 117 patients with AMI, 182 patients with AP and 100 age- and gender-matched controls. Six serum miRNAs, *miR-1*, *miR-134*, *miR-186*, *miR-208*, *miR-223* and *miR-499*, were identified as AMI biomarkers and presented significant differences between the AMI and AP cases. *miR-208* and *miR-499* showed higher expression in the AP cases than in the AMI cases[61].

Serum and exosome miRNAs and prognosis after MI: Using sera collected a median of 18 d after AMI onset, miRNAs were screened in 21 patients who experienced development of HF within 1 year after AMI and in 65 matched controls. *miR-192*, *miR-194* and *miR-34a*, all p53-responsive miRNAs, were coordinately increased, particularly in exosomes. The serum level of *miR-192* was significantly upregulated in AMI patients with develop-

ment of ischemic HF. *miR-194* and *miR-34a* expression levels were significantly correlated with the left ventricular (LV) end-diastolic dimension 1 year after AMI[62]. The prognostic impact of circulating miRNAs in patients who survived AMI was also analyzed by a high-throughput array consisting of 667 miRNAs. Eleven miRNAs were differentially expressed in the serum from patients at high-risk for cardiac death, and a subset of circulating miRNAs might be predictive for cardiac death in post-AMI patients. Serum levels of *miR-155* and *miR-380** were higher in patients who experienced cardiac death within 1 year after discharge[63].

Whole blood miRNAs as AMI biomarkers: After performing miRNA expression profiling in peripheral whole-blood samples of patients with AMI, 121 dysregulated miRNAs have been identified. These miRNAs possess a unique signature of 20 miRNAs predicting AMI with 96% specificity, 90% sensitivity and 93% accuracy. *miR-30c* and *miR-145* levels were expressed in correlation with infarct size, which was estimated by release of Troponin T (TnT). Identification of miRNAs that is not based solely on the release of miRNAs from a necrotic myocardium is important for understanding active processes involved in the pathogenesis of MI (inflammation, plaque, rupture and vascular injury). Dysregulated miRNAs in AMI might be equally derived from other cellular populations that play an active role in AMI pathophysiology[64]. To characterize temporal expression patterns of miRNAs in MI, another study was performed with miRNA expression levels measured at multiple time points (0, 2, 4, 12, 24 h after the initial presentation) in patients with acute MI. A subset of miRNAs was found to be significantly dysregulated both at the initial presentation and during the course of AMI. Novel miRNAs that are dysregulated early during MI were identified (*miR-1915* and *miR-181c**)[65].

Whole blood and plasma miRNAs as AMI biomarkers: The whole blood and plasma samples were obtained from 51 AMI patients and compared with 28 control subjects. Sample collection from AMI patients was performed within 24 h and 7 d after the onset of AMI. In plasma as well as in whole blood from AMI patients, elevated *miR-133* and *miR-328* levels was observed. Seven days after onset of AMI symptoms increased circulating *miR-133* and *miR-328* levels returned to control levels. There has also been observed a correlation between cardiac Troponin I (cTnI) and circulating *miR-133* or *miR-328*[66].

Plasma miRNAs as AMI biomarkers: In AMI rats, plasma samples were taken at 1, 3, 6, 12 and 24 h. At these time points, measurement of levels of *miR-1, miR-133a, miR-499* and *miR-208a* has been performed. All these miRNAs were significantly increased, at least at one time point. *miR-208a* was undetectable at time 0 h, increased 1 h after AMI and reached its peak at 3 h. At

time point 6-12 h, it began decreasing and at 24 h it was undetectable. At time point 1-3 h, *miR-1, miR-133a* and *miR-499* were elevated, at 3-12 h reached their peak and at 12-24 h finally decreased. *miR-1, miR-133a, miR-499* and *miR-208a* were present at very low levels or were absent in the plasma of healthy people, but were substantially higher in the plasma of 33 AMI patients compared with that of patients with other cardiovascular diseases, whereas *miR-208a* remained undetectable in patients with non-AMI heart diseases[67]. In another study, miRNAs were analyzed in human plasma, mouse plasma and mouse cardiac muscle. A microarray analysis showed 20 upregulated and 14 downregulated miRNAs in 17 healthy donors compared with 33 patients with AMI. *miR-1, miR-133a/b* and *miR-499-5p* were upregulated and *miR-122* and *miR-375* were downregulated 6-12 h after MI onset. Five days later, all miRNAs were back to basal plasma levels, except that *miR-122* was lower than in controls through day 30. Compared to cTnI, peak expression was observed at a similar time in MI patients for *miR-1* and *miR-133a/b,* but *miR-499-5p* showed a slower time course. In mice, the pattern of upregulated miRNAs was similar to that in MI patients, but reciprocal expression was observed in cardiac tissue 3-6 h after MI[68]. *miR-1* level was measured in a larger cohort of patients (159) with or without AMI. In the plasma from AMI patients, *miR-1* was significantly increased when compared with non-AMI patients. Its levels decreased to normal after medication. Statistical analysis revealed that elevated levels of circulating *miR-1* were not in correlation to patient characteristics (established biomarkers for AMI, concurrent disease as are blood pressure and diabetes mellitus or either age or gender)[69]. Increased *miR-1* and decreased *miR-126* expression were consistently observed in the plasma from 17 patients with AMI compared with 25 healthy subjects. cTnI, *miR-1* and *miR-126* expression levels showed the same trend[70]. *miR-1, miR-133a, miR-208b* and *miR-499* were further compared to cTnT for diagnostic value. Study has been performed on 67 patients with AMI and 32 healthy volunteers. The levels of all plasma miRNAs were significantly higher in AMI patients than in healthy volunteers. At the time of hospital discharge of AMI patients, expression of the cardiac-specific miRNAs was reduced to near baseline levels. However, it has turned out that for the diagnosis of AMI, the four plasma miRNAs were not superior to cTnT[71]. In another study, plasma samples were obtained from 18 patients with AMI and 30 healthy adults. In this cohort of samples, *miR-30a, miR-195* and *let-7b* levels were examined. At time points 4 h, 8 h and 12 h after the onset of AMI, circulating *miR-30a* was highly elevated. In AMI patients, *miR-195* was also highly expressed, when compared to control, but only at time points 8 h and 12 h. Through all the time points, *let-7b* was lower in AMI patients when compared to control samples. All three investigated circulating miRNAs, *miR-30a, miR-195* and *let-7b*, showed the peak expression at 8 h and were of significant diagnostic value for AMI[72].

Plasma miRNAs for differentiating MI: Plasma concentrations of cardiac-enriched *miR-208b* and *miR-499* were measured in a case-control study of 510 MI patients and 87 healthy controls. *miR-208b* and *miR-499* showed elevated expression in patients with MI and were nearly undetectable in healthy controls. In 397 patients with ST-elevation MI (STEMI), miRNAs had higher concentrations than in 113 patients with non-STEMI (NSTEMI)[73].

Plasma miRNAs for differentiating MI from other cardiovascular diseases: In all individuals with AMI, the concentration of plasma *miR-499* was shown to be increased; however it was below the detection limit in other groups of patient [control, chronic HF (CHF), and unstable AP][74]. The expression level of plasma *miR-133a* has been analyzed in 13 AMI patients, 176 AP patients and 127 control subjects for its relationship to the severity of coronary stenosis. The results showed that circulating *miR-133a* levels were significantly increased in AMI patients in a time-dependent manner, achieving a peak at 21.6 ± 4.5 h after the onset of AMI symptoms and showed a similar trend as the level of plasma cTnI. Importantly, the levels of circulating *miR-133a* positively correlated with the severity of coronary artery stenosis[75]. Another study showed that plasma levels of *miR-1*, *miR-133a*, *miR-208b* and *miR-499* (muscle- or cardiac-specific or enriched miRNAs), *miR-21* and *miR-29b* (fibrosis-related miRNAs) *miR-146*, *miR-155*, *miR-223* (leukocyte-associated miRNAs) are associated with different degrees of cardiac injury as are AMI, acute HF, diastolic dysfunction and even viral myocarditis. In the plasma of 32 patients with AMI, *miR-208b* and *miR-499* were highly elevated compared with control subjects and both correlated with plasma cTnT levels. Both miRNAs also showed significant but milder elevation in viral myocarditis. However, in patients with acute HF, only *miR-499* showed significant elevation, whereas no significant change was observed in diastolic dysfunction[76]. Another study group consisted of 17 patients with AMI, 4 with stable coronary artery disease (CAD) and 5 with no history of CAD. Expression of *miR-423-5p*, *miR-208* and *miR-1* was measured in plasma before percutaneous coronary intervention (PCI), at 6, 12 and 24 h. In stable CAD, the expression of *miR-1*, *miR-208a* and *miR-423-5p* did not show any significant differences at any time point. There was a higher number of *miR-423-5p* copies in patients with AMI before the PCI. However, 6, 12 and 24 h after PCI, the expression levels were similar to the control group and significantly lower than the baseline level. The expression levels of *miR-1* and *miR-208a* were not significantly different from the control group[77]. In another study, the increased expression levels of *miR-1*, *miR-21*, *miR-133a*, *miR-423-5p* and *miR-499-5p* has been showed in plasma of 92 patients with NSTEMI compared to 99 age-matched healthy control subjects. *miR-499-5p* and *miR-21* showed increased expression in NSTEMI compared to 81 patients with CHF. *mir-499-5p* also showed good diagnostic accuracy in differentiating patients with NSTEMI and CHF[78]. Takotsubo cardio-

myopathy (TTC) is clinically indistinguishable from AMI, and no established biomarkers are available for the early diagnosis of TTC and differentiation from AMI. After miRNA profiling, eight miRNAs were selected for verification in 36 patients with TTC, 27 patients with AMI and 28 healthy controls. Upregulation of *miR-16* and *miR-26a* was confirmed in patients with TTC compared with healthy subjects, and upregulation of *miR-16*, *miR-26a* and *let-7f* was observed in TTC compared with MI patients. Compared with healthy controls, *miR-1* and *miR-133a* showed upregulation in patients with MI, and *miR-133a* was substantially increased in patients with MI when compared with TTC. A unique signature comprising *miR-1*, *miR-16*, *miR-26a* and *miR-133a* differentiated TTC from healthy subjects and from MI patients[79].

Plasma miRNAs and prognosis after MI: Plasma *miR-1*, *miR-21*, *miR-29a*, *miR-133a* and *miR-208* were measured in 12 age-matched reference controls and 12 post-MI patients from day 2 through day 90 post-MI. After MI, a progressive increase of LV end-diastolic volume was accompanied by time-dependent changes in specific miRNAs. Two days post-MI, *miR-21* decreased and 5 d post-MI increased. At later time points its expression level reached the control values. Similarly, at day 5 post-MI, *miR-29a* increased and then decreased to the control level at later time points. *miR-208* showed elevated expression at day 5 post-MI and did not show any decrease up to day 90 post-MI[80]. *miR-1*, *miR-208b* and *miR-499-5p* were further measured in plasma samples from 424 patients for discrimination of a clinical diagnosis of MI and for association with 30-d mortality and for diagnosis of HF. Discrimination of MI was accurate for *miR-208b* and *miR-499-5p* but was considerably lower than for TnT. Increased miRNA levels were strongly associated with an increased risk of mortality or heart failure within 30 d for *miR-208b* and *miR-499-5p*, but the association was lost when adjusting for TnT[81]. In another study, circulating miRNAs were measured in 90 patients after AMI and several miRNAs were identified as potentially involved in LV remodeling. *miR-150* was downregulated in patients with remodeling compared with patients without. *miR-150* outperformed B-type natriuretic peptide (BNP) to predict remodeling and reclassified 54% of patients misclassified by BNP and 59% of patients misclassified by a multi-parameter clinical model[82]. Furthermore, plasma samples from 150 patients with AMI were obtained for determination of the levels of *miR-16*, *miR-27a*, *miR-101* and *miR-150*. A combination of the four miRNAs improved the prediction of LV contractility based on clinical variables. Patients with low levels of *miR-150* or *miR-101* and elevated levels of *miR-16* were at high risk for impaired LV contractility. The four-miRNA panel reclassified a significant proportion of patients, with a net reclassification improvement of 66%[83].

Plasma miRNAs and prospective study for MI: The association between baseline levels of miRNAs, the in-

cidence of MI, and the cellular origin of miRNAs was analyzed in 820 participants with 19 candidate miRNAs. Three miRNAs were consistently and significantly related to the incidence of MI; *miR-126* showed a positive association, *miR-223* and *miR-197* were negatively related to the risk of disease. Control group consisted of healthy volunteers, in who limb I/R was performed by thigh cuff inflation. After obtaining plasma samples at baseline, 10 min, 1 h, 5 h, 2 d, and 7 d, miRNA expression was analyzed. Six distinct miRNA clusters were identified by computational analysis, and one of them consisted of all miRNAs that were related to the risk of a future MI. This cluster included miRNAs predominantly expressed in platelets and its characteristic was activation 1 h post-I/R (early) and activation 7 d post-I/R (sustained). Platelets were suggested as being a major contributor to this miRNA expression pattern, since in subjects with a subsequent MI, dysregulated patterns of circulating miRNAs occurred with endothelium-enriched *miR-126*[84].

Plasma and urine miRNAs: In a pig I/R model, *miR-1, miR-133a* and *miR-208b* increased rapidly in plasma with a peak at 120 min, while *miR-499-5p* remained elevated longer. In humans, 25 patients with MI revealed that all four miRNAs were increased in plasma, with a peak at 12 h. Peak values of *miR-208b* correlated with peak troponin levels. *miR-1* and *miR-133a* both correlated strongly with renal elimination, which was confirmed by detection of *miR-1* and *miR-133a,* but not *miR-208b* or *miR-499-5p*, in the urine[85].

Urine miRNAs: Blood protein MI biomarkers (creatine phosphokinase-muscle band, TnT and TnI) are not typically filtered into urine. Urine *miR-1* was quickly increased in rats with a peak at 24 h after AMI and returned to the basal level 7 d after AMI. No *miR-208* was observed in normal urine; however, *miR-208* was easily detected in urine from rats with AMI. Serum exosomes from rats after AMI were isolated and injected into the circulating blood of normal rats; urine *miR-1* was significantly increased in the exosome-injected animals. The levels of urine *miR-1* were also significantly increased in patients with AMI[86].

In summary, it has been shown that miRNAs may be useful circulating biomarkers for the diagnosis of AMI, differentiating them from other cardiovascular diseases and prognoses after MI. However, two studies have shown that miRNAs are not useful circulating biomarkers for some aspects of MI, (1) for prognosis of patients with STEMI; or (2) for an incidence of LV remodeling 1 year after anterior AMI[87,88].

Therapeutic opportunities

All miRNAs as potential therapeutic targets were tested in mouse or rat models of MI. miRNAs and different therapeutic approaches analyzed in mouse model of MI are summarized in Figure 5 and miRNAs and different therapeutic approaches analyzed in rat model of MI are

overviewed Figure 6.

miR-181a and skeletal myoblast transplantation in rats with MI: A lentiviral siRNA against the loop region of *miR-181a* was shown to upregulate the skeletal myoblast (SKM) differentiation repressor Hox-A11 and reduce arrhythmias following SKM transplantation into ischemic myocardium of rats. Engraftments of SKMs with *miR-181a* knockdown improved cardiac function and significantly decreased the arrhythmogenic effect of SKM transplantation in rats with experimental MI[89].

miR-210 and treatment of ischemic heart disease: *miR-210* was highly expressed in mouse cardiomyocytes that survived 48 h after hypoxia exposure compared with apoptotic cardiomyocytes. Mice receiving a *miR-210* precursor showed significant improvement of LV fractional shortening after 8 wk. Histological analysis showed decreased cellular apoptosis and increased neovascularization. Two target genes involved in inhibition of angiogenesis/vascular remodeling and induction of apoptosis, Ephrin-A3 and Ptp1 (non-receptor phospho-tyrosine protein phosphatase), were confirmed. It has been shown that *miR-210* can improve angiogenesis, inhibit apoptosis and improve cardiac function in a mouse model of MI[90].

Phosphorinositide-3-kinase-regulated miRNA and mRNA: Activation of phosphorinositide-3-kinase (PIK3) is considered a new strategy for the treatment of heart failure and MI. To identify cardiac-selective miRNAs and mRNAs that mediate the protective properties of PIK3, experimental mice were used and identified growth factor receptor-bound protein (Grb14) gene expression that positively correlated with cardiac function. Grb14 is highly expressed in the mouse heart compared with other tissues. Three miRNAs were also highly correlated with Grb14, namely *miR-210*, *miR-34a* and *miR-222*[91].

Tanshinone and miR-1: Accumulating evidence suggests that tanshinone ⅡA can reduce the ischemic area and improve cardiac function and has been shown to suppress *miR-1* expression. Using a rat model of MI, tanshinone ⅡA was administered daily for 7 d before MI and lasted for 3 mo following MI. Tanshinone ⅡA was shown to relieve ischemia-induced injury, decrease the elevated *miR-1* levels in ischemic and hypoxic cardiomyocytes, and consequently restored the normal level of the *miR-1* target Cx43. In ischemic and hypoxic cardiomyocytes, tanshinone ⅡA also inhibited activated p38 MAPK, SRF and MEF2[92].

Ivabradine and _miR-1_ and _miR-133a_: Ivabradine is a selective inhibitor of the hyperpolarization-activated, cyclic nucleotide-gated pacemaker current. Its effect on electrophysiological remodeling of myocytes from post-MI rats was observed as a decrease in the transcription of HCN4, a target of *miR-1* and *miR-133a*. Both, *miR-1* and *miR-133* were significantly elevated in myocytes. The
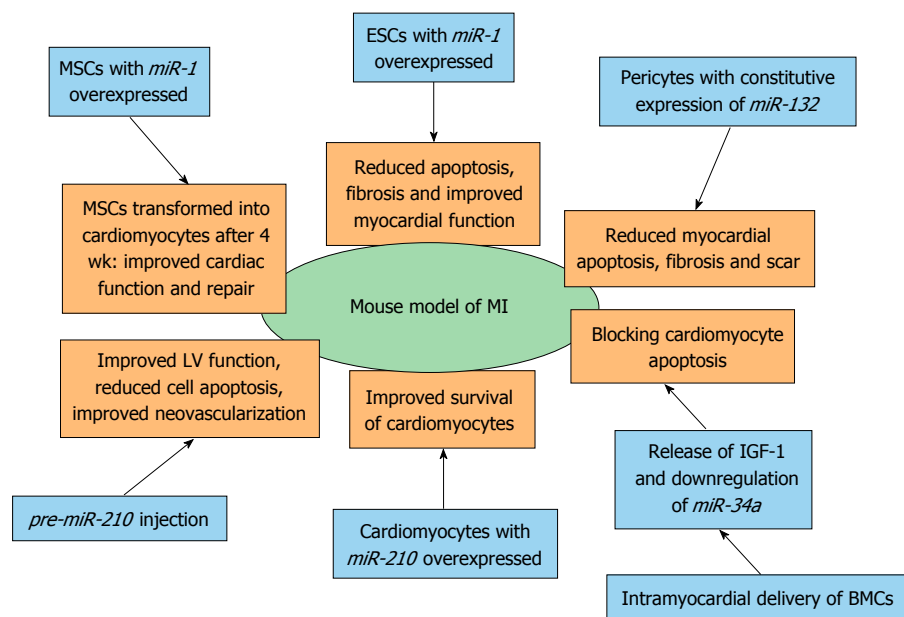
Figure 5 Therapeutic opportunities of miRNAs in myocardial infarction identified by using mouse model of myocardial infarction. BMC: Bone marrow cell; ESC: Embryonic stem cell; LV: Left ventricle; MSC: Mesenchymal stem cell; MI: Myocardial infarction; IGF-1: Insulin-like growth factor 1.
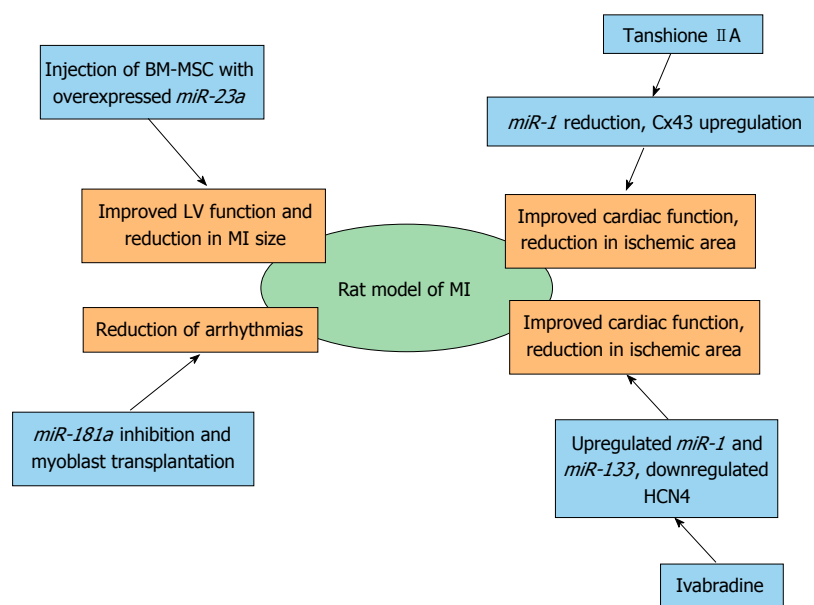

Figure 6 Therapeutic opportunities of miRNAs in myocardial infarction identified by using rat model of myocardial infarction. BM-MSC: Bone marrow-mesenchymal stem cell; LV: Left ventricle; MI: Myocardial infarction; Cx43: Gap junction alpha-1 protein; HCN4: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4.

beneficial effects of ivabradine may be due to the reversal of electrophysiological cardiac remodeling by reducing the overexpressed HCN channels in post-MI rats[93].

Embryonic stem cells and miRNAs: Embryonic stem cells (ESC) with overexpressed *mi*R-1 were transplanted into the infarcted myocardium of experimental animals, and reduced apoptosis was subsequently observed 4 wk post-MI. A significant elevation in p-Akt levels and diminished PTEN levels were also observed. The mice also had a significant improvement in some physiological car-

diac functions[94]. The same author investigated whether overexpression of *mi*R-1 in ESCs would enhance cardiac myocyte differentiation following transplantation into the infarcted myocardium. Two weeks after transplantation into the border zone of the infarcted heart, cardiac myocyte differentiation, adverse ventricular remodeling, and cardiac function were assessed. Overexpression of *mi*R-1 in transplanted ESCs protected the host myocardium from MI-induced apoptosis. A significant reduction in interstitial and vascular fibrosis was observed as well as significantly improved heart function[95].

Mesenchymal-stem cells and miRNAs: One week after MI, mice were intramyocardially injected at the heart infarcted zone with *miR-1*-transduced mesenchymal-stem cells (MSCs). At 4 wk post-transplantation, transplanted MSCs were able to differentiate into cardiomyocytes in the infarcted zone. Cardiac function with the *miR-1*-transduced MSCs was significantly improved, and treatment of MSCs expressing *miR-1* was more effective for cardiac repair, most likely by enhancing cell survival and cardiac myocyte differentiation compared with the MSCs without *miR-1*[96]. *In vitro* co-culture between cardiomyocytes and MSCs has been established to test whether MSCs deliver *miR-210* to host cardiomyocytes; this showed co-localization of *miR-210* with the gap-junction protein Cx43. *miR-210* has been proposed to be transferred through gap junctions. Higher survival rates of cardiomyocytes co-cultured with MSCs was observed with concomitant expression of caspase-8 associated protein 2 (CASP8AP2) suggesting that *miR-210* translocates from MSCs to protect host cardiomyocytes. Direct transfer of pro-survival *miR-210* from MSCs to host cardiomyocytes led to a functional recovery of the ischemic hearts of the experimental animals[97]. The clinical application of MSC-based therapy is restricted because of the poor survival of implanted cells. Using a tumor necrosis factor α-(TNF-α)-induced bone marrow (BM)-MSC injury model and a rat MI, it has been shown that *miR-23a* was involved in TNF-α-induced BM-MSC apoptosis through regulating caspase-7 and that the injection of BM-MSCs overexpressing *miR-23a* could improve LV function and reduce the infarct size in the rat MI model[98].

Bone marrow cells and *miR-34a*: Cell therapy with bone marrow cells (BMC) can improve the recovery of cardiac function after ischemia. I intra-myocardial delivery of BMCs in infarcted mice has been shown to regulate the expression of miRNAs in the heart and downregulate the expression of *miR-34a,* a pro-apoptotic miRNA. Transplanted BMCs regulate cardiac miRNAs by paracrine mode and thus contribute to the protective effects. IGF-1 inhibits the *miR-34a* processing and is released by BMCs, thereby blocking apoptosis in cardiomyocytes[99].

Pericytes and *miR-132*: Pericytes are key regulators of vascular maturation and therapeutic activity, and mechanistic targets of saphenous vein-derived pericyte progenitor cells (SVPs) have been investigated using a mouse MI model. Transplantation of SVPs into the peri-infarct zone of mice attenuated LV dilatation, reduced myocardial scar, cardiomyocyte apoptosis and interstitial fibrosis, and blood flow and neovascularization. *miR-132* was constitutively expressed and secreted by SVPs and markedly upregulated. Ras-GTPase activating protein and methyl-CpG-binding protein 2 were shown to be targets of *miR-132*. *miR-132* inhibition decreased SVP capacity to improve contractility, reparative angiogenesis, and interstitial fibrosis in infarcted hearts[100].

Telocytes and miRNAs: Telocytes (TCs) are a novel type of interstitial cell recently discovered in the myocardium. Rat experimental MI was investigated using electron microscopy, immunocytochemistry and analysis of several pro-angiogenic miRNAs that provided evidence for TC involvement in neo-angiogenesis after MI. TCs contain measurable quantities of angiogenic miRNAs (*let-7e*, *miR-10a*, *miR-21*, *miR-27b*, *miR-100*, *miR-126-3p*, *miR-130a*, *miR-143*, *miR-155* and *miR-503*)[101].

Bioinformatics analysis

Rationally designed bioinformatic analysis combined with experimental approaches to screen key therapeutic members of the IUPHAR database was conducted, following establishment of the whole genome protein interaction network and a comprehensive topological assessment. The number of validated and confidently predicted miRNAs regulating each gene encoding an ion channel or a gap junction protein was counted. Cx43 showed more intensive miRNA regulation compared with other ion channel and gap junction proteins[102].

My-Inflamome: One of crucial processes in cardiac repair after MI is inflammation. In a study, a network has been established that enhances understanding of the inflammatory responses and its interaction network in human MI. The network is called My-Inflamome and it assembles protein interactions that are associated to inflammation and related to prognosis after MI. Classification models were established based on microarray data of blood samples from patients after MI with various disease consequences. Significant associations were experimentally verified. Different biological processes included in the heart repair are organized into modules. Small set of miRNAs is also included in modules that are significantly associated with transcriptional regulation[103].

My-DTome: Another computational approach has been performed. It is based on different drug and protein interaction and it is called My-DTome (it is assembling the MI drug-target). It is also consisted of modules, which are related to the important molecular processes and pathways and to potential therapeutic approaches in MI that might be miRNAs-regulated. Non-cardiovascular drugs may also possess the cardiovascular effects and this systemic insight was established. This network might represent the basis for an investigation of new multidrug treatment and new targets MI[104].

Polymorphisms in miRNA binding sites

After searching across dbSNP and TargetScan, 10 SNPs in potential miRNA binding sites of 8 RAAS-related genes were identified and genotyped for risk for MI and blood pressure. It was found that nine SNPs in seven genes were prevalent. Of the nine SNPs, four in three genes were associated with blood pressure. The rare allele of the mineralocorticoid receptor (NR3C2) (SNP rs5534) was associated with a twofold increased risk of MI in men younger than 50 years of age. The reduction in miR-induced repression of gene expression was demonstrated[105].

CONCLUSION

In recent years miRNAs have been recognized as promising therapeutic, diagnostic and prognostic factors in the field of cardiovascular diseases. The usefulness of circulating miRNAs in the diagnosis and prognosis of MI has been established through numerous studies. Moreover, the therapeutic potential of miRNA has been established, especially in field of stem cell research. Heart tissue expression patterns examined in numerous experimental animals still need to be confirmed on human MI. Much more work is necessary before establishing routine use of miRNAs in clinical diagnosis, prognosis and therapy; however, the current findings are encouraging.

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