

Cardiac microRNAs: diagnostic and therapeutic potential

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Abstract

MicroRNAs are small non-coding post-translational biomolecules which, when expressed, modify their target genes. It is estimated that microRNAs regulate production of approximately 60% of all human proteins and enzymes that are responsible for major physiological processes. In cardiovascular disease pathophysiology, there are several cells that produce microRNAs, including endothelial cells, vascular smooth muscle cells, macrophages, platelets, and cardiomyocytes. There is a constant crosstalk between microRNAs derived from various cell sources. Atherosclerosis initiation and progression are driven by many pro-inflammatory and pro-thrombotic microRNAs. Atherosclerotic plaque rupture is the leading cause of cardiovascular death resulting from acute coronary syndrome (ACS) and leads to cardiac remodeling and fibrosis following ACS. MicroRNAs are powerful modulators of plaque progression and transformation into a vulnerable state, which can eventually lead to plaque rupture. There is a growing body of evidence which demonstrates that following ACS, microRNAs might inhibit fibroblast proliferation and scarring, as well as harmful apoptosis of cardiomyocytes, and stimulate fibroblast reprogramming into induced cardiac progenitor cells. In this review, we focus on the role of cardiomyocyte-derived and cardiac fibroblast-derived microRNAs that are involved in the regulation of genes associated with cardiomyocyte and fibroblast function and in atherosclerosis-related cardiac ischemia. Understanding their mechanisms may lead to the development of microRNA cocktails that can potentially be used in regenerative cardiology.

Key words: acute coronary syndrome, atherosclerosis, cardiac ischemia, cardiac regeneration, cardiomyocytes, fibroblasts, induced cardiomyocytes, microRNAs.

Definition of microRNAs, biogenesis and rationale for their use

MicroRNAs are small non-coding post-translational biomolecules which, when expressed, modify their target genes [1–3]. It is estimated that microRNAs regulate production of approximately 60% of all human proteins and enzymes that are responsible for major physiological processes [1, 2]. MicroRNAs are produced by a variety of cells [1–4]. In

cardiovascular disease pathophysiology, there are several cell lineages that produce microRNAs, including endothelial cells (ECs), vascular smooth muscle cells (VSMCs), macrophages, platelets, fibroblasts, and cardiomyocytes [3–5]. There is a constant crosstalk between microRNAs derived from various cell sources.

MicroRNAs regulate gene expression at the post-transcriptional level by binding to 3'- or less often to 5'-untranslated regions of target messenger RNAs (mRNAs), which in consequence leads to inhibited translation and/or induces degradation of targeted mRNA [1]. Through this mechanism, a single microRNA can alter the function of multiple mRNAs. Furthermore, circulating microRNAs in the serum are resistant to lysis and are stable against RNase, as they are hidden in microparticles, apoptotic bodies, etc. [1].

MicroRNA biogenesis is quite complex [2]. The primary microRNA (Pri-miRNA) is produced in the cell nucleus through the transcription of a DNA strand mediated by RNA polymerase II [2]. After transcription, Pri-miRNA is cleaved by the enzymatic complex DROSHA into a micro-RNA precursor (pre-miRNA). Pre-miRNA is exported to the cytoplasm by exportin-5 and cleaved by Dicer (an RNA degrading enzyme) and produces approximately 22 nucleotide RNA duplexes. A microRNA strand is transferred to the Argonaute complex (AGO), forming an RNA-induced silencing complex (RISC), and guides it to pair with the target mRNA through binding of the microRNA seed sequence with the microRNA recognition site on the mRNA. MicroRNAs are secreted out of cells via exosomes [2]. Presently, more than 2000 human microRNAs have been identified (www.mirbase.org), and some of them are involved in atherosclerotic processes [4, 5].

Atherosclerosis initiation and progression are driven by multiple pro-inflammatory and pro-thrombotic microRNAs that overcome microRNAs with protective functions against atherosclerosis [4–6]. Atherosclerotic plaque rupture is the leading cause of cardiovascular death resulting from acute coronary syndromes (ACS), both in ST-segment (STEMI) and non-ST segment elevation (NSTEMI) myocardial infarction, as well as cardiac remodeling and fibrosis following ACS [4–6].

In this review, we focus on the role of cardiomyocyte-derived and fibroblast-derived microRNAs that are involved in the regulation of genes associated with cardiomyocyte function and atherosclerosis-related cardiac ischemia. Furthermore, we discuss microRNAs which can be potentially used in regenerative cardiology. To understand the regulatory functions of cardiomyocyte-derived microRNAs, it is essential to discuss the structure of cardiac cells which comprise the heart.

The cardiac muscle

The cardiac muscle is a very complex organ; however, for the purpose of this review we will simplify our discussion regarding the structure and function of cardiomyocytes and fibroblasts in the human heart. The human heart is composed of region-specific cardiomyocytes (atrial or ventricular), ECs, VSMCs, fibroblasts which produce extracellular matrix, and blood vessels [7, 8]. The cardiomyocyte (cardiac muscle cell) is the fundamental unit responsible for heart contractility [7, 8]. Cardiomyocytes are involved in the contractile function of the heart with constant contraction and relaxation during the cardiac cycle throughout an individual's entire life span [7, 8]. Each human cardiomyocyte contains both contractile proteins, usually 1 or 2 nuclei, and large numbers of mitochondria which provide adequate levels of ATP required by the cells (Figure 1). The basement membrane of cardiomyocytes is composed of glycoproteins (laminin and fibronectin), type IV collagen, as well as proteoglycans [9]. This membrane is responsible for trapping calcium ions responsible for contractility and those that permit the mechanical and electrical coupling of adjacent cells.

Sarcomeres, the essential elements of cardiomyocytes, are composed of contractile proteins such as actin and myosin, and cytoskeletal proteins which maintain mechanical integrity of the cell [7, 8, 10]. Sarcomeres also contain the regulatory proteins troponin and tropomyosin. Cardiac troponins (cTn) I and C control the binding of myosin to actin and consequently take part in the contraction and relaxation of cardiac muscles (Figure 1). Myosin and actin generate the force of contraction, while thin filament proteins calibrate the force generated by contractile proteins. Also, titin, a myofilament protein, functions as a scaffold for sarcomeric assembly and as a molecular spring in striated muscle cells to regulate both systolic and diastolic function [11]. Titin molecules anchor to the Z-disc and M-line of the sarcomere. Alterations in titin isoform expression and titin proteolysis contribute to contractile dysfunction in dilated cardiomyopathy, ischemic heart injury, and COVID mRNA vaccination [12].

There are differences in the composition of myosin heavy chains and in fiber arrangement between atrial and ventricular regions. The β isoform of myosin heavy chain (MHC- β) is predominant in the adult ventricles, while the α isoform (MHC- α) is predominant in the atria [8]. In addition, there is a difference in myosin light chain isoforms between the atria and ventricles [10]. All cardiomyocytes comprise the fibers of the cardiac muscle, which communicate through gap junctions. The adjacent cells are anchored together by desmosomes.

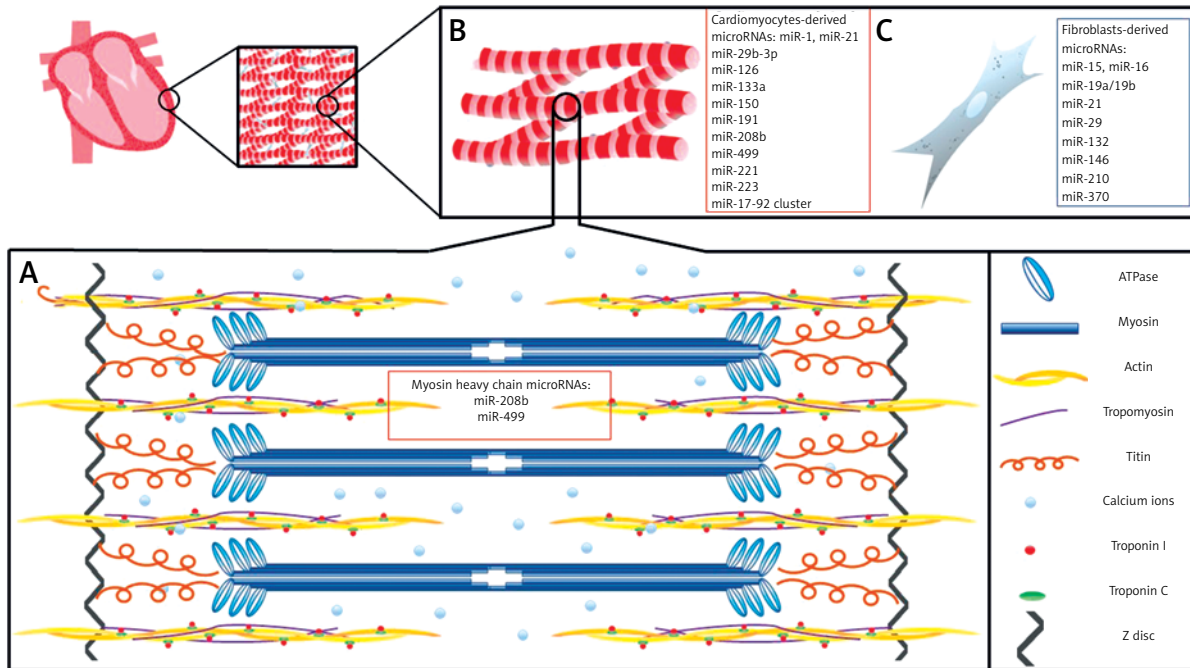


Figure 1. A basic schematic diagram of human left ventricular muscle structure and cardiac muscle-derived microRNAs. **A** – A sarcomere is composed of myofibrils, each containing myofilaments: the thick filaments are composed of myosin, while the thin filaments are composed of actin, tropomyosin, troponin C, and troponin I. Myosin contains two heads having ATPase activity. Calcium ions (Ca^{2+}) bind via troponin C. miR-208b and miR-499 regulate genes for myosin heavy chains. **B** – Cardiac muscle cells are striated, branched, involuntary, and usually contain a single nucleus. **C** – Fibroblasts provide support to cardiac muscle cells, as they are responsible for deposition of extracellular matrix in the heart

In the heart, cardiomyocytes co-exist with fibroblasts. Following cardiac injury, such as in myocardial infarction (MI), fibroblasts become activated and can transform into myofibroblasts, cells which exhibit the behavior of both a fibroblast (generating extracellular matrix) and a smooth muscle cell (ability to contract). Alternatively, they may proliferate as fibroblasts, contributing to scarring within the post-MI area.

The natural ability of cardiac muscle cells to regenerate is minimal. Studies have shown that approximately 1% of cardiac cells are renewed in young adults annually. However, this decreases to about 0.3–0.45% for individuals above 75 years of age [13]. Following cardiac injury there is an irreversible loss of cardiomyocytes and intense replacement of the cardiomyocyte space by excessive fibroblastic proliferation, which leads to scarring and post-MI acute and chronic complications.

Despite the progress which has been made in regenerative therapy in cardiology, such as stimulation of human pluripotent stem cell-derived cardiomyocytes or human embryonic stem cells, there is still room for further development of novel technologies in the prevention of adverse cardiac outcomes [10]. One possible direction is the employment of microRNAs that naturally regulate all physiological and pathophysiological processes in the heart [14].

MicroRNAs and heart development

MicroRNAs regulate all stages of embryonic cardiac development and heart functions throughout an individual's life [15]. This process is highly conserved in mammals [16]. The heart is the first organ which starts to develop, usually in the third week of gestation [16]. MicroRNAs appear during heart development at different stages and have diverse functions (Table I). During heart development, cardiomyocyte structure and proliferation are regulated by 2 critical microRNAs: miR-1 and miR-133 [17]. Their activity must be well balanced, as either over-expression or under-expression is associated with defects [18]. Studies suggest that miR-1 may regulate sarcomere formation in the mammalian heart [19]. A study involving a miR-133a double-knockout embryonic heart showed increased cardiomyocyte proliferation as well as apoptosis, disrupted sarcomere structure, and dysregulated expression of smooth muscle genes [20]. In the early stages of heart development, miR-128a stimulates the differentiation of cardiomyocyte progenitor cells into various subtypes of cardiomyocytes by modulating the differentiation of cardiac progenitor cell populations [21]. A cluster of miR-17-92 promotes cardiomyocyte proliferation in embryonic, postnatal, and adult

Table I. MicroRNAs involved in heart development (data from mouse studies, or human induced pluripotent stem cells) and their function

MicroRNAs involved in heart development	Subject of study	Activity/significance	Reference
miR-1	Mice	Promotes differentiation of embryonic stem cells into cardiomyocytes, regulates sarcomere formation in the mammalian heart, cardiomyocyte structure, proliferation, and cardiac conduction	[18, 19]
miR-133a	Mice	Inhibits differentiation of embryonic stem cells into cardiomyocytes, regulates sarcomere structure, cardiomyocyte proliferation and apoptosis, expression of smooth muscle genes	[20]
miR-128a	hiPSCs	Subtypes of cardiomyocytes, modulates differentiation of cardiac progenitor cell populations	[21]
A cluster of miR-17-92	Mice	Promotes cardiomyocyte proliferation in embryonic, postnatal, and adult hearts	[22, 23]
miR-15 miR-16	Mice	Decreases cardiomyocyte proliferation and induces apoptosis	[24]
miR-499	Mice	Production of myosin heavy chains, ventricular specification	[25, 26]
miR-208b	Mice	Production of myosin heavy chains	[27]
miR-145	Mice	Differentiation of multipotent cardiac neural crest stem cells into VSMCs	[15]
miR-21 miR-31 miR-103/107 miR-155 miR-200	Mice	Implicated in epicardial development	[15]
miR-218	Zebrafish	May control cardiac cell migration	[28]
miR-143/145	Mice	May be responsible for cell-cell contact	[29, 30]

hiPSCs – human induced pluripotent stem cells, VSMCs – vascular smooth muscle cells.

hearts, whereas members of the miR-15 family decrease cardiomyocyte proliferation and induce apoptosis [22–24]. Several microRNAs regulate the expression of cardiac myosin genes, including miR-208b (the most important) and miR-499 (dependent), which are involved in the regulation of genes responsible for producing myosin heavy chains [25]. Additionally, one study found that miR-499 was an important regulator of ventricular specification [26]. Expression of these microRNAs is also crucial in postnatal life; however, high expression levels of miR-208b do not lead to cardiomyocyte proliferation, but rather to cardiomyocyte hypertrophy, atrial fibrillation, and heart failure [27].

It is possible that miR-218 may control cardiac cell migration, whereas miR-143 (well-known for VSMC switching) may be responsible for cell-cell contact; however, this has not yet been confirmed in mammals [28]. In the early stages of murine cardiogenesis, miR-143 and miR-145 are highly expressed and are transcribed as a bicistronic cluster [29, 30]. MicroRNAs, such as miR-145, are involved in the differentiation of multipotent cardiac neural crest stem cells into VSMCs during heart organogenesis. Moreover, miR-145 is very important as it is capable of reprogramming adult fibroblasts into VSMCs [31]. MicroRNAs, including

miR-21, miR-31, miR-103/107, miR-155, and the miR-200 family, have been implicated in epicardial development [15].

All embryonic microRNAs act through their target genes, resulting in synthesis of proteins [32–70]. The microRNAs which appear to play a pivotal role in cardiac development include miR-1 (by promoting myogenesis and MHC differentiation) and miR-133 (by promoting mesoderm formation at the early stages of embryological development) (Table II) [32–35]. The expression of miR-1 and miR-133 in the embryonic heart is regulated by transcription factors SRF, MEF2, myogenic regulatory factor (MRF), and MyoD [34, 35]. In addition, miR-208b together with miR-499 drives muscle cell specification and slows MHC upregulation through targeting β -MHC and Sox6 genes [36–39].

During embryogenesis, the developmental potential of cells is gradually restricted as they become modified toward a specific function. As cell development continues, its function is determined by DNA methylation and chromatin remodeling, which is mostly regulated by transcription factors [40–44]. Cardiomyocytes being derived from pre-cardiac mesoderm are modulated by transcription factor MESP1 as well as other dependent factors, including GATA4, MEF2C, SRF, or ISL1 [40–44]. Different microRNAs target their specific

Table II. Target genes associated with cardiomyocyte-derived microRNAs

microRNA	Target gene	Activity/significance	Reference
miR-1	<i>HDAC4, MEF2C</i>	– Promote mesoderm formation and suppress non-muscle gene expression in ESC – Strongly enhance myogenesis by increase in expression of myogenin and MHCs, MyoD, MEF2 and skeletal α -actin	[19, 32]
miR-133a	<i>SRF</i>	– Promotes mesoderm formation and suppresses non-muscle gene expression in ESC – Enhances myoblast proliferation by repressing SRF	[20, 32, 34]
miR-128a	<i>Isl1, Sfrp5, Hcn4, Irx4</i>	– Postnatal heart growth by modulating cardiomyocyte proliferation during early cardiogenesis – Upregulating the transcription factors of cardiac progenitor cells – Marking ventricular progenitors	[21, 57, 58, 59]
cluster of miR-17-92	<i>PTEN, CDK1</i>	Regulation of cardiomyocyte proliferation in embryonic, postnatal, and adult hearts	[23, 60, 61]
miR-15 miR-16	<i>C/EBPβ, IGF1</i>	Protection/downregulation of cardiac hypertrophy	[54]
miR-208b	β -MHC, <i>Sox6, Purβ, Sp3, HP1β</i>	– Dominant role in the specification of muscle fiber identity by activating slow and repressing fast myofiber gene programs – Upregulation of slow MHCs in adult heart	[25] [37]
miR-499	<i>Sox6, cyclin D1</i>	Inhibition of cell proliferation and promotion of cell apoptosis in P19CL6 cells and cardiomyocytes	[38] [39]
miR-133b	<i>PTBP1, TAGLN2</i>	Alleviates apoptosis and cardiac fibrosis, modulates collagen deposition	[53]
miR-143/145	<i>HK2</i>	Addresses G6PD, PPP pathways and p62. Activation of these signaling pathways induces a reductive redox shift, resulting in cardiomyopathy	[55]
miR-155	<i>NEDD4</i>	Cardioprotective against cardiomyocyte apoptosis; modulates myofibroblast density	[56]
miR-218	<i>REST</i>	Regulates cardiac differentiation of embryonic stem cells by regulating the Wnt/ β -catenin signaling pathway and GATA4	[63]
miR-124	<i>STAT3</i>	– Downregulation of cardiac-specific markers-ANP, TnT, and α -MHC proteins; – Regulation of myogenic differentiation of BMSCs by targeting STAT3 mRNA	[64, 65]
miR-21	<i>Ajuba/Isl1</i>	Differentiation of BMSCs into cardiomyocyte-like cells; contributes to formation and maintenance of cell-cell connections and supports cell division and migration	[66]
miR-31	<i>CAMK2D, YWHAE</i>	Promotes loss of dystrophin and nNOS in human	[67]
miR-103/107	<i>PANKs, FADD</i>	Modulates mitochondrial concentration; regulates systemic glucose metabolism; H ₂ O ₂ -induced necrosis	[68, 69]
miR-200b	<i>GATA-4</i>	Transcription factor; regulation of developmental processes of the heart, cardiac myocyte proliferation, differentiation and survival	[70]

ANP – atrial natriuretic peptide, BMSCs – bone marrow-derived mesenchymal stem cells, CAMK2D – calcium/calmodulin dependent protein kinase II delta, CDK1 – cyclin dependent kinase 1, C/EBP β – CCAAT enhancer binding protein beta, ESCs – embryonic stem cells, FADD – Fas-associated via death domain, G6PD – glucose-6-phosphate dehydrogenase, GATA4 – GATA binding protein 4, Hcn4 – hyperpolarization activated cyclic nucleotide gated potassium channel 4, HDAC4 – histone deacetylase 4, HK 2 – hexokinase 2, HP1 β – heterochromatin protein 1 β , IGF1 – insulin-like growth factor-1, Irx4 – iroquois homeobox 4, MEF2 – myocyte-specific enhancer factor 2, MHC – myosin heavy chains, mRNA – messenger ribonucleic acid, MyoD – myoblast determination protein 1, NEDD4 – neural precursor cell expressed developmentally down-regulated protein 4, nNOS – neuronal nitric oxide synthase, PANKs – pantothenate kinases, PPP – pentose phosphate pathway, PTBP1 – polypyrimidine tract-binding protein 1, PTEN – phosphatase and tensin homolog, Pur β – purine rich element binding protein B, REST – RE1 silencing transcription factor, Sfrp5 – secreted frizzled related protein 5, Sox6 – SRY-box transcription factor 6, Sp3 – specificity protein 3, SRF – serum response factor, STAT3 – signal transducer and activator of transcription 3, TAGLN2 – transgelin 2, TnT – troponin T, YWHAE – tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon, Wnt – wingless/integrated.

genes, thus modifying the transcription factors and regulating cardiomyocyte development [40]. The molecular mechanisms regulating cardiomyocyte proliferation include core cell cycle proteins such as cyclins (cyclin D1, D2, B1), cyclin-dependent kinases (CDK1, CDK4), and nuclear proteins which regulate the cell cycle, such as Gata4, Meis1, and Hand2 [40–44]. In addition, signaling pathways including Hippo, Notch, and p38 kinase pathways are involved in cardiomyocyte proliferation [45–51]. Cardiomyocyte proliferation may also be stimulated by exogenous factors such as hypoxia or inhibited by thyroid hormones [52, 53].

MicroRNAs act through their target genes, and in this way are involved in the process of heart regeneration. After myocardial damage, the loss of cardiomyocytes is mostly irreversible, which results in scar formation and cardiac remodeling [53, 71, 72]. In this process, microRNAs can influence cardiac hypertrophy [54], especially by modulating glucose-6-phosphate dehydrogenase or pentose phosphate pathways, by stimulating cardiac cell apoptosis and subsequent fibrosis [53], or by modifying myofibroblast density [55, 56]. Tracing different cell lines and their potential to survive and regenerate after myocardial injury is the main issue of many current studies, as it can result in establishing genes or their target proteins as potential therapeutic points.

The fetal heart is an excellent model for the synchronized, often overlapping microRNA activity during development of the mature heart. To understand fibroblastic substitution following cardiomyocyte death in cardiac ischemia, it is important to elucidate the mechanisms involved in embryonic heart maturation. In regenerative cardiology, using microRNAs to stimulate fibrin production in cardiomyocytes, as well as keeping cardiomyocytes in situ (homing), is challenging. In addition, microRNA-based inhibition of excessive autophagy and apoptosis of cardiomyocytes should be well balanced. Finally, stimulation of fibroblasts into induced cardiomyocytes (iCMs) requires regulation of microRNAs.

Acute cardiac ischemia

Diagnostic microRNAs

Cardiomyocyte-derived microRNAs diagnostic for ACS

MicroRNAs contained in cardiomyocytes are released into the blood stream as a result of cardiac cell ischemia, leading to cell damage [73–76]. Among the cardiomyocyte-enriched microRNAs, miR-1, miR-21, miR-133a, miR-133b, miR-145, miR-208b, miR-223, and miR-499 are considered markers of myocardial damage and have been studied in the context of possible diagnostic bio-

markers for ACS [77–80]. These microRNAs are detectable in the serum, often before traditional markers of cardiac necrosis are detectable (Table III). Thus, microRNAs may be used to differentiate patients with stable angina from vulnerable patients. Some microRNAs might be better than others as they are characterized by their high diagnostic accuracy and rapid release preceding cardiac troponins (cTn) [76]. Studies showed that the diagnostic sensitivity and specificity of miR-499 was very high (between 98 and 100%) [77, 78]. In a study by Abdou *et al.*, diagnostic accuracy (AUC) for miR-499 was 0.90 (95% CI: 0.845–0.955) with 89% sensitivity and 83% specificity in STEMI patients [79]. Furthermore, 74% of patients had a significant increase in miR-499 expression levels compared to 65% positive cTn in the first 3 h of chest pain [79]. A meta-analysis of 14 observational studies showed that circulating miR-499 is a reliable biomarker for diagnosing acute myocardial infarction in patients [80]. Additionally, upregulation of miR-208a can be easily detected in 100% of ACS patients within 4 h of the onset of symptoms, whereas it remained undetectable in non-ACS patients [81]. A meta-analysis of 13 studies by Wang *et al.* involved a group of 1703 patients with ACS and a group of 1589 control patients [82]. They found that miR-208b is characterized by a high diagnostic accuracy for ACS (AUC = 0.93 (95% CI: 0.91–0.95), $p < 0.001$), with a pooled sensitivity of 83% and specificity of 97% [80]. All the microRNAs are placed in close proximity to the myosin and actin apparatus; thus, their high expression levels are associated with a greater loss of myofibrils, leading to an unfavorable reduction in left ventricular ejection fraction and post-MI remodeling [82–84]. An interesting issue is whether microRNAs can be considered potential biomarkers of acute coronary artery occlusion in an infarct-related artery (IRA). Occlusion of an IRA occurs both in STEMI and NSTEMI-ACSs [85]. A recent study by Gacón *et al.* showed promising results for miR-133b and miR-124, as they were associated with an increased risk of acute coronary artery thrombosis [85].

Prognostic microRNAs

MicroRNAs are prognostic for adverse cardiovascular events following ACS.

MicroRNAs associated with acute cardiac ischemia are often biomarkers of major cardiac and cerebral events following ACS [86–94]. Decreased miR-150 and increased serum levels of miR-1, miR-34a, miR-223, miR-146a, miR-197, and miR-208b are associated with increased cardiovascular mortality following ACS, major adverse cardiovascular events, heart failure (HF), and left ventricular remodeling [86–92] (Table III). In a study by Schulte

Table III. Cardiomyocyte-derived microRNAs diagnostic for acute coronary syndrome and prognostic for adverse cardiovascular events following acute coronary syndrome

microRNA	Period	Subject to study	Activity/significance	Ref.
Diagnostic for ACS	Study group(s)		MicroRNAs with the highest clinical diagnostic potential AUC (95% CI), <i>p</i> -value	
miR-208b miR-499	80 ACS patients and 50 healthy controls	Human serum	High diagnostic potential for ACS: miR-208b: 0.9996 (0.9983–1.001), <i>p</i> < 0.001 with a sensitivity of 98% and specificity of 100% miR-499: 0.9992 (0.9970–1.001), <i>p</i> < 0.001 with a sensitivity of 98% and specificity of 100% superior to cTn: 0.940 (0.8742–1.006), <i>p</i> < 0.001	[77]
miR-1 miR-133 miR-208 miR-499	145 NSTEMI patients and 30 healthy controls	Human serum	High diagnostic potential for NSTEMI-ACS: miR-1: 0.7731 (0.6865–0.8597), <i>p</i> < 0.05 miR-133: 0.928 (0.8857–0.9704), <i>p</i> < 0.05 miR-208: 0.994 (0.9869–1.001), <i>p</i> < 0.05 miR-499: 0.9945 (0.9863–1.003), <i>p</i> < 0.05 compared to cTn: 0.778, <i>p</i> < 0.05	[78]
miR-499	A meta-analysis of 14 studies including 1989 patients with ACS and 1732 control patients	Human serum	High diagnostic potential for ACS: miR-499: 0.98 (0.96–0.99), <i>p</i> < 0.001 with a pooled sensitivity of 84% and specificity of 97%	[80]
miR-208b	Meta-analysis of 13 studies including 1703 patients with ACS and 1589 control patients	Human serum	High diagnostic potential for ACS: miR-208b: 0.93 (0.91–0.95), <i>p</i> < 0.001 with a pooled sensitivity of 83% and specificity of 97%	[82]
miR-133b miR-124	27 patients with an occluded infarct-related coronary artery ACS vs. 16 patients with patent artery	Human serum	Good diagnostic potential for infarct-related coronary artery occlusion: miR-133b: 0.704 (0.547–0.860), <i>p</i> = 0.006 with a sensitivity of 63% and specificity of 81.3% miR-124: 0.787 (0.649–0.924), <i>p</i> < 0.001 with a sensitivity of 59.3% and specificity of 87.5%	[85]
Prognostic for MACCE after ACS				
miR-197 miR-223	340 patients with ACS	Human serum	Prognostic for CVD after ACS at 4 years miR-197: 2.24 (1.25–4.01), <i>p</i> = 0.006 miR-223: 4.94 (1.42–17.2), <i>p</i> = 0.012	[86]
miR-208b	21 patients with ACS	Human serum	Prognostic for CVD at 6 months miR-208b: 5.08 (1.13–22.82) <i>p</i> = 0.03	[87]
miR-223-3p	598 ACS patients randomized to ticagrelor vs. prasugrel treatment	Human plasma	miR-223 levels were prognostic for CVD/re-MI/IS at 30 days: 15.74 (2.07–119.9), <i>p</i> = 0.008 and at 1 year: 3.18 (1.40–7.19), <i>p</i> = 0.006	[88]

Table III. Cont.

microRNA	Period	Subject to study	Activity/significance	Ref.
miR-1-3p	142 patients with ACS or stroke	Human serum	Prognostic for CVD at 6 years miR-1-3p: 2.73 (1.22–6.12), $p = 0.014$	[89]
miR-34a miR-208b	359 patients with ACS	Human serum	Prognostic for CVD and left ventricular remodeling (LVEDV increase > 10%) at 6 months. miR-34a: 17.91 (2.07–98.81), $p = 0.003$ miR-208b: 4.18 (1.36–12.83), $p = 0.012$	[90]
miR-150	113 patients with ACS	Human serum	Prognostic for left ventricular remodeling. (LVEDV increase > 10%) at 1 year miR-150: 1.233 (1.125–1.352)	[91]
Responsive to antiplatelet therapy				
miR-223-3p	62 patients with NSTEMI	Human serum	Decreased circulating miR-223 is a marker of lower response to clopidogrel in NSTEMI. 0.111 (0.018–0.692), $p = 0.019$	[99]
miR-223-3p	21 male patients with NSTEMI taking clopidogrel ($n = 11$) or prasugrel/ticagrelor ($n = 10$) in addition to aspirin	Plasma serum	Plasma miR-223 was elevated with decreasing platelet reactivity	[101]
miR-21 miR-221 miR-223	272 ACS patients, including 21 extremely high responders and 18 extremely low responders	Human serum	miR-223, miR-221, and miR-21 are independently associated with clopidogrel antiplatelet responsiveness in ACS patients. However, the association could be influenced by the interaction with CYP2C19*2 genotype	[102]
Preventive of in-stent restenosis				
miR-22	Self-healable spongy coating stent with miR-22	Minipigs	PCEC@miR-22 coated stents showed reduced inflammation, low switching of VSMC phenotype, and low secretion of extracellular matrix, which significantly inhibited in-stent restenosis	[107]
miR-21	Animal model: suppression of vascular miR-21 correlated dose dependently with reduced luminal obliteration. Furthermore, anti-21 did not impede re-endothelialization	Rats	Compared with bare-metal stents, anti-21-coated stents effectively reduced in-stent restenosis. However, systemic anti-miR-21 had substantial off-target effects, lowering miR-21 expression in liver, heart, lung, and kidney with a concomitant increase in serum creatinine levels	[108]
miR-30b-5p	Detection of restenosis risk	Human serum	Downregulation of miR-30b-5p was predictive for coronary in-stent restenosis	[109]

ACEi/ARB – angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, ACS – acute coronary syndrome, AUC – area under the curve, CI – confidence interval, CVD – cardiovascular death, cTn – cardiac troponins, hiPSCs – human induced pluripotent stem cells, IS – ischemic stroke, LVEDV – left ventricular end diastolic volume, MACCE – major adverse cardiac and cerebral events, MI – myocardial infarction, MRA – mineralocorticoid receptor antagonists, VSMCs – vascular smooth muscle cells.

et al. which included 340 ACS patients, levels of miR-197 and miR-223 were predictive for cardiovascular death (CVD) [86]. In a study by Hromadka *et al.*, adding miR-223-3p into the model for calculating ischemic risk following ACS significantly increased the predictive accuracy for CVD (OR = 10.8, 95% CI: 1.37–85.07, $p = 0.024$), as well as the combined ischemic endpoint (CVD/re-MI/IS) within 30 days and 1 year [88]. In a study by Lin *et al.*, only the level of miR-150 differed between patients who developed HF (HR = 1.233; 95% CI: 1.125–1.352) from those who did not at a 1-year follow-up [90]. Some researchers have suggested that inflammation-related microRNAs such as miR-145, miR-146a, and miR-342 can serve as biomarkers of adverse prognosis following ACS [91, 92].

microRNAs modifying platelets' activity

Platelet response to dual antiplatelet treatment

A variety of therapeutic strategies were employed to diminish the risk of adverse cardiac events following ACS [95, 96]. The most important strategy includes urgent coronary percutaneous intervention (PCI) with restoration of IRA patency, preferably with provisional stenting [95, 96]. Undergoing this procedure in a timely manner limits the post-MI infarct size area, scarring, and incidence of malignant arrhythmia and episodes of HF.

Since PCI with stent implantation requires the usage of dual antiplatelet treatment (DAPT) for a prolonged period of time, it is associated with an increased risk of bleeding, particularly in elderly patients who also use anticoagulants for atrial arrhythmia [97]. MicroRNAs associated with platelet aggregation could be used as biomarkers of platelet response to DAPT treatment (Table III) [98–100]. Due to the limited value of platelet reactivity tests, this may offer a practical approach [75, 98]. MicroRNA-driven dosages of antiplatelet agents could enable personalization of antiplatelet treatment to prevent episodes of excessive bleeding or stent thrombosis [98–100]. High platelet reactivity during treatment is associated with decreased expression levels of circulating miR-233 [99]. In contrast, increased expression levels of miR-223 are associated with increased DAPT responsiveness [101]. In line with these observations, the expression of miR-21, miR-221, and miR-223 in platelets from ACS patients appeared to be decreased in non-responders who were receiving aspirin and clopidogrel [102]. Because results are not entirely consistent throughout the studies, investigations on the expression levels of microRNAs (such as miR-223 and miR-126) and properties of platelet coagulability are still ongoing [102].

MicroRNAs in the prevention of in-stent restenosis

The main drawback of stent implantation is stent restenosis [103, 104]. Data indicate that this complication could be overcome using coated stents with microRNA agonists (miR-22) or antagonists (anti-miR-21) (Table III) [105–108]. A study by Gutierrez-Carretero *et al.* found that low plasma levels of miR-30b-5p may have diagnostic potential for determining the risk of in-stent restenosis in coronary arteries [109].

Therapeutic approach based on microRNAs and their ability to modify target genes

Challenges in microRNA delivery

MicroRNAs target sites in mRNA mostly lie within the 3'-UTR and less frequently in 5'-UTRs or coding regions [1]. Natural microRNAs are stable and resistant to enzymatic lysis because they are hidden inside microvesicles and microparticles; therefore they are protected from quick enzymatic lysis [110, 111]. In contrast, exogenous microRNAs must obtain properties that would make them resistant to rapid lysis and increase their cellular adhesion, so they could exert their therapeutic actions. Clinical studies on micro-RNA-based therapies are feasible because novel technologies of microRNA delivery and encapsulation are developing. To decrease the expression of a specific microRNA, specific antisense oligonucleotides (ASOs), small interfering RNA (siRNA), and miRNA sponges are utilized [110–113]. ASOs (chemically modified) are preferred over natural oligonucleotides to reduce nuclease sensitivity and rapid renal clearance, but also to optimize delivery [110–112]. Genetic knockout and synthetic microRNA mimics or pre-miRNA in viral vehicles are used to enhance a specific microRNA level. Encapsulation includes adenovirus, lentivirus, AAV, microspheres, lipid nanoparticles, and liposomes [110–113].

ASOs for anti-miRs (antagomirs) are delivered as a single strand and do not seem to become part of the RISC, whereas mimics are usually applied as double strands to make them a substrate for Dicer and promote integration of one strand into the RISC. Agomir and antagomir are more stable in the blood stream, having a lasting effect between 1 and 6 weeks.

Also, various routes of microRNAs administration are used: subcutaneous injection, intravascular delivery, direct injection to the coronary circulation or into the heart, and devices coated with microRNAs. E.g. microRNA mimics of 4 cardiomyocyte-derived microRNAs are injected directly into the heart using nanocomplexes of branched polyethyleneimine-coated nitrogen-enriched carbon dots [114].

In the near future, further progress regarding the biomolecule mediated delivery of microRNAs is likely to be achieved by the development of aptamer conjugates [113]. Aptamers are single-stranded nucleic acids with high-affinity ligands of cellular surface receptors, facilitating their intracellular uptake by receptor-mediated transport [113]. They may be easily produced by standard *in vitro* synthesis techniques and able to be coupled to corresponding microRNA therapeutics by simple sticky-end annealing. Aptamer-conjugated microRNA agents, such as the GL21.T-miR-34c conjugate, are currently being tested in preclinical studies for selective targeting of tumor cells, including lung cancer cells.

MicroRNA-based strategies to prevent cardiac remodeling after ACS

Inhibition of extensive fibroblast proliferation resulting in scar formation

Extensive fibroblast proliferation after ACS leads to excessive left ventricle wall scarring that aggravates post-MI cardiac remodeling. microRNAs involved in the pathogenesis of post-MI left ventricular remodeling and HF are mostly fibroblast-derived [115]. Cardiac fibrosis is an interplay between pro-fibrotic microRNAs (miR-9, -15, -21, -26a, -34a, -92, -125b, 132, -155, 199b, -208a, -223, -433, and let-7c) and anti-fibrotic microRNAs (miR-7a/b, -19a, -19b, -22, -24, -29a, -29b, -30a-5p, -99, -101, -133a, -144, 146, -150, -210, -214, -370, and miR-384) [115]. Currently, microRNA-based strategies to prevent adverse outcomes following ACS are being investigated, focusing mainly on the inhibition of pro-fibrotic microRNAs. microRNA antagonists, at least in theory, should lead to the salvage of cardiac function through decreased fibroblast proliferation

Ongoing and terminated studies involving microRNA-based treatment in ACS patients

Before a micro-RNA-based treatment can be commenced, a microRNA has to come a long way, from bench genetic studies, cell cultures, animal models (mouse, pig, monkey) into first-in-human studies [110–113]. In the cardiovascular setting, a good example of a microRNA that makes a fast track career is miR-132-3p. Antagomir against miR-132-3p (known as CDR132L) prevented post-MI cardiac remodeling, which was confirmed in mouse models of HF, and later confirmed in pig models with ACS [110, 116, 117]. A phase I clinical trial in humans revealed good tolerability and evidence of a therapeutic benefit, such as preserved cardiac function and reversed cardiac remodeling, in HF patients (Table IV) [118]. The antagomir against miR-132-3p entered a phase II clinical

study in June 2022 (<https://clinicaltrials.gov/ct2/show/NCT05350969>) [110]. The antagomir against miR-132-3p is among a few microRNAs showing promise for their clinical utility. The other promising microRNA in cardiac ischemia and post-MI remodeling, potentially therapeutic, is antagomir-92a, which was found to reduce infarct size in mouse and pig models, and enhanced blood vessel growth and functional recovery of damaged tissues [116, 119, 120]. In a model of reperfused MI, a single dose of anti-miR-92a prevented cardiac remodeling without adverse effects in a porcine model [120].

In line with this observation, preclinical data encourage the use of miR-590-3p and miRNA-199a-3p, which regulate calcium signaling through HOMER1 [121]. They possess pro-proliferative effects in cardiomyocytes through DNA synthesis and increased cytokinesis. They are also involved in reducing fibrotic scar size and can improve cardiac function following MI in neonatal mice [121]. In addition, the miR-302-367 cluster can induce and maintain pluripotency, leading to an increase in cardiomyocyte mass, decreased myocardial fibrosis, and improved function in a failing myocardium [122]. In the preclinical stage, there are studies on the use of miR-29 mimics for the treatment of cardiac fibrosis, and studies on inhibitors of miR-15 [111]. Also, anti-miR-212 and anti-miR-652 demonstrated a good anti-fibrotic effect [116].

However, not all candidate microRNAs for therapy were so successful (Table IV) [123–146]. On contrary, there are more microRNAs that failed than those that succeeded. Clinical studies with anti-miR-103/107-3p (AZD4076), miR-34a mimics, and anti-miR-155-5p (cobomarsen) were terminated or halted by the sponsor for strategic reasons (severe immune-related side-effects) [110, 111, 123–125], although in preclinical studies they could be considered for therapy in cardiovascular disease [124, 125].

Confusing data regard studies with miR-21 antagonists and miR-21 mimics [126]. miR-21 function depends on its source, as it derives from diverse cell types, including ECs, platelets, VSMCs, cardiomyocytes, and fibroblasts. miR-21 participates in infarction injuries, cardiac remodeling, atherosclerosis, arrhythmias, and cardiomyopathy [126]. However, increased miR-21 expression caused by MI protected cardiomyocytes from apoptosis (positive action), along with enhancing the activation of cardiac fibroblasts (negative action) [126]. Furthermore, miR-21-targeted PPAR α resulted in increased inflammation owing to the ECs in the heart. Eventually, miR-21 therapy showed many off-target effects in the liver, lung, and kidney with a concomitant increase in serum creatinine levels [126].

Table IV. Review of candidate microRNAs tested as potential therapeutics in cardiac ischemia animal studies, phase I and phase II clinical trials in humans

microRNA	Role, rationale for use from previous research data	Studies in human	Randomized-controlled trial	Stage of the studies (the highest available)	Route of administration	Study design	Positive/negative/limitations	Ref.
miR-132-3p	Inhibition of miR-132 prevents cardiac fibrosis, and cardiac remodeling	Yes	Yes ASO antagomir against miR-132-3p (CDR132L)	Phase Ib (NCT04045405), 28 patients, completed	i.v. (0.32, 1, 3, and 10 mg/kg)	Chronic HF (LVEF \geq 30% and $<$ 50% or NT-proBNP $>$ 125 ng/l at screening	Completed. CDR132L was safe and well tolerated, confirmed linear plasma pharmacokinetics with no signs of accumulation, and suggested cardiac functional improvements	[118]
				HF-REVERT Phase II (NCT05350969), ongoing since June 2022	three i.v. infusions 28 days apart, 5 mg/kg, vs. 10 mg/kg, vs placebo	280 patients with HF and reduced LVEF after MI. Designed to evaluate the safety and efficacy of CDR132L	Ongoing (recruiting patients)	[110]
miR-103/107	Inhibition of miR-103/107 leads to improved glucose homeostasis and insulin sensitivity Inhibition of miR-103/107 reduces infarct size and prevents cardiomyocyte necrosis	Yes No	Yes miR-103/107-3p inhibitor (AZD4076 vs. placebo) No	Phase I (NCT02612662) Phase I/IIa (NCT02826525) 14 participants (early termination) ASO antagomiR-103-107 Healthy adult mice	s.c. 2 ml vs. placebo s.c.	Healthy male subjects with NASH Patients with diabetes type 2 and NAFLD Chronic treatment with antagomiR-103 and -107	Active, not recruiting (enrolled 40 participants) Immune-related side effects (halted for strategic reasons), off-target actions AntagomiR-103 and -107 decreased LVEF, cardiomyocyte size, and mitochondrial oxidative capacity in the absence of pathological stimuli. These data raise concern about the possible cardiac implications of the systemic use of antagomiR-103 and -107 in the clinical setting	[123] [124] [125]

Table IV. Cont.

microRNA	Role, rationale for use from previous research data	Studies in human	Randomized-controlled trial	Stage of the studies (the highest available)	Route of administration	Study design	Positive/negative/limitations	Ref.
miR-15-5p	Inhibition of miR-15b prevents cardiac remodeling	No	No LNA-modified anti-miR-15	Preclinical stage: mice, pig models	iv.	Mice and pig after ischemia-reperfusion surgery	Anti-miR-15 in mice reduces infarct size and cardiac remodeling and enhances cardiac function in response to MI	[127]
miR-16-5p	Loss of miR-15 and miR-16 is associated with unsuppressed growth in preclinical models of malignant pleural mesothelioma	Yes	Yes a double-stranded miR-16 mimic (TargomiRs)	Phase I (NCT02369198) completed	iv.	Malignant pleural mesothelioma Non-small cell lung cancer	Acceptable safety profile and early signs of activity of TargomiRs in patients with malignant pleural mesothelioma	[128]
	Suppression of miR-16 protects rat hearts from ischemic injury	Yes	No	Case-control study (12 healthy controls and 12 ICM patients)	Plasma samples	Plasma samples, AC16 Cell culture and transfection with miR-16 inhibitor	Inhibition of miR-16-5p has potential as a therapeutic approach to protect the heart against oxidative stress-induced injury	[129]
miR-17-5p	Inhibition of miR-17	Yes	Yes ASO anti-miR-17 (RGLS4326)	Phase Ib (NCT04536688) 19 participants (early termination)	s.c.	Autosomal dominant polycystic kidney disease	An interim analysis showed lack of efficacy	[130]
	Inhibition of miR-17 prevents cardiomyocyte apoptosis, reduces infarct area	No	No (Ad-Antagomir-17)	Rat study	Intramyocardial injection	MI model rats	MIR-17-5p silencing decreases the rate of apoptosis and repairs vascular injury	[131]

Table IV. Cont.

microRNA	Role, rationale for use from previous research data	Studies in human	Randomized-controlled trial	Stage of the studies (the highest available)	Route of administration	Study design	Positive/negative/limitations	Ref.
miR-21-5p	Inhibition of miR-21 prevents Alport nephropathy progression	Yes	Yes, ASO anti-miR-21 (Iademirsen)	HERA study Phase II (NCT02855268) 43 patients (closed)	s.c. once a week vs placebo	Alport syndrome	Interim analysis showed no meaningful improvement in kidney function compared with placebo	[132]
	Inhibition of miR-21 retains cardiac function	No	No LNA-antimiR-21	Pig study	Intracoronary		In a pig model of HF reduced cardiac fibrosis, hypertrophy and improved cardiac function	[133]
	Injection of miR-21-enriched EVs restores cardiac function	No	No	Mouse study	i.v. miR-21-enriched derived from Hek293T cells		Restores cardiac function in mice after MI	[134] [135]
miR-22-3p	Inhibition of miR-22-3p improves cardiac function	No	No	Rat study	Intra-myocardial injection;	KDM3A gene knockout rats;	miR-22-3p depletion activates cardiac autophagy, prevents post-MI remodeling, and reduces the infarct area.	[136]
				Mouse study	miR-22 loss-of-function genetic model in the mouse		Loss of miR-22 sensitized mice to the development of dilated cardiomyopathy	[137]
miR-25-3p	Inhibition of miR-25 ameliorates cardiac function	No	No	Mouse study	i.v.	Mouse heart failure model	Anti-miR-25 enhances cardiac contractility and function through SERCA2a restoration	[138]
miR-34a-5p	miR-34a is a naturally occurring tumor suppressor that is underexpressed in a broad range of tumor types	Yes	A liposomal double-stranded miR-34a mimic (MRX34)	Phase I (NCT01829971) 85 patients (terminated)	iv., different doses (70/90/110mg/m2) and various schemes	Adult patients with various solid tumors refractory to standard treatments	Terminated due to severe immune-mediated toxicities, resulting in four deaths related to miR-34a injection	[124]
	miR-34a deficiency improves survival of cardiomyocytes and VSMCs, revascularization, and prevents vascular calcification	No	No	Rat study	i.v.	Evaluation of miR-34a-5p knockdown on cardiac apoptosis and I/R injury after MI	The delivery of anti-miR-34a molecules is effective to treat vascular disease, but considering the tumor suppressor activity of miR-34a, systemic delivery could promote tumorigenesis	[125]

Table IV. Cont.

microRNA	Role, rationale for use from previous research data	Studies in human	Randomized-controlled trial	Stage of the studies (the highest available)	Route of administration	Study design	Positive/negative/limitations	Ref.
miR-92a	AntagomiR-92a reduces infarct size	No	No	Mouse, pig models	intracardiac	Model of acute MI	Reduced cardiac ischemia and post-MI remodeling, enhanced blood vessel growth and functional recovery of damaged tissues	[119, 120]
miR-126-5p	The 5p strand sustains endothelial integrity in the context of high shear stress and autophagy	Yes	No	Peripheral blood cells co-transfected with miR-126-5p mimic	Peripheral blood samples	30 patients with atherosclerosis	Prevented atherosclerosis, suppressed proliferation and migration of ox-LDL-VSMCs through upregulating miR-126-5p	[139] [140]
miR-148a-3p	Elevation of miR-148a preserves cardiac function	Yes	No	Mouse study	i.v.	Heart failure TAC surgery mouse model	Elevation of miR-148a expression protects the myocardium from dilation and cardiac dysfunction	[141]
miR-199-5p	Inhibition of miR-199b prevents cardiac remodeling	No	No	Mouse study	Cell culture of ECs of the heart	STAT3-KO spontaneous heart failure mouse model	Protective effects of downregulated miR-199a-5p on ischemic and hypoxic cardiomyocytes	[142]
miR-208a-3p	AntimiR prevents cardiac remodeling, preserves cardiac function	No	No	Rat and pig study	s.c.	Rat MI model and porcine ischemia-reperfusion model	Level of stress influences the level of target depression after anti-miR-208a treatment	[143]
miR-214-3p	miR-214-3p is an important repressor for fibroblast proliferation and fibroblast-to-myofibroblast transition	yes	No	Mouse study	i.v.	TAC-induced murine model	miR-214 deficiency impairs cardiomyocyte survival, increases cardiac fibrosis	[144]
miR-221/222-3p	miR-222 elevation protects from cardiac remodeling	No	No	Rat study	Intra-carotid infusion	Carotid injury rat model	Re-endothelialization was increased by miR-221/222 inhibition	[145] [146]

ASO – antisense oligonucleotide, EVs – extracellular vesicles, ICM – ischemic cardiomyopathy, i.v. – intravenously, LNA – locked nucleic acid modified, LVEF – left ventricular ejection fraction, NAFLD – non-alcoholic fatty liver disease, NASH – non-alcoholic steatohepatitis, NT-proBNP – amino terminal fragment of pro-brain natriuretic peptide.

Regenerative cardiology – reprogramming heart cells. Prevention of cardiomyocytes loss

During cardiac injury (e.g., resulting from MI), large numbers of cardiomyocytes are lost due to apoptosis, autophagy, or necrosis [14, 147–152]. The accompanying recruitment of monocytes results in the removal of any damaged/necrotic cardiomyocytes. This process results in cardiac muscle reduction, leading to HF, which is associated with malignant arrhythmia and increased mortality risk.

One approach is to inhibit the extensive apoptosis of cardiomyocytes, which can be accomplished via decreasing the expression of pro-apoptotic microRNAs (Table V). Among these, anti-miR-195 was shown to regulate anti-apoptotic genes; however, it is unclear whether miR-195 antagonists can reverse cell senescence or the apoptotic rate

[153]. miR-199a was shown to directly target *Clic5*, which is involved in the proliferation of adult cardiomyocytes [143, 150, 151]. Although miR-199a has been reported to improve cardiac function, its prolonged administration led to lethal cardiac arrest [150, 151]. Finally, miR-125b overexpression efficiently attenuated cardiac function injury of HF mice by targeting BAK1 through inhibiting cardiomyocyte apoptosis, suggesting that the miR-125b/BAK1 axis might be a potential target for the diagnosis or treatment of HF [152].

Another approach is the reprogramming of fibroblasts into induced cardiac progenitor cells (iCPCs) to obtain de novo cardiac lineages [108, 154–160]. Cardiomyocyte-derived microRNAs such as miR-1 (through the target gene *Bcl2*), miR-133a (many target genes), miR-208a (*APC*), miR-223 (*PARP-1*), miR-499, and miR-145 (*FRS2*) may play important roles in these processes [115, 150].

Table V. MicroRNA-based therapies in preclinical studies used for cardiomyocytes protection and proliferation

microRNA	Studied therapeutic agent	Subject to study	Activity/significance/side-effects	Reference
miR-195	Anti-miR-195 regulates anti-apoptotic genes, can decrease apoptosis after ACS	<i>In vitro</i> cultured myoblast	miR-195 inhibition did not affect cell ageing or rejuvenation of human skeletal muscle-derived stem/progenitor cells (SkMDS/PCs). It is unclear whether miR-195 antagonists can reverse cell senescence or the apoptotic rate	[153]
miR-199a	miR-199a was shown to directly target <i>Clic5</i> , which is involved in the proliferation of adult cardiomyocytes.	Mice	Although miR-199a has been reported to improve cardiac function, its prolonged administration led to lethal cardiac arrest	[143, 150]
miR-125b	miR-125b agonists	Mice	miR-125b overexpression efficiently attenuated cardiac function injury of heart failure mice by targeting BAK1 through inhibiting cardiomyocyte apoptosis, suggesting that the miR-125b/BAK1 axis might be a potential target for the diagnosis or treatment of HF	[152]
A cocktail of 4 microRNAs mimics (miR combo) miR-1, miR-133, miR-208, and miR-499	May be efficient in the induction of transformation of fibroblasts into induced cardiomyocytes (iCMs)	Adult human cardiac fibroblasts	The percentage of cTn-positive cells (former fibroblasts) 15 days after miR combo transfection was ~11%, as evaluated by flow cytometry. A potential new strategy for myocardial regeneration after ACS	[114]
A cocktail of 4 microRNAs mimics (miR combo) miR-1, miR-133, miR-208, and miR-499	miR combo effectively reprograms fibroblasts of any mammalian species into cardiomyocytes	Cardiac fibroblasts of pig, dog and fetal human cardiac fibroblasts	miR combo reprograms pig, dog, and fetal human cardiac fibroblasts into cardiomyocyte-like cells that induce the expression of sarcomere and cardiac ion channels. In a fetal human cardiac fibroblast model ~10% of cells had cardiomyocyte-like properties 14 days after transfection. This study validates the miR combo as a potential therapeutic modality for myocardial regeneration following ACS	[158]

A cocktail of 4 microRNAs called the miR combo (including mimics of miR-1, miR-133, miR-208, and miR-499) is proposed to be efficient in the induction of fibroblast transformation into induced cardiomyocytes (iCMs) (Table V) [154]. The researchers have also developed a delivery system to introduce the miR combo directly into the heart [155]. Yang *et al.* delivered a cocktail of 4 cardiomyocyte-derived microRNA mimics using nano-complexes of branched polyethyleneimine-coated nitrogen-enriched carbon dots, which were then injected into the heart [156]. These nanocomplexes led to the efficient direct reprogramming of fibroblasts into iCMs without genomic integration and resulted in effective recovery of cardiac function following MI [156, 157]. MicroRNAs have been used to enhance direct reprogramming alone or in combination with transcription factors. Studies by Jayawardena *et al.* and Jiang *et al.* reported that a transient non-viral transfection system of miR-combo including miR-1, miR-133, miR-208, and miR-499a was able to reprogram mouse cardiac fibroblasts into iCMs *in vitro* and *in vivo* [157]. Recently, Baksh *et al.* demonstrated that miR combo reprograms pig, dog, and fetal human cardiac fibroblasts into cardiomyocyte-like cells that induce the expression of sarcomere and cardiac ion channels [158]. In a fetal human cardiac fibroblast model, approximately 10% of cells had cardiomyocyte-like properties 14 days after transfection. This study is important as it validates the miR combo as a potential therapeutic modality for myocardial regeneration following ACS [158].

Combining microRNA-based therapies with other emerging therapies, such as cardiac stem cell or gene therapies.

Treating cardiovascular diseases with cardiac stem cells is a valid treatment. Cardiac stem cells can reproduce the myocardial cells [66, 161, 162]. Studies have shown that cardiac stem cell proliferation and differentiation are regulated by microRNAs [161, 162]. How microRNAs regulate cardiac stem cell behavior is an interesting area of research that can help us study and control the function of these cells *in vitro* – an achievement that may be beneficial for patients with cardiovascular diseases [161, 162].

Limitations of microRNA use.

It is important to understand the limitations of microRNAs. Many microRNAs can be used as diagnostic and prognostic markers without causing harm. However, even though microRNA mimics and antagonists are already being investigated in animal models, or even in human, their main limitations concern their therapeutic safety, sta-

bility in circulation, toxicity, unwanted off-target actions, and lack of therapeutic actions in humans that were previously described in cultures or animal models [110, 111]. Important issues to understand are their potential toxicity, off-target actions, and irreversible inhibition of mRNA. Some may act as oncogenes or exert dual/multiple roles, including opposite actions. Because of this, delivery systems were developed to administer microRNAs locally (e.g., directly into the coronary circulation or cardiac muscle) [110, 111]. To prevent their quick degradation in the blood stream, synthetic oligonucleotides that are more resistant to enzymatic lysis were produced.

Future directions and conclusions

Two approaches for the management of atherosclerosis can be considered for testing in the clinical setting. The first one, presented in this review, is associated with a reduction in atherosclerosis-related post-ischemic complications [108–110]. This approach attempts to inhibit fibroblast proliferation and scarring, harmful apoptosis, autophagy or necrosis of cardiomyocytes, and involves the reprogramming of fibroblasts into iCMs, which may offer better post-ACS recovery and a decreased rate of post-MI adverse events.

The second approach involves early intervention at the level of atherosclerotic growth initiation [4, 5, 74]. The optimal point for early intervention would be during the identification of intima-media complex thickening or the presence of early plaques [127–129]. Increasing thickness of the carotid intima-media complex and carotid plaques is related to the extent and severity of atherosclerosis, in addition to polyvascular atherosclerosis [163–171]. Also, the role of microRNAs in cardiovascular risk factor modification seems extremely important. microRNAs are highly expressed in patients with obesity, metabolic syndrome and familial hyperlipidemia [172, 173]. Particularly miR-150 showed associations with proprotein convertase subtilisin/kexin type 9 (PCSK9) [171]. PCSK9 regulates extracellular vesicle-derived microRNAs, especially those involved in inflammation and expression of the low-density lipoprotein receptor (LDLR) receptor. Studies on mice demonstrated a downregulation effect of immunotherapy with the PCSK9 peptide vaccine on the hepatic expression levels of miR-27a [174]. miR-27a has been shown to improve LDLR degradation by directly binding to its 3'-untranslated region (UTR) and indirectly by enhancing PCSK9 [174]. Therefore, respective microRNAs might be significantly regulated by statin therapy [175–177]. As mentioned before, also treatment with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, GLP-1, PCSK9 inhibitors,

and antiplatelets modulates expression levels of corresponding microRNAs [175–177].

Cellular and molecular biology is a rapidly growing field of research [178, 179]. Soon, it may provide novel solutions for personalized treatment, integrating knowledge from basic cardiological science to preclinical studies, and eventually to clinical practice. The clinical approaches are worthy of continued research and development. In both treatment strategies, microRNAs may play a critical role in the regeneration of the heart, as well as in the initiation and progression of atherosclerosis. While initial preclinical data are promising, there is still much work to be done in this field.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Yates LA, Norbury CJ, Gilbert RJ. The long and short of microRNA. *Cell* 2013; 153: 516-9.
2. Creemers EE, Tijssen AJ, Pinto YM. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? *Circ Res* 2012; 110: 483-95.
3. Ren J, Zhang J, Xu N, et al.. Signature of circulating microRNAs as potential biomarkers in vulnerable coronary artery disease. *Plos One* 2013; 8: e80738.
4. Sayed D, Abdellatif M. MicroRNAs in development and disease. *Physiol Rev* 2011; 91: 827-87.
5. Badacz R, Przewłocki T, Legutko J, Żmudka K, Kablak-Ziembicka A. microRNAs associated with carotid plaque development and vulnerability: the clinician's perspective. *Int J Mol Sci* 2022; 23: 15645.
6. Nie P, Yang F, Wan F, Jin S, Pu J. Analysis of microRNAs associated with carotid atherosclerotic plaque rupture with thrombosis. *Front Genet* 2021; 12: 599350.
7. Tsipis A. Introductory chapter: cardiomyocyte - fundamental unit of heart life and disease. In: *Visions of Cardiomyocyte – Fundamental Concepts of Heart Life and Disease*. 1st ed. IntechOpen: doi: 10.5772/intechopen.90103.
8. Reiser PJ, Portman MA, Ning XH, Schomisch Moravec C. Human cardiac myosin heavy chain isoforms in fetal and failing adult atria and ventricles. *Am J Physiol Heart Circ Physiol* 2001; 280: H1814-20.
9. Boland E, Quondamatteo F, Van Agtmael T. The role of basement membranes in cardiac biology and disease. *Biosci Rep* 2021; 41: BSR20204185..
10. Giacomelli E, Mummery CL, Bellin M. Human heart disease: lessons from human pluripotent stem cell-derived cardiomyocytes. *Cell Mol Life Sci* 2017; 74: 3711-39.
11. Chan BYH, Roczkowsky A, Cho WJ, et al. MMP inhibitors attenuate doxorubicin cardiotoxicity by preventing intracellular and extracellular matrix remodelling. *Cardiovasc Res* 2021; 117: 188-200.
12. Sergi CM. mRNA vaccination in children and youth: a cautionary note. *Arch Med Sci* 2022; 18: 1404-6.
13. Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. *Science* 2009; 324: 98-102.
14. Ouyang Z, Wei K. miRNA in cardiac development and regeneration. *Cell Regen* 2021; 10: 14.
15. Yan S, Jiao K. Functions of miRNAs during mammalian heart development. *Int J Mol Sci* 2016; 17: 789.
16. Tan CMJ, Lewandowski AJ. The transitional heart: from early embryonic and fetal development to neonatal life. *Fetal Diagn Ther* 2020; 47: 373-86.
17. Kalayinia S, Arjmand F, Maleki M, Malakootian M, Singh CP. MicroRNAs: roles in cardiovascular development and disease. *Cardiovasc Pathol* 2021; 50: 107296.
18. Zhao Y, Ransom JF, Li A, et al. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell* 2007; 129: 303-17.
19. Heidersbach A, Saxby C, Carver-Moore K, et al. microRNA-1 regulates sarcomere formation and suppresses smooth muscle gene expression in the mammalian heart. *Elife* 2013; 2: e01323.
20. Liu N, Bezprozvannaya S, Williams AH, et al. microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. *Genes Dev* 2008; 22: 3242-54.
21. Hoelscher SC, Stich T, Diehm A, et al. miR-128a acts as a regulator in cardiac development by modulating differentiation of cardiac progenitor cell populations. *Int J Mol Sci* 2020; 21: 1158.
22. Wang J, Greene SB, Bonilla-Claudio M, et al. Bmp signaling regulates myocardial differentiation from cardiac progenitors through a microRNA-mediated mechanism. *Dev Cell* 2010; 19: 903-12.
23. Chen J, Huang ZP, Seok HY, et al. mir-17-92 cluster is required for and sufficient to induce cardiomyocyte proliferation in postnatal and adult hearts. *Circ Res* 2013; 112: 1557-66.
24. Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci* 2005; 102: 13944-9.
25. Van Rooij E, Quiat D, Johnson BA, et al. A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. *Dev Cell* 2009; 17: 662-73.
26. Fu JD, Rushing SN, Lieu DK, et al. Distinct roles of microRNA-1 and -499 in ventricular specification and functional maturation of human embryonic stem cell-derived cardiomyocytes. *PLoS One* 2011; 6: e27417.
27. Hupfeld J, Ernst M, Knyrim M, et al. miR-208b reduces the expression of Kcnj5 in a cardiomyocyte cell line. *Biomedicines* 2021; 9: 719.
28. Fish JE, Wythe JD, Xiao T, et al. Slit/miR-218/Robo regulatory loop is required during heart tube formation in zebrafish. *Development* 2011; 138: 1409-19.
29. Deacon DC, Nevis KR, Cashman TJ, et al. The miR-143-adducin3 pathway is essential for cardiac chamber morphogenesis. *Development* 2010; 137: 1887-96.
30. Vacante F, Denby L, Sluimer JC, Baker AH. The function of miR-143, miR-145 and the MiR-143 host gene in cardiovascular development and disease. *Vascul Pharmacol* 2019; 112: 24-30.

31. Boettger T, Beetz N, Kostin S, et al. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the mir143/145 gene cluster. *J Clin Investig* 2009; 119: 2634-47.
32. Chen JF, Mandel EM, Thomson JM, et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 2006; 38: 228-33.
33. Wystub K, Besser J, Bachmann A, Boettger T, Braun T. miR-1/133a clusters cooperatively specify the cardiomyogenic lineage by adjustment of myocardin levels during embryonic heart development. *PLoS Genet* 2013; 9: e1003793.
34. Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature* 2005; 436: 214-20.
35. Ivey KN, Muth A, Arnold J, et al. MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. *Cell Stem Cell* 2008; 2: 219-29.
36. Quiat D, Voelker KA, Pei J, et al. Concerted regulation of myofiber-specific gene expression and muscle performance by the transcriptional repressor Sox6. *Proc Natl Acad Sci USA* 2011; 108: 10196-201.
37. van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN. Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* 2007; 316: 575-9.
38. Sluijter JP, van Mil A, van Vliet P, et al. MicroRNA-1 and -499 regulate differentiation and proliferation in human-derived cardiomyocyte progenitor cells. *Arterioscler Thromb Vasc Biol* 2010; 30: 859-68.
39. Li X, Wang J, Jia Z, et al. MiR-499 regulates cell proliferation and apoptosis during late-stage cardiac differentiation via Sox6 and cyclin D1. *PLoS One* 2013; 8: e74504.
40. Pasumarthi KB, Nakajima H, Nakajima HO, Soonpaa MH, Field LJ. Targeted expression of cyclin D2 results in cardiomyocyte DNA synthesis and infarct regression in transgenic mice. *Circ Res* 2005; 96: 110-8.
41. Rojas A, Kong SW, Agarwal P, Gilliss B, Pu WT, Black BL. GATA4 is a direct transcriptional activator of cyclin D2 and Cdk4 and is required for cardiomyocyte proliferation in anterior heart field-derived myocardium. *Mol Cell Biol* 2008; 28: 5420-31.
42. Mohamed TMA, Ang YS, Radzinsky E, et al. Regulation of cell cycle to stimulate adult cardiomyocyte proliferation and cardiac regeneration. *Cell* 2018; 173: 104-16. e112.
43. Schindler AJ, Baugh LR, Sherwood DR. Identification of late larval stage developmental checkpoints in *Caenorhabditis elegans* regulated by insulin/IGF and steroid hormone signaling pathways. *PLoS Genet* 2014; 10: e1004426.
44. Mahmoud AI, Kocabas F, Muralidhar SA, et al. Meis1 regulates postnatal cardiomyocyte cell cycle arrest. *Nature* 2013; 497: 249-53.
45. Engel FB, Schebesta M, Duong MT, et al. p38 MAP kinase inhibition enables proliferation of adult mammalian cardiomyocytes. *Genes Dev* 2005; 19: 1175-87.
46. Campa VM, Gutierrez-Lanza R, Cerignoli F, et al. Notch activates cell cycle reentry and progression in quiescent cardiomyocytes. *J Cell Biol* 2008; 183: 129-41.
47. Bersell K, Arab S, Haring B, Kühn B. Neuregulin1/ErbB4 signaling induces cardiomyocyte proliferation and repair of heart injury. *Cell* 2009; 138: 257-70.
48. Gemberling M, Karra R, Dickson AL, Poss KD. Nrg1 is an injury-induced cardiomyocyte mitogen for the endogenous heart regeneration program in zebrafish. *Elife* 2015; 4: e05871.
49. Heallen T, Zhang M, Wang J, et al. Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. *Science* 2011; 332: 458-61.
50. Xin M, Kim Y, Sutherland LB, et al. Hippo pathway effector Yap promotes cardiac regeneration. *Proc Natl Acad Sci USA* 2013; 110: 13839-13844.
51. Heallen T, Morikawa Y, Leach J, et al. Hippo signaling impedes adult heart regeneration. *Development* 2013; 140: 4683-90.
52. Kimura W, Xiao F, Canseco DC, et al. Hypoxia fate mapping identifies cycling cardiomyocytes in the adult heart. *Nature* 2015; 523: 226-30.
53. Hirose K, Payumo AY, Cutie S, et al. Evidence for hormonal control of heart regenerative capacity during endothermy acquisition. *Science* 2019; 364: 184-8.
54. Guo H, Ma K, Hao W, et al. miR15a/mir16-1 cluster and its novel targeting molecules negatively regulate cardiac hypertrophy. *Clin Transl Med* 2020; 10: e242.
55. Ogawa K, Noda A, Ueda J, et al. Forced expression of miR-143 and -145 in cardiomyocytes induces cardiomyopathy with a reductive redox shift. *Cell Mol Biol Lett* 2020; 25: 40.
56. Hu C, Liao J, Huang R, Su Q, He L. MicroRNA-155-5p in serum derived-exosomes promotes ischaemia-reperfusion injury by reducing CypD ubiquitination by NEDD4. *ESC Heart Fail* 2023; 10: 1144-57.
57. Li JY, Li XC, Tang YL. Upregulation of miR-128 mediates heart injury by activating Wnt/ β -catenin signaling pathway in heart failure mice. *Organogenesis* 2021; 17: 27-39.
58. Witman N, Heigwer J, Thaler B, Lui WO, Morrison JJ. miR-128 regulates non-myocyte hyperplasia, deposition of extracellular matrix and Islet1 expression during newt cardiac regeneration. *Dev Biol* 2013; 383: 253-63.
59. Huang W, Feng Y, Liang J, et al. Loss of microRNA-128 promotes cardiomyocyte proliferation and heart regeneration. *Nat Commun* 2018; 9: 700.
60. Xiang R, Lei H, Chen M, et al. The miR-17-92 cluster regulates FOG-2 expression and inhibits proliferation of mouse embryonic cardiomyocytes. *Braz J Med Biol Res* 2012; 45: 131-8.
61. Ventura A, Young AG, Winslow MM, et al. Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. *Cell* 2008; 132: 875-86.
62. Katz MG, Fargnoli AS, Kendle AP, Hajjar RJ, Bridges CR. The role of microRNAs in cardiac development and regenerative capacity. *Am J Physiol Heart Circ Physiol* 2016; 310: H528-41.
63. Liu JJ, Zhao CM, Li ZG, et al. miR-218 involvement in cardiomyocyte hypertrophy is likely through targeting REST. *Int J Mol Sci* 2016; 17: 848.
64. Cai B, Li J, Wang J, et al. microRNA-124 regulates cardiomyocyte differentiation of bone marrow-derived mesenchymal stem cells via targeting STAT3 signaling. *Stem Cells* 2012; 30: 1746-55.
65. Cheng XJ, Li L, Xin BQ. MiR-124 regulates the inflammation and apoptosis in myocardial infarction rats by targeting STAT3. *Cardiovasc Toxicol* 2021; 21: 710-20.
66. Yang W, Zhao L, Xue, Liu J, Shan J. MiRNA-21 promotes differentiation of bone marrow mesenchymal stem cells into cardiomyocyte-like cells by regulating the Ajaiba/Is11 axis pathway. *Arch Med Sci* 2022; 18: 1672-7.

67. Zhang H, Yang G, Zhong N, et al. Possible key microRNAs and corresponding molecular mechanisms for atrial fibrillation. *Anatol J Cardiol* 2020; 23: 324-33.
68. Rech M, Kuhn AR, Lumens J, et al. AntagomiR-103 and -107 treatment affects cardiac function and metabolism. *Molecular therapy. Nucleic Acids* 2019; 14: 424-37.
69. Wang JX, Zhang XJ, Li Q, et al. MicroRNA-103/107 regulate programmed necrosis and myocardial ischemia/reperfusion injury through targeting FADD. *Circ Res* 2015; 117: 352-63.
70. Yao CX, Wei QX, Zhang YY, et al. miR-200b targets GATA-4 during cell growth and differentiation. *RNA Biol* 2013; 10: 465-80.
71. Vichova T, Waldauf P, Karpisek M, et al. Oxidative stress markers, thioredoxin 1, 8-isoprostane, in relation to ischemic time in patients with ST segment elevation myocardial infarction treated by primary percutaneous coronary intervention. *Pol Arch Intern Med* 2021; 131: 755-8.
72. Chabior A, Gąsecka A, Marchel M, et al. Expression of versican mRNA transcript to predict cardiac remodeling after myocardial infarction. *Kardiol. Pol* 2021; 79: 833-40.
73. Morelli MB, Shu J, Sardu C, Matarese A, Santulli G. Cardiosomal microRNAs are essential in post-infarction myofibroblast phenocconversion. *Int J Mol Sci* 2020; 21: 201.
74. Kablak-Ziembicka A, Badacz R, Przewłocki T. Clinical application of serum microRNAs in atherosclerotic coronary artery disease. *J Clin Med* 2022; 11: 6849.
75. Navickas R, Gal D, Laucevicius A, Taparauskaite A, Zdanyte M, Holvoet P. Identifying circulating microRNAs as biomarkers of cardiovascular disease: a systematic review. *Cardiovasc Res* 2016; 111: 322-37.
76. Lee CK, Hsieh YP, Hsu SW, Lan SJ. Exploring diagnostic and prognostic predictive values of microRNAs for acute myocardial infarction: a PRISMA-compliant systematic review and meta-analysis. *Medicine* 2021; 100: e26627.
77. Agiannitopoulos K, Pavlopoulou P, Tsamis K, et al. Expression of miR-208b and miR-499 in Greek patients with acute myocardial infarction. *In Vivo* 2018; 32: 313-8.
78. Liu G, Niu X, Meng X, Zhang Z. Sensitive miRNA markers for the detection and management of NSTEMI acute myocardial infarction patients. *J Thorac Dis* 2018; 10: 3206-15.
79. Abdou DM, Aziz EE, Kazem YS, Elgabrty S, Taha HS. The diagnostic value of circulating microRNA-499 versus high-sensitivity cardiac troponin T in early diagnosis of ST segment elevation myocardial infarction. *Comp Clin Pathol* 2021; 30: 565-70.
80. Zhao J, Yu H, Yan P, Zhou X, Wang Y, Yao Y. Circulating microRNA-499 as a diagnostic biomarker for acute myocardial infarction: a meta-analysis. *Dis Markers* 2019; 2019: 6121696.
81. Wang GK, Zhu JQ, Zhang JT, et al. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J* 2010; 31: 659-66.
82. Wang J, Xu L, Tian L, Sun Q. Circulating microRNA-208 family as early diagnostic biomarkers for acute myocardial infarction: a meta-analysis. *Medicine* 2021; 100: e27779.
83. Gidlof O, Smith JG, Miyazu K, et al. Circulating cardio-enriched microRNAs are associated with long-term prognosis following myocardial infarction. *BMC Cardiovasc Disord* 2013; 13: 12.
84. Dutka M, Bobiński R, Korbecki J. The relevance of microRNA in post-infarction left ventricular remodeling and heart failure. *Heart Fail Rev* 2019; 24: 575-86.
85. Gacoń J, Kablak-Ziembicka A, Stepień E, et al. Decision-making microRNAs (miR-124, -133a/b, -34a and -134) in patients with occluded target vessel in acute coronary syndrome. *Kardiol Pol* 2016; 74: 280-8.
86. Schulte C, Molz S, Appelbaum S, et al. miRNA-197 and miRNA-223 predict cardiovascular death in a cohort of patients with symptomatic coronary artery disease. *PLoS One* 2015; 10: e0145930.
87. Alavi-Moghaddam M, Chehraz M, Alipoor SD, et al. A preliminary study of microRNA-208b after acute myocardial infarction: impact on 6-month survival. *Dis Markers* 2018; 2018: 2410451.
88. Hromadka M, Motovska Z, Hlinomaz O, et al. MiR-126-3p and MiR-223-3p as biomarkers for prediction of thrombotic risk in patients with acute myocardial infarction and primary angioplasty. *J Pers Med* 2021; 11: 508.
89. Badacz R, Kleczyński P, Legutko J, et al. Expression of miR-1-3p, miR-16-5p and miR-122-5p as possible risk factors of secondary cardiovascular events. *Biomedicines* 2021; 9: 1055.
90. Lin X, Zhang S, Huo Z. Serum circulating miR-150 is a predictor of post-acute myocardial infarction heart failure. *Int Heart J* 2019; 60: 280-6.
91. Lv P, Zhou M, He J, et al. Circulating miR-208b and miR-34a are associated with left ventricular remodeling after acute myocardial infarction. *Int J Mol Sci* 2014; 15: 5774-88.
92. Xiao S, Xue T, Pan Q, et al. MicroRNA-146a serves as a biomarker for adverse prognosis of ST-segment elevation myocardial infarction. *Cardiovasc Ther* 2021; 2021: 2923441.
93. Badacz R, Przewłocki T, Pieniążek P, et al. MicroRNA-134-5p and the extent of arterial occlusive disease are associated with risk of future adverse cardiac and cerebral events in diabetic patients undergoing carotid artery stenting for symptomatic carotid artery disease. *Molecules* 2022; 27: 2472.
94. Ziaee S, Hosseindokht M, Cheraghi S, et al. Predictive inflammation-related microRNAs for cardiovascular events following early-onset coronary artery disease. *Arch Med Res* 2021; 52: 69-75.
95. Simsek B, Cinar T, Tanik VO, et al. The association of acute-to-chronic glycemic ratio with no-reflow in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Kardiol Pol* 2021; 79: 170-8.
96. Jankowski P, Topór-Mądry R, Gąsior M, et al. Management and predictors of clinical events in 75 686 patients with acute myocardial infarction. *Kardiol Pol* 2022; 80: 468-75.
97. Bogacki P, Kablak-Ziembicka A, Bryniarski K, et al. Triple anticoagulation therapy in patients with atrial fibrillation undergoing percutaneous coronary intervention – real life assessment. *Adv Interv Cardiol* 2016; 12: 303-13.
98. Procyk G, Klimczak-Tomaniak D, Sygitowicz G, Tomaniak M. Circulating and platelet microRNAs in cardiovascular risk assessment and antiplatelet therapy monitoring. *J Clin Med* 2022; 11: 1763.
99. Zhang YY, Zhou X, Ji WJ, et al. Decreased circulating microRNA-223 level predicts high on-treatment platelet reactivity in patients with troponin-negative non-ST elevation acute coronary syndrome. *J Thromb Thrombolysis* 2014; 38: 65-72.

100. Chyrchel B, Totoń-Zurańska J, Kruszelnicka O, et al. Association of plasma miR-223 and platelet reactivity in patients with coronary artery disease on dual antiplatelet therapy: a preliminary report. *Platelets* 2015; 26: 593-7.
101. Peng L, Liu J, Qin L, et al. Interaction between platelet-derived microRNAs and CYP2C19*2 genotype on clopidogrel antiplatelet responsiveness in patients with ACS. *Thromb Res* 2017; 157: 97-102.
102. Xie W, Yin Q, Zhang M, Li S, Chen S. Leukocyte miR-223-3p is not associated with altered platelet responses to clopidogrel in patients with coronary artery disease. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2018; 43: 421-7.
103. Tekieli Ł, Musiałek P, Kabtak-Ziembicka A, et al. Severe, recurrent in-stent carotid restenosis: endovascular approach, risk factors. Results from a prospective academic registry of 2637 consecutive carotid artery stenting procedures (TARGET-CAS). *Adv Interv Cardiol* 2019; 15: 465-71.
104. Paszek E, Zajdel W, Musiałek P, et al. Percutaneous management of long and diffused coronary lesions using newer generation drug-eluting stents in routine clinical practice: long-term outcomes and complication predictors. *Pol Arch Intern Med* 2019; 129: 392-8.
105. Santulli G. microRNAs distinctively regulate vascular smooth muscle and endothelial cells: functional implications in angiogenesis, atherosclerosis, and in-stent restenosis. *Adv Exp Med Biol* 2015; 887: 53-77.
106. Dai H, Wang J, Shi Z, Ji X, Huang Y, Zhou R. Predictive value of miRNA-21 on coronary restenosis after percutaneous coronary intervention in patients with coronary heart disease. A protocol for systematic review and meta-analysis. *Medicine* 2021; 100: e24966.
107. Wang J, Qian HL, Chen SY, et al. miR-22 eluting cardiovascular stent based on a self-healable spongy coating inhibits in-stent restenosis. *Bioact Mater* 2021; 6: 4686-96.
108. Wang D, Deuse T, Stubbendorff M, et al. Local MicroRNA modulation using a novel anti-miR-21-eluting stent effectively prevents experimental in-stent restenosis. *Arterioscler Thromb Vasc Biol* 2015; 35: 45-1953.
109. Gutierrez-Carretero E, Mayoral-González I, Jesús Morón F, et al. miR-30b-5p downregulation as a predictive biomarker of coronary in-stent restenosis. *Biomedicines* 2021; 9: 354.
110. Lagerbauer B, Engelhardt S. MicroRNAs as therapeutic targets in cardiovascular disease. *J Clin Invest* 2022; 132: e159179.
111. Charkaborty C, Sharma AR, Sharma G, Lee SS. Therapeutic advances of microRNAs: a preclinical and clinical update. *J Adv Res* 2021; 28: 127-38.
112. Scarlatescu AI, Micheu MM, Popa-Fotea NM, Dorobantu M. MicroRNAs in acute ST-elevation myocardial infarction – a new tool for diagnosis and prognosis: therapeutic implications. *Int J Mol Sci* 2021; 22: 4799.
113. Diener C, Keller A, Meese E. Emerging concepts of miRNA therapeutics: from cells to clinic. *Trends Genetics* 2022; 38: 613-26.
114. Paoletti C, Divieto C, Chiono V. Direct reprogramming of adult human cardiac fibroblasts into induced cardiomyocytes using miRcombo. *Methods Mol Biol* 2022; 2573: 31-40.
115. Varzideh F, Kansakar U, Donkor K, et al. Cardiac remodeling after myocardial infarction: functional contribution of microRNAs to inflammation and fibrosis. *Front Cardiovasc Med* 2022; 9: 863238.
116. Zhou SS, Jin JF, Wang J, et al. miRNAs in cardiovascular diseases: potential biomarkers, therapeutic targets and challenges. *Acta Pharmacol Sin* 2018; 39: 1073-84.
117. Batkai S, Genschel C, Viereck J, et al. CDR132L improves systolic and diastolic function in a large animal model of chronic heart failure. *Eur Heart J* 2021; 42: 192-201.
118. Täubel J, Hauke W, Rump S, et al. Novel antisense therapy targeting microRNA-132 in patients with heart failure: results of a first-in-human phase 1b randomized, double-blind, placebo-controlled study. *Eur Heart J* 2021; 42: 178-88.
119. Bonauer A, Carmona G, Iwasaki M, et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. *Science* 2009; 324: 1710-3.
120. Lesizza P, Prosdocimo G, Martinelli V, Sinagra G, Zacchigna S, Giacca M. Single-dose intracardiac injection of pro-regenerative microRNAs improves cardiac function after myocardial infarction. *Circ Res* 2017; 120: 1298-304.
121. Eulalio A, Mano M, Ferro MD, et al. Functional screening identifies miRNAs inducing cardiac regeneration. *Nature* 2021; 492: 376-81.
122. Gao Z, Zhu X, Dou Y. The miR-302-367 cluster: a comprehensive update on its evolution and functions. *Open Biol* 2015; 5: 150138.
123. Rech M, Kuhn AR, Lumens J, et al. AntagomiR-103 and -107 treatment affects cardiac function and metabolism. *Mol Ther Nucleic Acids* 2019; 14: 424-37.
124. Zhang S, Cheng Z, Wang Y, Han T. The risks of miRNA therapeutics: in a drug target perspective. *Drug Des Devel Ther* 2021; 15: 721-33.
125. Hong DS, Kang YK, Borad M, et al. Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. *Br J Cancer* 2020; 122: 1630-7.
126. Wang Z, Wang Z, Wang T, Yuan J, Wang X, Zhang Z. Inhibition of miR-34a-5p protected myocardial ischemia reperfusion injury-induced apoptosis and reactive oxygen species accumulation through regulation of Notch Receptor 1 signaling. *Rev Cardiovasc Med* 2019; 20: 187-97.
127. Dai B, Wang F, Nie X, et al. The cell type-specific functions of miR-21 in cardiovascular diseases. *Front Genet* 2020; 11: 563166.
128. Hullinger TG, Montgomery RL, Seto AG, et al. Inhibition of miR-15 protects against cardiac ischemic injury. *Circ Res* 2012; 110: 71-81.
129. van Zandwijk N, Pavlakis N, Kao SC, et al. Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: a first-in-man, phase 1, open-label, dose-escalation study. *Lancet Oncol* 2017; 18: 1386-96.
130. Toro R, Pérez-Serra A, Mangas A, et al. miR-16-5p suppression protects human cardiomyocytes against endoplasmic reticulum and oxidative stress-induced injury. *Int J Mol Sci* 2022; 23: 1036.
131. Lee EC, Valencia T, Allerson C, et al. Discovery and preclinical evaluation of anti-miR-17 oligonucleotide RGLS4326 for the treatment of polycystic kidney disease. *Nat Commun* 2019; 10: 4148.
132. Yang S, Fan T, Hu Q, et al. Downregulation of microRNA-17-5p improves cardiac function after myocardial infarction via attenuation of apoptosis in endothelial cells. *Mol Genet Genomics* 2018; 293: 883-94.
133. Gomez IG, MacKenna DA, Johnson BG, et al. Anti-microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. *J Clin Invest* 2015; 125: 141-56.

134. Yan MW, Chen C, Gong W, et al. miR-21-3p regulates cardiac hypertrophic response by targeting histone deacetylase-8. *Cardiovasc Res* 2015; 105: 340-52.
135. Holland A, Enrick M, Diaz A, Yin L. Is miR-21 a therapeutic target in cardiovascular disease? *Int J Drug Discov Pharmacol* 2023; 2: 26-36.
136. Song Y, Zhang C, Zhang JX, et al. Localized injection of miRNA-21-enriched extracellular vesicles effectively restores cardiac function after myocardial infarction. *Theranostics* 2019; 9: 2346-60.
137. Zhang BF, Jiang H, Chen J, et al. LncRNA H19 ameliorates myocardial infarction-induced myocardial injury and maladaptive cardiac remodeling by regulating KDM3A. *J Cell Mol Med* 2020; 24: 1099-115.
138. Huang ZP, Chen J, Seok HY, et al. MicroRNA-22 regulates cardiac hypertrophy and remodeling in response to stress. *Circ Res* 2013; 112: 1234-43.
139. Jeong D, Yoo J, Lee P, et al. miR-25 Tough decoy enhances cardiac function in heart failure. *Mol Ther* 2018; 26: 718-29.
140. Chen Z, Pan X, Sheng Z, Yan G, Chen L, Ma G. Baicalin suppresses the proliferation and migration of Ox-LDL-VSMCs in atherosclerosis through upregulating miR-126-5p. *Biol Pharm Bull* 2019; 42: 1517-23.
141. Schober A, Nazari-Jahantigh M, Wei Y, et al. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. *Nat Med* 2014; 20: 368-76.
142. Raso A, Dirx E, Philippen LE, et al. Therapeutic delivery of miR-148a suppresses ventricular dilation in heart failure. *Mol Ther* 2019; 27: 584-99.
143. Haghikia A, Missol-Kolka E, Tsikas D, et al. Signal transducer and activator of transcription 3-mediated regulation of miR-199a-5p links cardiomyocyte and endothelial cell function in the heart: a key role for ubiquitin-conjugating enzymes. *Eur Heart J* 2011; 32: 1287-97.
144. Eding JE, Demkes CJ, Lynch JM, et al. The efficacy of cardiac anti-miR-208a therapy is stress dependent. *Mol Ther* 2017; 25: 694-704.
145. Yang K, Shi J, Hu Z, Hu X. The deficiency of miR-214-3p exacerbates cardiac fibrosis via miR-214-3p/NLR5 axis. *Clin Sci* 2019; 133: 1845-56.
146. Qi J, Wang J, Katayama H, Sen S, Liu SM. Circulating microRNAs (cmRNAs) as novel potential biomarkers for hepatocellular carcinoma. *Neoplasma* 2013; 60: 135-42.
147. Liu X, Cheng Y, Yang J, Xu L, Zhang C. Cell-specific effects of miR-221/222 in vessels: molecular mechanism and therapeutic application. *J Mol Cell Cardiol* 2012; 52: 245-55.
148. López-Muneta L, Miranda-Arrubla J, Carvajal-Vergara X. The future of direct cardiac reprogramming: any GMT cocktail variety? *Int J Mol Sci* 2020; 21: 7950.
149. Yamakawa H, Ieda M. Cardiac regeneration by direct reprogramming in this decade and beyond. *Inflamm Regeneration* 2021; 41: 20.
150. Li Z, Song Y, Liu L, et al. miR-199a impairs autophagy and induces cardiac hypertrophy through mTOR activation. *Cell Death Differ* 2017; 24: 1205-13.
151. Jiang L, Liang J, Huang W, Wu Z, Paul C, Wang Y. Strategies and challenges to improve cellular programming-based approaches for heart regeneration therapy. *Int J Mol Sci* 2020; 21: 7662.
152. Zhang B, Mao S, Liu X, et al. MiR-125b inhibits cardiomyocyte apoptosis by targeting BAK1 in heart failure. *Mol Med* 2021; 27: 72.
153. Nowaczyk M, Malcher A, Zimna A, Rozwadowska N, Kurpisz M. Effect of miR-195 inhibition on human skeletal muscle-derived stem/progenitor cells. *Kardiol Pol* 2022; 80: 813-24.
154. Gabisonia K, Prosdocimo G, Aquaro GD, et al. MicroRNA therapy stimulates uncontrolled cardiac repair after myocardial infarction in pigs. *Nature* 2019; 569: 418-22.
155. Nicoletti L, Paoletti C, Tarricone G, et al. Lipoplexes for effective in vitro delivery of microRNAs to adult human cardiac fibroblasts for perspective direct cardiac cell reprogramming. *Nanomedicine* 2022; 45: 102589.
156. Yang L, Xue S, Du M, Lian F. Highly efficient microRNA delivery using functionalized carbon dots for enhanced conversion of fibroblasts to cardiomyocytes. *Int J Nanomed* 2021; 16: 3741-54.
157. Jayawardena TM, Egemnazarov B, Finch EA, et al. MicroRNA-mediated in vitro and in vivo direct reprogramming of cardiac fibroblasts to cardiomyocytes. *Circ Res* 2012; 110: 1465-73.
158. Baksh SS, Hidgkinson CP. Conservation of miR combobased direct cardiac reprogramming. *Biochem Biophys Rep* 2022; 31: 101310.
159. Fu JD, Stone NR, Liu L, et al. Direct reprogramming of human fibroblasts toward a cardiomyocyte-like state. *Stem Cell Rep* 2013; 1: 235-47.
160. Mohamed TM, Stone NR, Berry EC, et al. Chemical enhancement of in vitro and in vivo direct cardiac reprogramming. *Circulation* 2017; 135: 978-95.
161. Maleki B, Alani B, Tamehri Zadeh SS, et al. MicroRNAs and exosomes: cardiac stem cells in heart diseases. *Pathol Res Pract* 2022; 229: 153701.
162. González-Melgoza L, Ruiz-Manriquez LM, De Donato M, et al. Recent insights into the microRNA and long non-coding RNA-mediated regulation of stem cell populations. *3 Biotech* 2022; 12: 270.
163. Kablak-Ziembicka A, Przewłocki T. Clinical significance of carotid intima-media complex and carotid plaque assessment by ultrasound for the prediction of adverse cardiovascular events in primary and secondary care patients. *J Clin Med* 2021; 10: 4628.
164. Puz P, Urbanek T, Ziaja D, et al. Factors associated with the symptomatic status of carotid artery stenosis: identification in a cross-sectional study and development of a scoring system. *Pol Arch Intern Med* 2021; 131: 17-25.
165. Gacoń J, Przewłocki T, Podolec J, et al. Prospective study on the prognostic value of repeated carotid intima-media thickness assessment in patients with coronary and extra coronary steno-occlusive arterial disease. *Pol Arch Intern Med* 2019; 129: 808-17.
166. Kadoglou NP, Khattab E, Velidakis N, Patsourakos N, Lambadiari V. A new approach of statin therapy in carotid atherosclerosis: targeting indices of plaque vulnerability on the top of lipid-lowering. A narrative review. *Kardiol Pol* 2022; 80: 880-90.
167. Formanowicz D, Krawczyk JB, Perek B, Lipski D, Tykarski A. Management of high-risk atherosclerotic patients by statins may be supported by logistic model of intima-media thickening. *J Clin Med* 2021; 10: 2876.
168. Kablak-Ziembicka A, Przewłocki T, Pieniazek P, et al. The role of carotid intima-media thickness assessment in cardiovascular risk evaluation in patients with polyvascular atherosclerosis. *Atherosclerosis* 2010; 209: 125-30.
169. Motek P, Żmudzki P, Machnik A, et al. Thin-cap fibroatheroma and increased coronary intima-media thickness

- are associated with an altered balance of arginine metabolites. *Pol Arch Intern Med* 2023; 133: 16536.
170. Peczyńska J, Klonowska B, Żelazowska-Rutkowska B, et al. The relationship between selected inflammation and oxidative stress biomarkers and carotid intima-media thickness (IMT) value in youth with type 1 diabetes co-existing with early microvascular complications. *J Clin Med* 2022; 11: 4732.
 171. Kablak-Ziembicka A, Przewlocki T, Tracz W, et al. Prognostic value of carotid intima-media thickness in detection of coronary atherosclerosis in patients with calcified aortic valve stenosis. *J Ultrasound Med* 2005; 24: 461-7.
 172. Macchi C, Greco MF, Favero C, et al. Associations among PCSK9 levels, atherosclerosis-derived extracellular vesicles, and their miRNA content in adults with obesity. *Front Cardiovasc Med* 2022; 8: 785250.
 173. Klisic A, Radoman Vujacic I, Munjas J, Ninic A, Kotur-Stevuljevic J. Micro-ribonucleic acid modulation with oxidative stress and inflammation in patients with type 2 diabetes mellitus – a review article. *Arch Med Sci* 2022; 18: 870-80.
 174. Ataei S, Ganjali S, Banach M, Karimi E, Sahebkar A. The effect of PCSK9 immunization on the hepatic level of microRNAs associated with the PCSK9/LDLR pathway. *Arch Med Sci* 2023; 19: 203-8.
 175. Radbakhsh S, Sathyapalan T, Banach M, Sahebkar A. Incretins and microRNAs: interactions and physiological relevance. *Pharmacol Res* 2020; 153: 104662.
 176. Mohajeri M, Banach M, Atkin SL, et al. MicroRNAs: novel molecular targets and response modulators of statin therapy. *Trends Pharmacol Sci* 2018; 39: 967-81.
 177. Paseban M, Marjaneh RM, Banach M, Riahi MM, Bo S, Sahebkar A. Modulation of microRNAs by aspirin in cardiovascular disease. *Trends Cardiovasc Med* 2020; 30: 249-54.
 178. Sabatel-Pérez F, Sánchez-Prieto J, Becerra-Muñoz VM, et al. Improving familial hypercholesterolemia index case detection: sequential active screening from centralized analytical data. *J Clin Med* 2021; 10: 749.
 179. Wu C, Liu B, Wang R, Li G. The regulation mechanisms and clinical application of microRNAs in myocardial infarction: a review of the recent 5 years. *Front Cardiovasc Med* 2022; 8: 809580.