Non-coding RNAs as therapeutic targets for preventing myocardial ischemia-reperfusion injury

Structured Abstract

Introduction: New treatments are required to improve clinical outcomes in patients presenting with acute myocardial infarction (AMI), in order to reduce myocardial infarct (MI) size and prevent heart failure. Following AMI, the heart is subjected to the detrimental effects of acute ischemia/reperfusion injury (IRI), which result in cardiomyocyte death and impaired cardiac function. Emerging studies have implicated a fundamental role for non-coding RNAs (microRNAs [miRNA], and more recently long non-coding RNAs [lncRNA]) in the setting of acute myocardial IRI.

Areas covered: In this article, we discuss the roles of miRNAs and lncRNAs as potential biomarkers and therapeutic targets for the detection and treatment of AMI, review their roles as mediators and effectors of cardioprotection against acute myocardial IRI, particularly in the settings of interventions such as ischemic pre- and post-conditioning (IPC & IPost) as well as remote ischemic conditioning (RIC), and highlight future strategies for targeting these ncRNAs as potential novel therapies for reducing MI size and preventing heart failure following AMI.

Expert opinion: Investigating the roles of miRNAs and lncRNAs in the setting of AMI has provided new insights into the pathophysiology underlying acute myocardial IRI, and has identified novel biomarkers and therapeutic targets for detecting and treating AMI. Pharmacological and genetic manipulation of these ncRNAs has the therapeutic potential to improve clinical outcomes in AMI patients.

Keywords*: Ischemia-reperfusion injury; long non-coding RNA; microRNAs; Non-coding RNAs; Acute myocardial infarction; Cardioprotection; Ischemic conditioning.*

1. Introduction

Despite current therapy, acute myocardial infarction (AMI) and the heart failure which often follows, remain the leading causes of death and disability worldwide. As such new therapeutic strategies are required to protect the heart against the detrimental effects of acute ischemia/reperfusion injury (IRI), in order to prevent cardiomyocyte death and reduce myocardial infarct (MI) size, preserve left ventricular (LV) function, and prevent the onset of heart failure [1,2]. Compared with drugs with a broad-spectrum mechanism of action, agents specifically targeting mitochondrial function – which has been perceived as a crucial mediator of reperfusion injury, have failed to demonstrate significant beneficial clinical outcomes [2-5]. Thus, a multi-targeted approach (combining mitochondrial-targeting with other components of the IRI pathway) may confer better efficacy compared to a single-target intervention. In this regard, emerging studies have implicated a fundamental role for non-coding RNAs (ncRNA) in both cardiac development (cardiogenesis [6]) and disease (left ventricular hypertrophy [7], heart failure [8], and acute myocardial infarction [9,10]).

Only 2% of the human genome is made up of protein-coding regions, with the majority of transcripts comprising non-coding RNAs (ncRNAs), such as microRNA (miRNA) and long non-coding RNAs (lncRNAs). MiRNAs are short (21-23 nucleotides in length), single-stranded ncRNAs, that modulate gene expression by inhibiting mRNA translation or promoting mRNA degradation (for recent reviews see [11]). The newly defined class of ncRNAs, lncRNAs (over 200 nucleotides in length), have been shown to regulate gene expression through a versatile array of post-transcriptional, translational, and epigenetic modes of action in cardiac development and disease (for a detailed review please see [12]).

In this article, we provide an overview of the roles of miRNA and lncRNA, as potential biomarkers and therapeutic targets for the detection and treatment of AMI, review their roles as mediators and effectors of cardioprotection against acute myocardial IRI, and highlight future strategies for

targeting ncRNAs as potential novel therapies for reducing MI size and preventing heart failure following AMI (refer to Figure 1). The role of ncRNAs as potential therapeutic targets for preventing post-AMI adverse LV remodeling will not be specifically discussed in this article, as the main focus of this article will be on those ncRNAs involved in the first few hours following AMI [11,12].

2. MiRNAs in acute myocardial ischemia/reperfusion injury

Over the last decade, miRNAs have been extensively investigated in the setting of acute myocardial IRI. A large number of studies have shown early changes in myocardial expression of miRNAs (either increasing or decreasing) in response to acute myocardial IRI. Genetic or pharmacological manipulation of these miRNAs has been shown to modulate the sensitivity of the myocardium to acute IRI, thereby implicating miRNAs as therapeutic targets for cardioprotection (see Table 1 for summary of the major studies).

One of the first studies to investigate the role of miRNAs in the setting of AMI, was by Rooij et al [13] who investigated the changes in miRNA expression in the infarct border zones in murine and human hearts. They observed changes in a large number of miRNAs, including upregulation of miR-15b, miR-21, miR-199, and miR-214, and down-regulation of miR-29c and miR-150. Interestingly, many of these miRNAs had been previously shown to be dysregulated in the settings of left ventricular hypertrophy (LVH) and heart failure. They went on to demonstrate that down-regulation of miR-29 in cardiac fibroblasts following AMI increased the expression of a number of fibrosis genes due to de-repression. The over-expression of miR-29 was able to decrease the expression of these fibrosis genes, implicating this miRNA as a therapeutic target for reducing cardiac fibrosis following AMI.

Activation or inhibition of a number of proteins have been implicated as downstream targets for miRNA in the setting of acute myocardial IRI. As expected many of the downstream targets impact on cell death pathways such as apoptosis, autophagy, and more recently

necroptosis (see Table 1 for summary). More recently, downstream targets affecting calcium signaling, inflammation, mitochondrial protective pathways [14,15], and other cardioprotective pathways have been implicated.

3. MiRNAs as mediators of cardioprotection

An increasing number of experimental studies have demonstrated that endogenous miRNAs within the heart are able to mediate the protection against acute IRI conferred by pharmacological agents and endogenous cardioprotective strategies such as ischemic conditioning (see Figure 2). This appears to be mediated by downstream targets involved in cell death and cell survival pathways underlying acute myocardial IRI and cardioprotection, respectively.

3.1. MiRNAs as mediators of ischemic preconditioning

The heart can be endogenously protected against acute IRI by applying brief non-lethal episodes of ischemia and reperfusion either prior to the index ischemic episode – a phenomenon which was first described in 1986 [16], and has been termed 'ischemic preconditioning' (IPC). The IPC stimulus is known to induce 2 windows of cardioprotection – the first, immediately following the stimulus and lasting 2-3 hours (termed 'classical IPC'), and the second, appearing 12-24 hours after the IPC stimulus and lasting 48-72 hours (termed 'delayed IPC', and requiring the synthesis of new proteins) [17-19]. Unravelling the molecular pathways mediating IPC has identified novel therapeutic targets and strategies for protecting the heart against AMI. The role of miRNAs as mediators and effectors of IPC cardioprotection has been investigated over the last decade, and an overview is given here.

A number of experimental studies have demonstrated the following changes in miRNA expression in heart tissue associated with IPC: increased expression of miR-21, miR-1,[20,21], miR-144/451 [22], miR-487b, miR-139-5p, miR-192, and miR-212 [23]; and downregulated expression of miR-1 [24] and miR-199a [25]. From these studies, it appears that the role of miR-1 in IPC is unclear

with either upregulation or downregulation in this miRNA – interestingly this was also observed with IPost (see next section), and the difference may relate to the timing of the tissue sampling and the experimental methods used.

In terms of showing that the miRNA actually contributes to IPC-cardioprotection, this has only been shown for miR-21 and miR-451. Cheng et al [20] found that intramyocardial injection of the antagomiR-21, 24 hours prior to acute myocardial IRI, abolished the increased expression of miR-21 and abrogated the MI-limiting effects of IPC. Injection of miR-21 was able to recapitulate IPC-cardioprotection and attenuate apoptotic cell death by downregulating programmed cell death 4 (PDCD4). Wang et al [22] demonstrated that mice deficient in the miR-144/451 complex were not amenable to IPC, and this effect was associated with increased expression of NADPH oxidase (NOX) and Ras-related C3 botulinum toxin substrate 1 (RAC1), and elevated levels of reactive oxygen species (ROS). Interestingly, using antagomiRs to miR-144 and miR-451, the MI-limiting effect of IPC was only blocked when miR-451 was ablated, suggesting that in this miRNA complex miR-451 rather than miR-144 is required for IPC. Rane et al [25] have shown that IPC can downregulate miR-199a resulting in activation of the HIF-1α/Sirt1 pro-survival pathway [26,27].

The role of miRNAs as mediators of delayed IPC has also been investigated, with upregulation of miR-1, miR-21 and miR-24 demonstrated 24 hours after application of the IPC stimulus, effects which were associated with upregulation of eNOS, heat shock transcription factor-1 (HSF-1) and hsp70, known mediators of delayed IPC [28]. Interestingly, the injection of these miRNAs 48 hours prior to acute myocardial IRI was able to limit MI size in naïve murine hearts, suggesting that these miRNAs could mimic the cardioprotection elicited by delayed IPC. The mechanisms through which IPC modulates the expression of miRNAs in the heart are not known, although Rane et al [29] have suggested that the pro-survival kinase, Akt, and the pro-injurious beta-adrenergic cascades may converge on miR-199a, providing a potential point of point of regulation for determining cell death and survival in the setting of acute myocardial IRI.

The mechanistic pathways through which IPC actually regulates myocardial miRNA expression is not known and requires further study.

3.2. MiRNAs as mediators of ischemic postconditioning

The major limitation of IPC as a cardioprotective strategy, is that it needs to be applied prior to the index ischemic event, which is not possible in AMI patients. This can be overcome by the phenomenon of 'ischemic postconditioning' (IPost) [30-32], in which myocardial reperfusion is interrupted by brief cycles of myocardial ischemia, allowing the protective stimulus to be applied at the onset of reperfusion in AMI patients treated by PPCI. The mechanisms underlying IPost are similar but not identical to those mediating IPC [33,34]. The role of miRNAs as mediators of IPost cardioprotection have been recently investigated, and are reviewed in this section.

A number of experimental studies have investigated changes in myocardial miRNA expression following IPost, and have reported IPost cardioprotection to be associated with: increased expression of miR-1, miR-133a, miR-214, microRNA-1, microRNA let-7i, and microRNA let-7e in rodent hearts [23,35-37]; downregulation of miR-208 [23]; enhanced expression of miR-29b, miR-133a, and miR-146b in pig hearts [38]; down-regulation of miR-1 in the rat heart [21]; and downregulation of miR-1 and upregulation of miR-21 in atrial tissue harvested from patients undergoing cardiac valve surgery [39]. From these studies, it can be seen that the role of miR-1 in IPost has produced mixed data with either upregulation or downregulation in this miRNA with IPost. In this regard, Bian et al [40] found agomir-1 increased MI size and reduced Cx43 expression, whereas IPost downregulated miR-1 and preserved Cx43 expression in the rat heart.

Experimental evidence for miRNAs actually contributing to the cardioprotective effects of IPost have only been shown for miR-21 and miR-499. Tu et al [36] found that IPost markedly upregulated (by 5-fold compared to sham) the myocardial expression of miR-21 in the murine heart, and the effect of IPost on reducing MI size, limiting apoptotic cell death and upregulating Akt phosphorylation and BCl2 expression, were all abrogated by knock-down of miR-21 with an

antagomir-21, confirming the contribution of this miRNA to IPost cardioprotection. Similarly, Zhu et al [41] demonstrated that antagomir-mediated knock-down of miR-4199 abolished IPost cardioprotection, suggesting that miR-499 may also contribute to IPost by targeting PDCD4. Again, the actual mechanisms through which IPost changes the myocardial expression of these miRNAs is not known and needs to be investigated.

3.3. MiRNAs as mediators of remote ischemic conditioning

Both IPC and IPost require the protective conditioning stimulus to be applied directly to the heart, thereby limiting their application to patients. This can be avoided by the phenomenon of 'remote ischemic conditioning' (RIC), in which the protective conditioning stimulus is applied to an organ or tissue away from the heart [42-45]. Indeed, the ability to induce RIC by applying brief cycles of ischemia and reperfusion to the limb [46-48], has greatly facilitated the translation of RIC into the clinical setting – this can be easily achieved both non-invasively and at low-cost with the use of a standard blood pressure cuff places on the upper arm or thigh to induce brief cycles of ischemia and reperfusion [49-52]. However, the underlying mechanisms through which the cardioprotective signal is relayed from the 'conditioned' limb to the heart remains unclear. It is thought to involve a neuro-hormonal pathway generating circulating cardioprotective factor or factors, the identity of which remain unclear [44,45,53,54]. A number of studies have investigated the role of miRNAs as potential mediators of limb RIC cardioprotection, and are reviewed here.

Experimental studies have investigated changes in myocardial expression of miRNAs following limb RIC (brief cycles of hind-limb ischemia/reperfusion) in the rodent heart, and have shown reduced expression of miR-1 and downstream targets such as PCDC4 [21], but not brain-derived neurotrophic factor (BDNF) [55]. Interestingly, after 6 hours of reperfusion, myocardial expression of miR-1 was reported to be elevated by limb RIC compared to control ones [55], making it possible that the upregulation of miR-1 at this time-point contributed to the delayed cardioprotective effect of limb RIC. The effect of limb RIC on miR-1 has also been demonstrated

in human heart tissue by Slagsvold et al [56] who found that RIC reduced the myocardial expression of miR-1 in right atrial appendage tissue harvested from patients undergoing coronary artery bypass graft (CABG) surgery, findings which were associated with preserved mitochondrial function, and a lower incidence of post-operative atrial fibrillation. In addition, this patient study found increased myocardial expression of miR-338-3p in patients subjected to limb RIC when compared to control, but the relevance of this needs to be further investigated [56]. Similarly, Hu et al [57] found that limb RIC down-regulated miR-1 and miR-195 in right atrial appendage tissue harvested from patients undergoing heart valve replacement surgery, findings which were associated with upregulation of the anti-apoptotic factor, Bcl-2, less apoptotic cell death, and reduced peri-operative myocardial injury. Interestingly, in another study, Slagsvold et al [58] failed to show any change in miRNA expression in left ventricular tissue harvested from CABG patients following limb RIC, suggesting differential expression of miRNAs within the heart following limb RIC.

The role of circulating miRNAs as potential mediators of limb RIC cardioprotection has also been investigated. Li et al [59] found that limb RIC increased circulating plasma levels of miR-144 in mice and human volunteers, suggesting that plasma miR-144 levels may be used as a biomarker to assess the efficacy of the limb RIC protocol. In addition, myocardial expression of miR-144 in murine hearts following limb RIC, and the cardioprotective effect of limb RIC was blocked by inhibiting miR-144 using an antisense oligonucleotide. Finally, exogenous administration of a miR-144 homologue oligonucleotide reduced MI size in the murine heart, a finding which was associated with increased phosphorylation of known cardioprotective factors, Akt and Erk1/2, decreased levels of mTOR, and enhanced myocardial autophagy [59]. The mechanism through which miR-144 is transported in the plasma from the conditioned limb to the heart was thought to be due to the Argonaute-2 (Ago-2) protein rather than circulating exosomes [59]. Whether circulating exosomes actually contribute to the cardioprotective effect of limb RIC, and whether this is mediated by miRNAs transported in exosomes is inconclusive [60,61]. A

subsequent clinical study has confirmed the increase in plasma miR-144 following limb RIC in patients with stable coronary artery disease, an effect which was not associated with any changes in myocardial perfusion in ischemic and non-ischemic areas of the heart [62].

Interestingly, it has been reported that limb RIC, repeated daily for one month, prevented adverse LV remodeling following AMI in the rodent heart [63]. Yamaguchi et al [64] have subsequently reported that repeated limb RIC was associated with increased expression of miR-29a in circulating exosomes, and augmented expression of both miR-29a and miR-30a in myocardial tissue. As such, whether miR-29a generated in the limb following RIC, is then carried to the heart, where it activates cardioprotective signaling pathways is not known.

3.4. MiRNAs as mediators of pharmacological cardioprotection

A number of other cardioprotective strategies and pharmacological agents have been shown to reduce cell death or MI size, by modifying the expression of miRNAs within the heart (see Table 2 for summary). In general, these other cardioprotective strategies and agents are associated with either downregulation of pro-injurious miRNAs and/or upregulation of protective miRNAs.

One of the first studies to investigate the role of endogenous miRNAs as mediators of cardioprotection was by Yin et al [10], who demonstrated that total body heat stress (an endogenous cardioprotective stimulus which mimics IPC) increased the expression of miR-1, miR-21 and miR-24 in the murine heart. Interestingly, miRNAs isolated from the heat-stressed mice and injected into naïve animals were able to reduce MI size, and this transferrable protective effect was associated with the downregulation of pro-apoptotic genes (caspases 1, 2, 8 and 14, Bid, Bcl10, Cidea, Ltbr, Trp53 and Fas ligand), and the upregulation of anti-apoptotic proteins (Bag3, and Prdx2). Finally, this study demonstrated that the administration of chemically synthesised miR-21 limited MI size, and this beneficial effect could be abrogated using a miR-21 inhibitor.

From Table 2, it is clear that a diverse variety of cardioprotective strategies and agents have been shown to protect the heart against acute IRI. Although some of these to appear act by

modulating cardioprotective signaling pathways underlying ischemic conditioning, others appear to target other cell survival and signaling pathways. In many cases, whether the miRNA is actually responsible for mediating the beneficial effects elicited by the cardioprotective strategy or agent has not been demonstrated. Moreover, in most cases the mechanisms through which the cardioprotective strategy of agent modulates miRNA expression is not known.

4. LncRNAs in acute myocardial ischemia/reperfusion injury

The lncRNAs have emerged as important regulators of cell function in both cardiac development and disease, and have been shown to regulate both gene expression and protein translation Nuclear-localized lncRNAs can regulate gene expression at both the epigenetic and transcriptional levels in either *cis* or *trans* position*,* according to whether the lncRNA gene is in close proximity or distant to their target genes, respectively. Cytosol-based lncRNAs can modify protein translation by blocking, stabilizing/destabilizing, or sponging miRNAs. LncRNAs can be classified into different groups based on their functional roles including acting as a: signal (expressed at a specific time or in a particular place following various stimuli); decoy (repressing transcription by sequestering transcription factors, chromatin remodelers); guide (binding transcription factors to their target sites); enhancer (inducing a chromosomal loop to bring together enhances and promoter regions); or scaffold (bringing multiprotein complexes together) [12].

LncRNAs have been investigated primarily in cardiac development and some cardiac diseases (left ventricular hypertrophy and heart failure), and have only recently been explored in the setting of acute myocardial IRI. Prior studies in liver [65] and brain [66], have described roles for lncRNAs in liver and brain tissue following acute IRI. Dharap et al [66] found that lncRNAs in brain tissue were highly dysregulated following acute IRI in a murine model of stroke. Chen et al [65] demonstrated that siRNA knock-down of lncRNA AK139328 protected the murine liver against

acute IRI by reducing caspase 3 activation and preserving levels of phosphorylated Akt, GSK-3 and eNOS.

One of the first studies to investigate the role of lncRNAs in the setting of acute IRI in the heart, was by Liu et al [67] who demonstrated marked upregulation of 5 lncRNAs (AK035396, ENSMUST00000156081, AK005401, ENSMUST00000118172 and ENSMUST00000118702), and downregulation of 5 lncRNAs (uc007prv.1, AK080112, ENSMUST00000170410, AK156124 and ENSMUST00000166777), in the infarcted murine heart at 2.5 hours of reperfusion. Changes in myocardial expression of lncRNAs were found to be associated with modulation of the mRNAs (CXCL1, CCL9, CXCL12, EDA, TNFAIP3 and BIRC3), targets which are known to be relevant to acute myocardial IRI. Using a similar approach in the porcine heart subjected to acute IRI, Kaikkonen et al [68] have shown changes in a large number of lncRNAs in infarcted tissue when compared to remote myocardium including lncRNAs directed to myocardial transcription factors GATA-binding protein 4, GATA-binding protein 6, and Krüppel-like factor 6. These changes were associated with the induction of inflammatory mediators and dampening of peroxisome proliferator-activated receptor signalling and oxidative phosphorylation, the ischemic region, and differential expression of transcriptional factors linked to ischemia including Krüppel-like factor, MEF2C, ETS, NFY, ATF, E2F2, and NRF1. Saddic et al [69] have recently investigated the changes in myocardial lncRNA expression in patients undergoing CABG surgery following acute ischemia in left ventricular biopsies taken at initiation of cardiopulmonary bypass, and after a median time of 74 minutes ischemia. Some of the major findings from this clinical study included: upregulation of 97 lncRNAs including RP11-64B16.4 (the extent of which was related to the ischemic time), and downregulation of 13 lncRNAs, changes which were associated with mRNA implicated in the stress and immune response to ischemia.

The role of lncRNAs as mediators of acute IRI and potential targets for cardioprotection has been recently investigated. Wang et al [70] found that the lncRNA, necrosis-related factor (NRF), prevented cell death induced by acute myocardial IRI, by binding to and repressing miR-

873, and attenuating RIPK1/RIPK3-mediated programmed cell necrosis, highlighting NRF as a novel target for cardioprotection. It has been shown in H9c2 cells that simulated IRI upregulated the lncRNA, maternally expressed gene 3 (MEG3), and siRNA knockdown of MEG3 reduced cell death and attenuated apoptosis [71]. The mechanism of protection was linked to miR-183-induced suppression of p27 through activation of the PI3K/AKT/FOXO3a signaling cascade. Finally, it has been shown that siRNA knockdown of the lncRNA, NONRATT021972, prevented the upregulation of $P2X_7$ in cervical sympathetic neurones resulting is dampening of the hemodynamic changes and less cell death induced by acute myocardial IRI [72,73]. Whether lncRNAs mediate the cardioprotection elicited by pharmacological agents and endogenous strategies such as ischemic conditioning, as is the case with miRNAs, is not known and needs to be investigated.

5. Cross-talk between miRNAs and lncRNAs

Emerging studies have shown a functional interaction between miRNAs and lncRNAs. The mutual interaction of miRNAs and lncRNAs can regulate each other's activities, and can be categorized into 4 types depending on the nature of the interaction and its effects: miRNAs inducing lncRNA decay; lncRNAs acting as miRNA sponges/decoys; lncRNAs and miRNAs competing for mRNAs; and lncRNAs acting as precursors for miRNAs [74,75].

During acute myocardial IRI, increased levels of the lncRNA, autophagy-promoting factor (APF), have been demonstrated to result in direct binding with miR-188-3p, which inhibited the latter's activity leading to enhanced cardiac autophagy, and greater MI injury [76]. Cardiac apoptosis-related lncRNA (CARL), a lncRNA that is important in the regulation of mitochondrial dynamics, cardiac apoptosis, and cardiac dysfunction, has been demonstrated to act as a ''sponge'' to miR-539 [77], a miRNA which binds PHB2 mRNA and inhibits the latter's activity. The presence of CARL thus upregulates the expression of PHB2 thereby suppressing mitochondrial fission and apoptosis. MiR-484 which is required for the inhibition of mitochondrial fission-

mediated apoptotic cell death can be bound by nuclear miR-361. The mitochondrial dynamic-related lncRNA (MDRL) has been shown to act as an endogenous sponge by binding to miR-361, downregulating its expression and inhibiting mitochondrial fission and apoptosis in cardiomyocytes [78]. Finally, the lncRNA, H19, can act as a sponge to block miR-103/107 expression, and in the presence of ischemia however, H19 expression is downregulated thereby increasing myocardial injury [79].

6. MiRNAs and lncRNAs as biomarkers in AMI patients

Although cardiac magnetic resonance imaging (MRI) remains the gold standard of imaging modality to assess the consequences of acute IRI in AMI patients in terms of MI size and adverse LV remodeling [80-82], this technique is limited in terms of ease of access and availability of skilled personnel to maneuver. The presence of ncRNAs in the serum plasma and bodily fluids, however, offer a window of opportunity to serve as biomarkers in AMI patients [83-85]. Several clinical studies have measured changes in circulating levels of miRNAs and lncRNAs from AMI patients soon after presentation, and have investigated their roles as novel biomarkers of myocardial injury for diagnosing AMI, and their ability to predict those AMI patients at risk of developing adverse post-MI modelling (reviewed in [86]). Although the plasma contains RNases, circulating ncRNAs especially circular lncRNAs have been shown to be stable in this environment indicating that they are relatively resistant to nucleolytic degradation, making them potentially useful as a circulating biomarker for AMI. Following AMI, myocardial necrosis induced by acute IRI results in the release of cardiac biomarkers such as Troponins (T and I) and CK-MB into the circulation, the presence of which has been shown to diagnose AMI and predict clinical outcomes.

Several clinical studies have demonstrated the release of miRNAs into the circulation in AMI patients suggesting that they can be used for the early diagnosis of AMI. The first clinical studies to investigate the utility of circulating cardiac- and muscle-enriched miRNAs following AMI demonstrated elevated levels of miR-1, miR-133a/b, miR-499, miR-423-5p, miR-208a/b [87-89], and

depressed levels of miR-122 and miR-375 levels [89], when compared to control subjects. Subsequent studies have shown that these miRNAs correlate with other cardiac biomarkers of AMI such as Troponin and CK-MB, with miR-208 having the highest specificity and sensitivity for AMI. However, their performance in detecting AMI are not superior to current cardiac biomarkers such as Troponin, and therefore their value may be enhanced by measuring multiple miRNAs in combination with conventional cardiac biomarkers. Elevated levels of miR-208b and miR-133a early after AMI have been demonstrated to predict patients at risk of developing adverse left ventricular remodeling after AMI, and correlate with increased risk of mortality or HF at 6 months [90-92], suggesting the miRNAs may be used to risk stratify AMI patients.

A number of non-cardiac miRNAs have also been implicated as biomarkers of myocardial injury and predictors of clinical outcomes following AMI. MiR-633b and miR-1291 have been shown to detect AMI with high specificity and sensitivity [93], and miR-150 and miR-486 have been shown to be able to distinguish STEMI from non-STEMI patients [94]. Larger clinical studies with extended follow-up have shown that elevated levels of miR-126, miR-197, miR-223, miR-155, miR-380*, miR-192, miR-194, miR-34a, miR-328, miR-134, miR16, miR-27a [95-100], and low levels of miR-223, miR-197, miR-150, miR-101 [95,97], were associated with risk of heart failure and cardiovascular death. The source of these circulating non-cardiac miRNAs is not clear and further work is required to elucidate their contribution to the pathophysiology of AMI and subsequent clinical outcomes.

One of the first clinical studies to investigate the role of circulating lncRNAs in AMI patients, was by Vausort et al [101], who demonstrated elevations in plasma of the following lncRNAs; hypoxia inducible factor 1A antisense RNA 2 (aRNA), potassium voltage-gated channel KQT-like subfamily, member 1 opposite strand/antisense transcript 1 (KCNQ1OT1), myocardial infarction-associated transcript (MIAT]), and metastasis associated lung adenocarcinoma transcript 1 [MALAT1]). In contrast, the lncRNA, plasma levels of cyclin-dependent kinase inhibitor 2B antisense RNA 1 (ANRIL) was decreased following AMI. Interestingly, plasma levels of ANRIL

and KCNQ1OT1 were found to be predictive of LV dysfunction in AMI patients. In a subsequent experimental study, Li et al [102] demonstrated that downregulation of the KCNQ1OT1 lncRNA, protected H9C2 cardiac cells from simulated IRI, and this protective effect was mediated via the adiponectin receptor and suppression of the p38 MAPK/NF-kB signal cascade.

In another clinical study [103], plasma levels of the circular lncRNA, urothelial carcinoma-associated 1 (UCA1), were found to be depressed soon after AMI (within 2-6 hours), and increased at day 3, changes which were inversely related to miRNA, suggesting that it may have a role as a novel cardiac biomarker, although its ability to diagnose AMI was not superior to the current standard cardiac biomarkers, Troponin I and CK-MB. Zhang et al [104] demonstrated reciprocal changes in the lncRNAs, Zinc finger antisense 1 (ZFAS1) and Cdr1 antisense (CDR1AS), in AMI patients, with lower levels of ZFAS1 and elevated levels of CDR1AS in blood samples taken 3.5 hours after onset of acute myocardial ischemia. Both these lncRNAs were shown to independently predict AMI. Another clinical study has reported elevations in the lncRNA Myosin Heavy Chain Associated RNA Transcripts (MHRT) in the blood from AMI patients, when compared to controls, and went onto show that siRNA knock-down of MHRT in neonatal rat cardiomyocytes reduce cell death following oxidative stress, suggesting a cardioprotective role for this MHRT [105]. Finally, in a recent clinical study, reduced plasma levels of the lncRNAs (ENST00000416860.2, ENST00000421157.1 and TCONS_00025701) were demonstrated, but the impact of these changes on MI size or subsequent remodeling was not investigated [106].

7. Therapeutic targeting of ncRNA as a cardioprotective strategy

7.1. Delivery of ncRNA to the ischemic heart

Non-coding RNA or the antisense molecules may be delivered to the ischemic heart using a number of approaches including intravenous or intramyocardial injections or carriers such as viruses, nanoparticles, or exosomes. Nanoparticles, which can be made heart tissue-specific, can be used to improve the bioavailability of ncRNA genes or the antisense molecules, to the ischemic

tissue following AMI [106]. The administration of exosomes have been shown to confer cardioprotection against acute myocardial IRI in experimental animal studies. In addition, delivery of miRNAs to the ischemic heart using exosomes has been shown to reduce MI size following AMI [107,108]. Exosomes secreted by a variety of cells (induced pluripotent stem cells [iPS], H9c2 cells, and cardiosphere-derived cells [CDCs]) have been shown to contain miRNAs which contribute to the cardioprotective effect elicited by the exosomes following AMI. Wang et al [109] demonstrated that exosomes harvested from mouse cardiac fibroblast-derived iPS cells protected H9c2 cells against hydrogen-peroxide induced oxidative stress and inhibited caspases 3 and 7 via miR-21 and miR-210. Zhang et al [110] found that hypoxia modified the expression of several miRNAs in exosomes generated by H9c2 cells, and these exosomal miRNAs (including miR-21-5p, miR-378-3p, miR-152-3p, and let-7i-5p, were able to protect naïve H9c2 cell against simulated IRI and prevent apoptosis through HIF-1, TNF, MAPK, and mTOR signaling pathways. Furthermore, a luciferase reporter assay confirmed that Atg12 and Fas ligand were the targets of miR-152-3p and let-7i-5p, respectively. Finally, Couto et al [111] have recently demonstrated that administering exosomes containing miR-181b harvested from CDCs reduced MI size in rat and pig models of myocardial infarction, and this protected effect was mediated through the PKC-δ and polarization of macrophages to an anti-inflammatory phenotype.

7.2. Therapeutic targeting of ncRNAs

Given that a number of miRNAs have been shown to have detrimental effects during acute myocardial IRI, therapeutic inhibition of these miRNAs may provide a potential therapeutic strategy for cardioprotection, although the timing of intervention is crucial to elicit either protection against reperfusion injury or circumvent negative remodeling post-MI A number of different approaches have been used to inhibit miRNAs including antisense oligonucleotides and locked nucleic acid (LNA)-modified oligonucleotides. Injection of chemically modified, cholesterol-conjugated, single-stranded RNA analogs complementary to miRNAs (termed “antagomirs”) have

been used to therapeutically silence detrimental miRNAs *in vivo* following AMI, in mainly rodent models of acute myocardial IRI (see Table 1). In terms of eventually translating this therapeutic approach into the clinical setting, some experimental studies have demonstrated that inhibiting detrimental miRNAs such as to miR-15 and miR-92a at the onset of reperfusion reduced MI size in large animal (porcine) models of acute myocardial IRI (see Table 1). This approach is technically feasible, as only a single dose of the antisense oligonucleotide is required to reduce acute MI size. However, in order to achieve long-term or chronic effects (for example following post-MI cardiac remodeling or heart failure), multiple doses of antagomirs will be required for sustained inhibition of miRNA, which presents a challenge in terms of delivery and off-target effects. LNA-modified oligonucleotides are more stable than antagomirs and have enhanced specificity toward complementary RNA or DNA, hence allowing for shorter molecules. LNA-modified oligonucleotides have been shown to provide both sustained and potent silencing of cardiac expressed miRNAs, irrespective of the method of delivery.

Delivery of ncRNA mimics to the ischemic heart may be used to target miRNAs or lncRNAs which are known to be beneficial for cardioprotection, but are downregulated following AMI. Finally, an alternative approach to upregulate cardioprotective miRNAs, can be to use a small molecule therapeutic strategy. By screening small molecules on their ability to induce miR-182, Lee et al [112] identified the hit compound Kenpaullone as a cardioprotective small molecule capable of increasing myocaridal expression of miR-182 and protecting against acute IRI.

8. Conclusions

A number of miRNAs and more recently lncRNAs have been investigated in the setting of acute myocardial IRI, many of which have been shown to be biomarkers of cardiac disease or potential therapeutic targets for protecting against AMI preventing heart failure. In this setting, ncRNAs may be targeted either directly using ncRNA mimetics and/or antagomiRs, although the stability and specificity of these compounds require further investigation. An alternative will be indirect

targeting using pharmacological agents or endogenous cardioprotective strategies such as ischemic conditioning. In addition, further work is required to investigate the interplay of ncRNAs with known cardioprotective signaling pathways underlying ischemic preconditioning, postconditioning, and remote ischemic conditioning. Finally, more research is required to optimize the delivery of ncRNA to the ischemic heart in order to translate the targeting ncRNA as a cardioprotective strategy for reducing MI size and preventing heart failure following AMI, particularly with the possible issue of uptake by non-target tissues and off-target effects. In this regard, ongoing studies are investigating the use of nanoparticles, viruses and exosomes to improve the cardiac-specific delivery, and increase bioavailability of ncRNAs to the ischemic heart.

Expert Opinion

The therapeutic targeting of ncRNA in cardiac disease is only beginning to be unraveled and much more remains to be learned. Nonetheless, ncRNAs as important regulators during cardiac disease together with the feasibility to potently inhibit or upregulate specific ncRNAs, makes them exciting new candidates to target in the setting of ischemic heart disease. Analogous to protein kinases/phosphatases, the ncRNAs can work independently or in concert to achieve their diverse effects on cell survival and death. Further complicating the scenario is the fact that multiple upstream genes can exert their actions on the ncRNAs, thus rendering the understanding of the regulatory network more difficult. In addition, different ncRNAs exert different effects at different locations at the site of injury in the heart, e.g. remote vs border vs infarct area. Yet, it is the dynamism of the ncRNAs that may potentially allow them to act as biomarkers to predict AMI occurrence, severity of injury and future degree of recovery, provided sample collection and testing protocols for the ncRNAs are standardized. Interventions using stable ncRNA mimetics and/or antagomirs are likely to emerge as therapeutic strategies for protecting against AMI although the off-target effects and non-target tissue uptake of the ncRNAs remain extremely

relevant prior to clinical trial testing. In summary, key features in harnessing ncRNAs to combat cardiac disease require: (1) identifying novel ncRNAs that serve as biomarkers for AMI and predict clinical outcomes following AMI; and (2) defining the optimum conditions, e.g. cell type specificity, timing of delivery, method of delivery, for the use of ncRNAs and their inhibitors for reducing acute myocardial IRI and preventing heart failure. In addition to serving its potential as a therapeutic target for AMI, the ncRNAs may hold further potential for alternative therapies such as cellular therapy or even cardiac regeneration to repair the damaged heart

Article highlights box

- Once considered “genomic junk”, non-coding regions of the genome are shown to produce ncRNAs which play critical roles in the regulation of cardiac development and cardiac diseases such as left ventricular hypertrophy, heart failure, and acute myocardial infarction.
- Both miRNAs and lncRNAs play important role in the pathogenesis of acute myocardial ischemia-reperfusion injury.
- Modulation of miRNAs and lncRNAs provides a potential therapeutic strategy for cardioprotection.
- Emerging evidence supports the existence of cross-talk between miRNAs and lncRNAs that regulate distinct molecular events during acute myocardial IRI.

Reference List

1. Cabrera-Fuentes HA, Aragones J, Bernhagen J, Boening A, Boisvert WA, Botker HE, Bulluck H, Cook S, Di LF, Engel FB, Engelmann B, Ferrazzi F, Ferdinandy P, Fong A, Fleming I, Gnaiger E, Hernandez-Resendiz S, Kalkhoran SB, Kim MH, Lecour S, Liehn EA, Marber MS, Mayr M, Miura T, Ong SB, Peter K, Sedding D, Singh MK, Suleiman MS, Schnittler HJ, Schulz R, Shim W, Tello D, Vogel CW, Walker M, Li QO, Yellon DM, Hausenloy DJ, Preissner KT. From basic mechanisms to clinical applications in heart protection, new players in cardiovascular diseases and cardiac theranostics: meeting report from the third international symposium on "New frontiers in cardiovascular research". *Basic Res Cardiol*. 2016;111:69. 10.1007/s00395-016-0586-x [doi];10.1007/s00395-016-0586-x [pii]

2. Hausenloy DJ, Garcia-Dorado D, Botker HE, Davidson SM, Downey J, Engel FB, Jennings R, Lecour S, Leor J, Madonna R, Ovize M, Perrino C, Prunier F, Schulz R, Sluijter JPG, van Laake LW, Vinten-Johansen J, Yellon DM, Ytrehus K, Heusch G, Ferdinandy P. Novel targets and future strategies for acute cardioprotection: Position Paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart. *Cardiovasc Res*. 2017;113:564-585. 3074260 [pii];10.1093/cvr/cvx049 [doi]

3. Campo G, Pavasini R, Morciano G, Lincoff AM, Gibson CM, Kitakaze M, Lonborg J, Ahluwalia A, Ishii H, Frenneaux M, Ovize M, Galvani M, Atar D, Ibanez B, Cerisano G, Biscaglia S, Neil BJ, Asakura M, Engstrom T, Jones DA, Dawson D, Ferrari R, Pinton P, Ottani F. Clinical benefit of drugs targeting mitochondrial function as an adjunct to reperfusion in ST-segment elevation myocardial infarction: A meta-analysis of randomized clinical trials. *Int J Cardiol*. 2017;244:59-66. S0167-5273(17)31985-X [pii];10.1016/j.ijcard.2017.06.040 [doi]

4. Campo G, Pavasini R, Morciano G, Lincoff MA, Gibson C, Kitakaze M, Lonborg J, Ahluwalia A, Ishii H, Frenneaux M, Ovize M, Galvani M, Atar D, Ibanez B, Cerisano G, Biscaglia S, Neil BJ, Asakura M, Engstrom T, Jones DA, Dawson D, Ferrari R, Pinton P, Ottani F. Data on administration of cyclosporine, nicorandil, metoprolol on reperfusion related outcomes in ST-segment Elevation Myocardial Infarction treated with percutaneous coronary intervention. *Data Brief*. 2017;14:197-205. 10.1016/j.dib.2017.07.033 [doi];S2352-3409(17)30335-9 [pii]

5. Hausenloy DJ, Botker HE, Engstrom T, Erlinge D, Heusch G, Ibanez B, Kloner RA, Ovize M, Yellon DM, Garcia-Dorado D. Targeting reperfusion injury in patients with ST-segment elevation myocardial infarction: trials and tribulations. *Eur Heart J*. 2017;38:935-941. ehw145 [pii];10.1093/eurheartj/ehw145 [doi]

6. Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature*. 2005;436:214-220. nature03817 [pii];10.1038/nature03817 [doi]

7. Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Hoydal M, Autore C, Russo MA, Dorn GW, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G. MicroRNA-133 controls cardiac hypertrophy. *Nat Med*. 2007;13:613-618. nm1582 [pii];10.1038/nm1582 [doi]

8. van RE, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, Richardson JA, Olson EN. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc Natl Acad Sci U S A*. 2006;103:18255-18260. 0608791103 [pii];10.1073/pnas.0608791103 [doi]

9. Stieger P, Daniel JM, Tholen C, Dutzmann J, Knopp K, Gunduz D, Aslam M, Kampschulte M, Langheinrich A, Fischer S, Cabrera-Fuentes H, Wang Y, Wollert KC, Bauersachs J, Braun-Dullaeus R, Preissner KT, Sedding DG. Targeting of Extracellular RNA Reduces Edema Formation and Infarct Size and Improves Survival After Myocardial Infarction in Mice. *J Am Heart Assoc*. 2017;6. JAHA.116.004541 [pii];10.1161/JAHA.116.004541 [doi]

10. Yin C, Wang X, Kukreja RC. Endogenous microRNAs induced by heat-shock reduce myocardial infarction following ischemia-reperfusion in mice. *FEBS Lett*. 2008;582:4137-4142. S0014-5793(08)00922-8 [pii];10.1016/j.febslet.2008.11.014 [doi]

11. Poller W, Dimmeler S, Heymans S, Zeller T, Haas J, Karakas M, Leistner DM, Jakob P, Nakagawa S, Blankenberg S, Engelhardt S, Thum T, Weber C, Meder B, Hajjar R, Landmesser U. Non-coding RNAs in cardiovascular diseases: diagnostic and therapeutic perspectives. *Eur Heart J*. 2017. 3738809 [pii];10.1093/eurheartj/ehx165 [doi]

12. Bar C, Chatterjee S, Thum T. Long Noncoding RNAs in Cardiovascular Pathology, Diagnosis, and Therapy. *Circulation*. 2016;134:1484-1499. CIRCULATIONAHA.116.023686 [pii];10.1161/CIRCULATIONAHA.116.023686 [doi]

13. van RE, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc Natl Acad Sci U S A*. 2008;105:13027-13032. 0805038105 [pii];10.1073/pnas.0805038105 [doi]

14. Hall AR, Burke N, Dongworth RK, Hausenloy DJ. Mitochondrial fusion and fission proteins: novel therapeutic targets for combating cardiovascular disease. *Br J Pharmacol*. 2014;171:1890-1906. 10.1111/bph.12516 [doi]

15. Burke N, Hall AR, Hausenloy DJ. OPA1 in Cardiovascular Health and Disease. *Curr Drug Targets*. 2015;16:912-920. CDT-EPUB-64308 [pii]

16. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986;74:1124-1136.

17. Marber MS, Latchman DS, Walker JM, Yellon DM. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation*. 1993;88:1264-1272.

18. Kuzuya T, Hoshida S, Yamashita N, Fuji H, Oe H, Hori M, Kamada T, Tada M. Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. *Circ Res*. 1993;72:1293-1299.

19. Hausenloy DJ, Yellon DM. The Second Window of Preconditioning (SWOP) Where Are We Now? *Cardiovasc Drugs Ther*. 2010.

20. Cheng Y, Zhu P, Yang J, Liu X, Dong S, Wang X, Chun B, Zhuang J, Zhang C. Ischaemic preconditioning-regulated miR-21 protects heart against ischaemia/reperfusion injury via anti-apoptosis through its target PDCD4. *Cardiovasc Res*. 2010;87:431-439. cvq082 [pii];10.1093/cvr/cvq082 [doi]

21. Duan X, Ji B, Wang X, Liu J, Zheng Z, Long C, Tang Y, Hu S. Expression of microRNA-1 and microRNA-21 in different protocols of ischemic conditioning in an isolated rat heart model. *Cardiology*. 2012;122:36-43. 000338149 [pii];10.1159/000338149 [doi]

22. Wang X, Zhu H, Zhang X, Liu Y, Chen J, Medvedovic M, Li H, Weiss MJ, Ren X, Fan GC. Loss of the miR-144/451 cluster impairs ischaemic preconditioning-mediated cardioprotection by targeting Rac-1. *Cardiovasc Res*. 2012;94:379-390. cvs096 [pii];10.1093/cvr/cvs096 [doi]

23. Varga ZV, Zvara A, Farago N, Kocsis GF, Pipicz M, Gaspar R, Bencsik P, Gorbe A, Csonka C, Puskas LG, Thum T, Csont T, Ferdinandy P. MicroRNAs associated with ischemia-reperfusion injury and cardioprotection by ischemic pre- and postconditioning: protectomiRs. *Am J Physiol Heart Circ Physiol*. 2014;307:H216-H227. ajpheart.00812.2013 [pii];10.1152/ajpheart.00812.2013 [doi]

24. Cheng Y, Tan N, Yang J, Liu X, Cao X, He P, Dong X, Qin S, Zhang C. A translational study of circulating cell-free microRNA-1 in acute myocardial infarction. *Clin Sci (Lond)*. 2010;119:87-95. CS20090645 [pii];10.1042/CS20090645 [doi]

25. Rane S, He M, Sayed D, Vashistha H, Malhotra A, Sadoshima J, Vatner DE, Vatner SF, Abdellatif M. Downregulation of miR-199a derepresses hypoxia-inducible factor-1alpha and Sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. *Circ Res*. 2009;104:879-886. CIRCRESAHA.108.193102 [pii];10.1161/CIRCRESAHA.108.193102 [doi]

26. Ong SG, Hausenloy DJ. Hypoxia-inducible factor as a therapeutic target for cardioprotection. *Pharmacol Ther*. 2012;136:69-81. S0163-7258(12)00130-1 [pii];10.1016/j.pharmthera.2012.07.005 [doi]

27. Ong SG, Lee WH, Theodorou L, Kodo K, Lim SY, Shukla DH, Briston T, Kiriakidis S, Ashcroft M, Davidson SM, Maxwell PH, Yellon DM, Hausenloy DJ. HIF-1 reduces ischaemia-reperfusion injury in the heart by targeting the mitochondrial permeability transition pore. *Cardiovasc Res*. 2014;104:24-36. cvu172 [pii];10.1093/cvr/cvu172 [doi]

28. Yin C, Salloum FN, Kukreja RC. A novel role of microRNA in late preconditioning: upregulation of endothelial nitric oxide synthase and heat shock protein 70. *Circ Res*. 2009;104:572-575. CIRCRESAHA.108.193250 [pii];10.1161/CIRCRESAHA.108.193250 [doi]

29. Rane S, He M, Sayed D, Yan L, Vatner D, Abdellatif M. An antagonism between the AKT and beta-adrenergic signaling pathways mediated through their reciprocal effects on miR-199a-5p. *Cell Signal*. 2010;22:1054-1062. S0898-6568(10)00061-6 [pii];10.1016/j.cellsig.2010.02.008 [doi]

30. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol*. 2003;285:H579-H588.

31. Tsang A, Hausenloy DJ, Mocanu MM, Yellon DM. Postconditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway. *Circ Res*. 2004;95:230-232.

32. Tsang A, Hausenloy DJ, Yellon DM. Myocardial postconditioning: reperfusion injury revisited. *Am J Physiol Heart Circ Physiol*. 2005;289:H2-H7.

33. Hausenloy DJ. Cardioprotection techniques: preconditioning, postconditioning and remote con-ditioning (basic science). *Curr Pharm Des*. 2013;19:4544-4563. CPD-EPUB-20121221-5 [pii]

34. Hausenloy DJ, Yellon DM. Ischaemic conditioning and reperfusion injury. *Nat Rev Cardiol*. 2016;13:193-209. nrcardio.2016.5 [pii];10.1038/nrcardio.2016.5 [doi]

35. He B, Xiao J, Ren AJ, Zhang YF, Zhang H, Chen M, Xie B, Gao XG, Wang YW. Role of miR-1 and miR-133a in myocardial ischemic postconditioning. *J Biomed Sci*. 2011;18:22. 1423-0127-18-22 [pii];10.1186/1423-0127-18-22 [doi]

36. Tu Y, Wan L, Fan Y, Wang K, Bu L, Huang T, Cheng Z, Shen B. Ischemic postconditioning-mediated miRNA-21 protects against cardiac ischemia/reperfusion injury via PTEN/Akt pathway. *PLoS One*. 2013;8:e75872. 10.1371/journal.pone.0075872 [doi];PONE-D-13-17169 [pii]

37. Wan DY, Zhang Z, Yang HH. Cardioprotective effect of miR-214 in myocardial ischemic postconditioning by down-regulation of hypoxia inducible factor 1, alpha subunit inhibitor. *Cell Mol Biol (Noisy -le-grand)*. 2015;61:1-6.

38. Baars T, Skyschally A, Klein-Hitpass L, Cario E, Erbel R, Heusch G, Kleinbongard P. microRNA expression and its potential role in cardioprotection by ischemic postconditioning in pigs. *Pflugers Arch*. 2014;466:1953-1961. 10.1007/s00424-013-1429-3 [doi]

39. Gao Y, Huang R, Chen R, Li J, Luo W. Ischemic postconditioning altered microRNAs in human valve replacement. *J Surg Res*. 2016;200:28-35. S0022-4804(15)00751-9 [pii];10.1016/j.jss.2015.07.010 [doi]

40. Bian B, Yu XF, Wang GQ, Teng TM. Role of miRNA-1 in regulating connexin 43 in ischemia-reperfusion heart injury: a rat model. *Cardiovasc Pathol*. 2017;27:37-42. S1054-8807(16)30255-1 [pii];10.1016/j.carpath.2016.12.006 [doi]

41. Zhu J, Yao K, Wang Q, Guo J, Shi H, Ma L, Liu H, Gao W, Zou Y, Ge J. Ischemic Postconditioning-Regulated miR-499 Protects the Rat Heart Against Ischemia/Reperfusion Injury by Inhibiting Apoptosis through PDCD4. *Cell Physiol Biochem*. 2016;39:2364-2380. 000452506 [pii];10.1159/000452506 [doi]

42. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation*. 1993;87:893-899.

43. Hausenloy DJ, Yellon DM. Remote ischaemic preconditioning: underlying mechanisms and clinical application. *Cardiovasc Res*. 2008;79:377-386.

44. Pickard JM, Botker HE, Crimi G, Davidson B, Davidson SM, Dutka D, Ferdinandy P, Ganske R, Garcia-Dorado D, Giricz Z, Gourine AV, Heusch G, Kharbanda R, Kleinbongard P, MacAllister R, McIntyre C, Meybohm P, Prunier F, Redington A, Robertson NJ, Suleiman MS, Vanezis A, Walsh S, Yellon DM, Hausenloy DJ. Remote ischemic conditioning: from experimental observation to clinical application: report from the 8th Biennial Hatter Cardiovascular Institute Workshop. *Basic Res Cardiol*. 2015;110:453. 10.1007/s00395-014-0453-6 [doi]

45. Sivaraman V, Pickard JM, Hausenloy DJ. Remote ischaemic conditioning: cardiac protection from afar. *Anaesthesia*. 2015;70:732-748. 10.1111/anae.12973 [doi]

46. Oxman T, Arad M, Klein R, Avazov N, Rabinowitz B. Limb ischemia preconditions the heart against reperfusion tachyarrhythmia. *Am J Physiol*. 1997;273:H1707-H1712.

47. Birnbaum Y, Hale SL, Kloner RA. Ischemic preconditioning at a distance: reduction of myocardial infarct size by partial reduction of blood supply combined with rapid stimulation of the gastrocnemius muscle in the rabbit. *Circulation*. 1997;96:1641-1646.

48. Kharbanda RK, Mortensen UM, White PA, Kristiansen SB, Schmidt MR, Hoschtitzky JA, Vogel M, Sorensen K, Redington AN, MacAllister R. Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation*. 2002;106:2881-2883.

49. Candilio L, Hausenloy DJ, Yellon DM. Remote ischemic conditioning: a clinical trial's update. *J Cardiovasc Pharmacol Ther*. 2011;16:304-312. 16/3-4/304 [pii];10.1177/1074248411411711 [doi]

50. Candilio L, Malik A, Hausenloy DJ. Protection of organs other than the heart by remote ischemic conditioning. *J Cardiovasc Med (Hagerstown)*. 2013;14:193-205. 10.2459/JCM.0b013e328359dd7b [doi]

51. Hausenloy DJ, Candilio L, Evans R, Ariti C, Jenkins DP, Kolvekar S, Knight R, Kunst G, Laing C, Nicholas J, Pepper J, Robertson S, Xenou M, Clayton T, Yellon DM. Remote Ischemic Preconditioning and Outcomes of Cardiac Surgery. *N Engl J Med*. 2015;373:1408-1417. 10.1056/NEJMoa1413534 [doi]

52. White SK, Frohlich GM, Sado DM, Maestrini V, Fontana M, Treibel TA, Tehrani S, Flett AS, Meier P, Ariti C, Davies JR, Moon JC, Yellon DM, Hausenloy DJ. Remote ischemic conditioning reduces myocardial infarct size and edema in patients with ST-segment

elevation myocardial infarction. *JACC Cardiovasc Interv*. 2015;8:178-188. S1936-8798(14)01073-5 [pii];10.1016/j.jcin.2014.05.015 [doi]

53. Hausenloy DJ, Iliodromitis EK, Andreadou I, Papalois A, Gritsopoulos G, Anastasiou-Nana M, Kremastinos DT, Yellon DM. Investigating the signal transduction pathways underlying remote ischemic conditioning in the porcine heart. *Cardiovasc Drugs Ther*. 2012;26:87-93. 10.1007/s10557-011-6364-y [doi]

54. Pickard JM, Davidson SM, Hausenloy DJ, Yellon DM. Co-dependence of the neural and humoral pathways in the mechanism of remote ischemic conditioning. *Basic Res Cardiol*. 2016;111:50. 10.1007/s00395-016-0568-z [doi];10.1007/s00395-016-0568-z [pii]

55. Brandenburger T, Grievink H, Heinen N, Barthel F, Huhn R, Stachuletz F, Kohns M, Pannen B, Bauer I. Effects of remote ischemic preconditioning and myocardial ischemia on microRNA-1 expression in the rat heart in vivo. *Shock*. 2014;42:234-238. 10.1097/SHK.0000000000000201 [doi]

56. Slagsvold KH, Rognmo O, Hoydal M, Wisloff U, Wahba A. Remote ischemic preconditioning preserves mitochondrial function and influences myocardial microRNA expression in atrial myocardium during coronary bypass surgery. *Circ Res*. 2014;114:851-859. CIRCRESAHA.114.302751 [pii];10.1161/CIRCRESAHA.114.302751 [doi]

57. Hu Q, Luo W, Huang L, Huang R, Chen R. Apoptosis-related microRNA changes in the right atrium induced by remote ischemic perconditioning during valve replacement surgery. *Sci Rep*. 2016;6:18959. srep18959 [pii];10.1038/srep18959 [doi]

58. Slagsvold KH, Moreira JB, Rognmo O, Hoydal M, Bye A, Wisloff U, Wahba A. Remote ischemic preconditioning preserves mitochondrial function and activates pro-survival protein kinase Akt in the left ventricle during cardiac surgery: a randomized trial. *Int J Cardiol*. 2014;177:409-417. S0167-5273(14)01947-0 [pii];10.1016/j.ijcard.2014.09.206 [doi]

59. Li J, Rohailla S, Gelber N, Rutka J, Sabah N, Gladstone RA, Wei C, Hu P, Kharbanda RK, Redington AN. MicroRNA-144 is a circulating effector of remote ischemic preconditioning. *Basic Res Cardiol*. 2014;109:423. 10.1007/s00395-014-0423-z [doi]

60. Giricz Z, Varga ZV, Baranyai T, Sipos P, Paloczi K, Kittel A, Buzas EI, Ferdinandy P. Cardioprotection by remote ischemic preconditioning of the rat heart is mediated by extracellular vesicles. *J Mol Cell Cardiol*. 2014;68:75-78. S0022-2828(14)00014-5 [pii];10.1016/j.yjmcc.2014.01.004 [doi]

61. Vicencio JM, Yellon DM, Sivaraman V, Das D, Boi-Doku C, Arjun S, Zheng Y, Riquelme JA, Kearney J, Sharma V, Multhoff G, Hall AR, Davidson SM. Plasma exosomes protect the myocardium from ischemia-reperfusion injury. *J Am Coll Cardiol*. 2015;65:1525-1536. S0735-1097(15)00619-1 [pii];10.1016/j.jacc.2015.02.026 [doi]

62. Pryds K, Nielsen RR, Hoff CM, Tolbod LP, Bouchelouche K, Li J, Schmidt MR, Redington AN, Frokiaer J, Botker HE. Effect of remote ischemic conditioning on myocardial perfusion in patients with suspected ischemic coronary artery disease. *J Nucl Cardiol*. 2016. 10.1007/s12350-016-0709-7 [doi];10.1007/s12350-016-0709-7 [pii]

63. Wei M, Xin P, Li S, Tao J, Li Y, Li J, Liu M, Li J, Zhu W, Redington AN. Repeated remote ischemic postconditioning protects against adverse left ventricular remodeling and improves survival in a rat model of myocardial infarction. *Circ Res*. 2011;108:1220-1225. CIRCRESAHA.110.236190 [pii];10.1161/CIRCRESAHA.110.236190 [doi]

64. Yamaguchi T, Izumi Y, Nakamura Y, Yamazaki T, Shiota M, Sano S, Tanaka M, Osada-Oka M, Shimada K, Miura K, Yoshiyama M, Iwao H. Repeated remote ischemic conditioning attenuates left ventricular remodeling via exosome-mediated intercellular communication on chronic heart failure after myocardial infarction. *Int J Cardiol*. 2015;178:239-246. S0167-5273(14)02081-6 [pii];10.1016/j.ijcard.2014.10.144 [doi]

65. Chen Z, Jia S, Li D, Cai J, Tu J, Geng B, Guan Y, Cui Q, Yang J. Silencing of long noncoding RNA AK139328 attenuates ischemia/reperfusion injury in mouse livers. *PLoS One*. 2013;8:e80817. 10.1371/journal.pone.0080817 [doi];PONE-D-13-35181 [pii]

66. Dharap A, Nakka VP, Vemuganti R. Effect of focal ischemia on long noncoding RNAs. *Stroke*. 2012;43:2800-2802. STROKEAHA.112.669465 [pii];10.1161/STROKEAHA.112.669465 [doi]

67. Liu Y, Li G, Lu H, Li W, Li X, Liu H, Li X, Li T, Yu B. Expression profiling and ontology analysis of long noncoding RNAs in post-ischemic heart and their implied roles in ischemia/reperfusion injury. *Gene*. 2014;543:15-21. S0378-1119(14)00416-8 [pii];10.1016/j.gene.2014.04.016 [doi]

68. Kaikkonen MU, Halonen P, Liu OH, Turunen TA, Pajula J, Moreau P, Selvarajan I, Tuomainen T, Aavik E, Tavi P, Yla-Herttuala S. Genome-Wide Dynamics of Nascent Noncoding RNA Transcription in Porcine Heart After Myocardial Infarction. *Circ Cardiovasc Genet*. 2017;10. CIRCGENETICS.117.001702 [pii];10.1161/CIRCGENETICS.117.001702 [doi]

69. Saddic LA, Sigurdsson MI, Chang TW, Mazaika E, Heydarpour M, Shernan SK, Seidman CE, Seidman JG, Aranki SF, Body SC, Muehlschlegel JD. The Long Noncoding RNA Landscape of the Ischemic Human Left Ventricle. *Circ Cardiovasc Genet*. 2017;10. CIRCGENETICS.116.001534 [pii];10.1161/CIRCGENETICS.116.001534 [doi]

70. Wang K, Liu F, Liu CY, An T, Zhang J, Zhou LY, Wang M, Dong YH, Li N, Gao JN, Zhao YF, Li PF. The long noncoding RNA NRF regulates programmed necrosis and myocardial injury during ischemia and reperfusion by targeting miR-873. *Cell Death Differ*. 2016;23:1394-1405. cdd201628 [pii];10.1038/cdd.2016.28 [doi]

71. Gong L, Xu H, Chang H, Tong Y, Zhang T, Guo G. Knockdown of long non-coding RNA MEG3 protects H9c2 cells from hypoxia-induced injury by targeting microRNA-183. *J Cell Biochem*. 2017. 10.1002/jcb.26304 [doi]

72. Tu G, Zou L, Liu S, Wu B, Lv Q, Wang S, Xue Y, Zhang C, Yi Z, Zhang X, Li G, Liang S. Long noncoding NONRATT021972 siRNA normalized abnormal sympathetic activity mediated by the upregulation of P2X7 receptor in superior cervical ganglia after myocardial ischemia. *Purinergic Signal*. 2016;12:521-535. 10.1007/s11302-016-9518-3 [doi];10.1007/s11302-016-9518-3 [pii]

73. Zou L, Tu G, Xie W, Wen S, Xie Q, Liu S, Li G, Gao Y, Xu H, Wang S, Xue Y, Wu B, Lv Q, Ying M, Zhang X, Liang S. LncRNA NONRATT021972 involved the pathophysiologic processes mediated by P2X7 receptors in stellate ganglia after myocardial ischemic injury. *Purinergic Signal*. 2016;12:127-137. 10.1007/s11302-015-9486-z [doi];10.1007/s11302-015-9486-z [pii]

74. Yoon JH, Abdelmohsen K, Gorospe M. Functional interactions among microRNAs and long noncoding RNAs. *Semin Cell Dev Biol*. 2014;34:9-14. S1084-9521(14)00170-0 [pii];10.1016/j.semcdb.2014.05.015 [doi]

75. Li N, Ponnusamy M, Li MP, Wang K, Li PF. The Role of MicroRNA and LncRNA-MicroRNA Interactions in Regulating Ischemic Heart Disease. *J Cardiovasc Pharmacol Ther*. 2016. 1074248416667600 [pii];10.1177/1074248416667600 [doi]

76. Wang K, Liu CY, Zhou LY, Wang JX, Wang M, Zhao B, Zhao WK, Xu SJ, Fan LH, Zhang XJ, Feng C, Wang CQ, Zhao YF, Li PF. APF lncRNA regulates autophagy and myocardial infarction by targeting miR-188-3p. *Nat Commun*. 2015;6:6779. ncomms7779 [pii];10.1038/ncomms7779 [doi]

77. Wang K, Long B, Zhou LY, Liu F, Zhou QY, Liu CY, Fan YY, Li PF. CARL lncRNA inhibits anoxia-induced mitochondrial fission and apoptosis in cardiomyocytes by impairing miR-539-dependent PHB2 downregulation. *Nat Commun*. 2014;5:3596. ncomms4596 [pii];10.1038/ncomms4596 [doi]

78. Wang K, Sun T, Li N, Wang Y, Wang JX, Zhou LY, Long B, Liu CY, Liu F, Li PF. MDRL lncRNA regulates the processing of miR-484 primary transcript by targeting miR-361. *PLoS Genet*. 2014;10:e1004467. 10.1371/journal.pgen.1004467 [doi];PGENETICS-D-13-03236 [pii]

79. Wang JX, Zhang XJ, Li Q, Wang K, Wang Y, Jiao JQ, Feng C, Teng S, Zhou LY, Gong Y, Zhou ZX, Liu J, Wang JL, Li PF. MicroRNA-103/107 Regulate Programmed Necrosis and Myocardial Ischemia/Reperfusion Injury Through Targeting FADD. *Circ Res*. 2015;117:352-363. CIRCRESAHA.117.305781 [pii];10.1161/CIRCRESAHA.117.305781 [doi]

80. Perazzolo MM, Lima JA, Iliceto S. MRI in acute myocardial infarction. *Eur Heart J*. 2011;32:284-293. ehq409 [pii];10.1093/eurheartj/ehq409 [doi]

81. Hausenloy DJ, Bulluck H. Risk Stratification by Cardiovascular Magnetic Resonance After Reperfused ST-Segment Elevation Myocardial Infarction: Ready for Prime Time? *JACC Cardiovasc Imaging*. 2017. S1936-878X(17)30622-8 [pii];10.1016/j.jcmg.2017.05.025 [doi]

82. Bulluck H, Hammond-Haley M, Weinmann S, Martinez-Macias R, Hausenloy DJ. Myocardial Infarct Size by CMR in Clinical Cardioprotection Studies: Insights From Randomized Controlled Trials. *JACC Cardiovasc Imaging*. 2017;10:230-240. S1936-878X(17)30065-7 [pii];10.1016/j.jcmg.2017.01.008 [doi]

83. Xuan L, Sun L, Zhang Y, Huang Y, Hou Y, Li Q, Guo Y, Feng B, Cui L, Wang X, Wang Z, Tian Y, Yu B, Wang S, Xu C, Zhang M, Du Z, Lu Y, Yang BF. Circulating long non-coding

RNAs NRON and MHRT as novel predictive biomarkers of heart failure. *J Cell Mol Med*. 2017;21:1803-1814. 10.1111/jcmm.13101 [doi]

84. Xu Y, Huang R, Gu J, Jiang W. Identification of long non-coding RNAs as novel biomarker and potential therapeutic target for atrial fibrillation in old adults. *Oncotarget*. 2016;7:10803-10811. 7514 [pii];10.18632/oncotarget.7514 [doi]

85. Wang P, Fu H, Cui J, Chen X. Differential lncRNAmRNA coexpression network analysis revealing the potential regulatory roles of lncRNAs in myocardial infarction. *Mol Med Rep*. 2016;13:1195-1203. 10.3892/mmr.2015.4669 [doi]

86. Viereck J, Thum T. Circulating Noncoding RNAs as Biomarkers of Cardiovascular Disease and Injury. *Circ Res*. 2017;120:381-399. CIRCRESAHA.116.308434 [pii];10.1161/CIRCRESAHA.116.308434 [doi]

87. Wang GK, Zhu JQ, Zhang JT, Li Q, Li Y, He J, Qin YW, Jing Q. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J*. 2010;31:659-666. ehq013 [pii];10.1093/eurheartj/ehq013 [doi]

88. Ai J, Zhang R, Li Y, Pu J, Lu Y, Jiao J, Li K, Yu B, Li Z, Wang R, Wang L, Li Q, Wang N, Shan H, Li Z, Yang B. Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. *Biochem Biophys Res Commun*. 2010;391:73-77. S0006-291X(09)02161-5 [pii];10.1016/j.bbrc.2009.11.005 [doi]

89. D'Alessandra Y, Devanna P, Limana F, Straino S, Di CA, Brambilla PG, Rubino M, Carena MC, Spazzafumo L, De SM, Micheli B, Biglioli P, Achilli F, Martelli F, Maggiolini S, Marenzi G, Pompilio G, Capogrossi MC. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. *Eur Heart J*. 2010;31:2765-2773. ehq167 [pii];10.1093/eurheartj/ehq167 [doi]

90. Gidlof O, Andersson P, van der Pals J, Gotberg M, Erlinge D. Cardiospecific microRNA plasma levels correlate with troponin and cardiac function in patients with ST elevation myocardial infarction, are selectively dependent on renal elimination, and can be detected in urine samples. *Cardiology*. 2011;118:217-226. 000328869 [pii];10.1159/000328869 [doi]

91. Widera C, Gupta SK, Lorenzen JM, Bang C, Bauersachs J, Bethmann K, Kempf T, Wollert KC, Thum T. Diagnostic and prognostic impact of six circulating microRNAs in acute coronary syndrome. *J Mol Cell Cardiol*. 2011;51:872-875. S0022-2828(11)00276-8 [pii];10.1016/j.yjmcc.2011.07.011 [doi]

92. Lv P, Zhou M, He J, Meng W, Ma X, Dong S, Meng X, Zhao X, Wang X, He F. Circulating miR-208b and miR-34a are associated with left ventricular remodeling after acute myocardial infarction. *Int J Mol Sci*. 2014;15:5774-5788. ijms15045774 [pii];10.3390/ijms15045774 [doi]

93. Peng L, Chun-guang Q, Bei-fang L, Xue-zhi D, Zi-hao W, Yun-fu L, Yan-ping D, Yang-gui L, Wei-guo L, Tian-yong H, Zhen-wen H. Clinical impact of circulating miR-133, miR-1291 and miR-663b in plasma of patients with acute myocardial infarction. *Diagn Pathol*. 2014;9:89. 1746-1596-9-89 [pii];10.1186/1746-1596-9-89 [doi]

94. Zhang R, Lan C, Pei H, Duan G, Huang L, Li L. Expression of circulating miR-486 and miR-150 in patients with acute myocardial infarction. *BMC Cardiovasc Disord*. 2015;15:51. 10.1186/s12872-015-0042-0 [doi];10.1186/s12872-015-0042-0 [pii]

95. Zampetaki A, Willeit P, Tilling L, Drozdov I, Prokopi M, Renard JM, Mayr A, Weger S, Schett G, Shah A, Boulanger CM, Willeit J, Chowienczyk PJ, Kiechl S, Mayr M. Prospective study on circulating MicroRNAs and risk of myocardial infarction. *J Am Coll Cardiol*. 2012;60:290-299. S0735-1097(12)01588-4 [pii];10.1016/j.jacc.2012.03.056 [doi]

96. He F, Lv P, Zhao X, Wang X, Ma X, Meng W, Meng X, Dong S. Predictive value of circulating miR-328 and miR-134 for acute myocardial infarction. *Mol Cell Biochem*. 2014;394:137-144. 10.1007/s11010-014-2089-0 [doi]

97. Devaux Y, Vausort M, McCann GP, Kelly D, Collignon O, Ng LL, Wagner DR, Squire IB. A panel of 4 microRNAs facilitates the prediction of left ventricular contractility after acute myocardial infarction. *PLoS One*. 2013;8:e70644. 10.1371/journal.pone.0070644 [doi];PONE-D-13-16789 [pii]

98. Schulte C, Molz S, Appelbaum S, Karakas M, Ojeda F, Lau DM, Hartmann T, Lackner KJ, Westermann D, Schnabel RB, Blankenberg S, Zeller T. miRNA-197 and miRNA-223 Predict Cardiovascular Death in a Cohort of Patients with Symptomatic Coronary Artery Disease. *PLoS One*. 2015;10:e0145930. 10.1371/journal.pone.0145930 [doi];PONE-D-15-36143 [pii]

99. Matsumoto S, Sakata Y, Nakatani D, Suna S, Mizuno H, Shimizu M, Usami M, Sasaki T, Sato H, Kawahara Y, Hamasaki T, Nanto S, Hori M, Komuro I. A subset of circulating microRNAs are predictive for cardiac death after discharge for acute myocardial infarction. *Biochem Biophys Res Commun*. 2012;427:280-284. S0006-291X(12)01779-2 [pii];10.1016/j.bbrc.2012.09.039 [doi]

100. Matsumoto S, Sakata Y, Suna S, Nakatani D, Usami M, Hara M, Kitamura T, Hamasaki T, Nanto S, Kawahara Y, Komuro I. Circulating p53-responsive microRNAs are predictive indicators of heart failure after acute myocardial infarction. *Circ Res*. 2013;113:322-326. CIRCRESAHA.113.301209 [pii];10.1161/CIRCRESAHA.113.301209 [doi]

101. Vausort M, Wagner DR, Devaux Y. Long noncoding RNAs in patients with acute myocardial infarction. *Circ Res*. 2014;115:668-677. CIRCRESAHA.115.303836 [pii];10.1161/CIRCRESAHA.115.303836 [doi]

102. Li X, Dai Y, Yan S, Shi Y, Han B, Li J, Cha L, Mu J. Down-regulation of lncRNA KCNQ1OT1 protects against myocardial ischemia/reperfusion injury following acute myocardial infarction. *Biochem Biophys Res Commun*. 2017. S0006-291X(17)31541-3 [pii];10.1016/j.bbrc.2017.08.005 [doi]

103. Yan Y, Zhang B, Liu N, Qi C, Xiao Y, Tian X, Li T, Liu B. Circulating Long Noncoding RNA UCA1 as a Novel Biomarker of Acute Myocardial Infarction. *Biomed Res Int*. 2016;2016:8079372. 10.1155/2016/8079372 [doi]

104. Zhang Y, Sun L, Xuan L, Pan Z, Li K, Liu S, Huang Y, Zhao X, Huang L, Wang Z, Hou Y, Li J, Tian Y, Yu J, Han H, Liu Y, Gao F, Zhang Y, Wang S, Du Z, Lu Y, Yang B. Reciprocal

Changes of Circulating Long Non-Coding RNAs ZFAS1 and CDR1AS Predict Acute Myocardial Infarction. *Sci Rep*. 2016;6:22384. srep22384 [pii];10.1038/srep22384 [doi]

105. Zhang J, Gao C, Meng M, Tang H. Long Noncoding RNA MHRT Protects Cardiomyocytes against H2O2-Induced Apoptosis. *Biomol Ther (Seoul)*. 2016;24:19-24. 10.4062/biomolther.2015.066 [doi];bt-24-019 [pii]

106. Ong SB, Lu S, Katwadi K, Ismail NI, Kwek XY, Hausenloy DJ. Nanoparticle delivery of mitoprotective agents to target ischemic heart disease. *Future Cardiol*. 2017;13:195-198. 10.2217/fca-2017-0012 [doi]

107. Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, Lim SK, de Kleijn DP. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Res*. 2013;10:301-312. S1873-5061(13)00003-2 [pii];10.1016/j.scr.2013.01.002 [doi]

108. Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP, Lim SK. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res*. 2010;4:214-222. S1873-5061(09)00141-X [pii];10.1016/j.scr.2009.12.003 [doi]

109. Wang Y, Zhang L, Li Y, Chen L, Wang X, Guo W, Zhang X, Qin G, He SH, Zimmerman A, Liu Y, Kim IM, Weintraub NL, Tang Y. Exosomes/microvesicles from induced pluripotent stem cells deliver cardioprotective miRNAs and prevent cardiomyocyte apoptosis in the ischemic myocardium. *Int J Cardiol*. 2015;192:61-69. S0167-5273(15)01039-6 [pii];10.1016/j.ijcard.2015.05.020 [doi]

110. Zhang J, Ma J, Long K, Qiu W, Wang Y, Hu Z, Liu C, Luo Y, Jiang A, Jin L, Tang Q, Wang X, Li X, Li M. Overexpression of Exosomal Cardioprotective miRNAs Mitigates Hypoxia-Induced H9c2 Cells Apoptosis. *Int J Mol Sci*. 2017;18. ijms18040711 [pii];10.3390/ijms18040711 [doi]

111. de CG, Gallet R, Cambier L, Jaghatspanyan E, Makkar N, Dawkins JF, Berman BP, Marban E. Exosomal MicroRNA Transfer Into Macrophages Mediates Cellular Postconditioning. *Circulation*. 2017;136:200-214. CIRCULATIONAHA.116.024590 [pii];10.1161/CIRCULATIONAHA.116.024590 [doi]

112. Lee SY, Lee S, Choi E, Ham O, Lee CY, Lee J, Seo HH, Cha MJ, Mun B, Lee Y, Yoon C, Hwang KC. Small molecule-mediated up-regulation of microRNA targeting a key cell death modulator BNIP3 improves cardiac function following ischemic injury. *Sci Rep*. 2016;6:23472. srep23472 [pii];10.1038/srep23472 [doi]

113. Tang Y, Zheng J, Sun Y, Wu Z, Liu Z, Huang G. MicroRNA-1 regulates cardiomyocyte apoptosis by targeting Bcl-2. *Int Heart J*. 2009;50:377-387. JST.JSTAGE/ihj/50.377 [pii]

114. Pan Z, Sun X, Ren J, Li X, Gao X, Lu C, Zhang Y, Sun H, Wang Y, Wang H, Wang J, Xie L, Lu Y, Yang B. miR-1 exacerbates cardiac ischemia-reperfusion injury in mouse models. *PLoS One*. 2012;7:e50515. 10.1371/journal.pone.0050515 [doi];PONE-D-12-13388 [pii]

115. Bostjancic E, Zidar N, Stajer D, Glavac D. MicroRNAs miR-1, miR-133a, miR-133b and miR-208 are dysregulated in human myocardial infarction. *Cardiology*. 2010;115:163-169. 000268088 [pii];10.1159/000268088 [doi]

116. Chaudhuri AD, Choi DC, Kabaria S, Tran A, Junn E. MicroRNA-7 Regulates the Function of Mitochondrial Permeability Transition Pore by Targeting VDAC1 Expression. *J Biol Chem*. 2016;291:6483-6493. M115.691352 [pii];10.1074/jbc.M115.691352 [doi]

117. Hullinger TG, Montgomery RL, Seto AG, Dickinson BA, Semus HM, Lynch JM, Dalby CM, Robinson K, Stack C, Latimer PA, Hare JM, Olson EN, van RE. Inhibition of miR-15 protects against cardiac ischemic injury. *Circ Res*. 2012;110:71-81. CIRCRESAHA.111.244442 [pii];10.1161/CIRCRESAHA.111.244442 [doi]

118. Dong S, Cheng Y, Yang J, Li J, Liu X, Wang X, Wang D, Krall TJ, Delphin ES, Zhang C. MicroRNA expression signature and the role of microRNA-21 in the early phase of acute myocardial infarction. *J Biol Chem*. 2009;284:29514-29525. M109.027896 [pii];10.1074/jbc.M109.027896 [doi]

119. Sayed D, He M, Hong C, Gao S, Rane S, Yang Z, Abdellatif M. MicroRNA-21 is a downstream effector of AKT that mediates its antiapoptotic effects via suppression of Fas ligand. *J Biol Chem*. 2010;285:20281-20290. M110.109207 [pii];10.1074/jbc.M110.109207 [doi]

120. Qin Y, Yu Y, Dong H, Bian X, Guo X, Dong S. MicroRNA 21 inhibits left ventricular remodeling in the early phase of rat model with ischemia-reperfusion injury by suppressing cell apoptosis. *Int J Med Sci*. 2012;9:413-423. 10.7150/ijms.4514 [doi];ijmsv09p0413 [pii]

121. Chen Z, Qi Y, Gao C. Cardiac myocyte-protective effect of microRNA-22 during ischemia and reperfusion through disrupting the caveolin-3/eNOS signaling. *Int J Clin Exp Pathol*. 2015;8:4614-4626.

122. Yang J, Chen L, Yang J, Ding J, Li S, Wu H, Zhang J, Fan Z, Dong W, Li X. MicroRNA-22 targeting CBP protects against myocardial ischemia-reperfusion injury through anti-apoptosis in rats. *Mol Biol Rep*. 2014;41:555-561. 10.1007/s11033-013-2891-x [doi]

123. Qian L, van Laake LW, Huang Y, Liu S, Wendland MF, Srivastava D. miR-24 inhibits apoptosis and represses Bim in mouse cardiomyocytes. *J Exp Med*. 2011;208:549-560. jem.20101547 [pii];10.1084/jem.20101547 [doi]

124. Wang Y, Men M, Yang W, Zheng H, Xue S. MiR-31 Downregulation Protects Against Cardiac Ischemia/Reperfusion Injury by Targeting Protein Kinase C Epsilon (PKCepsilon) Directly. *Cell Physiol Biochem*. 2015;36:179-190. 000374062 [pii];10.1159/000374062 [doi]

125. Hinkel R, Penzkofer D, Zuhlke S, Fischer A, Husada W, Xu QF, Baloch E, van RE, Zeiher AM, Kupatt C, Dimmeler S. Inhibition of microRNA-92a protects against ischemia/reperfusion injury in a large-animal model. *Circulation*. 2013;128:1066-1075. CIRCULATIONAHA.113.001904 [pii];10.1161/CIRCULATIONAHA.113.001904 [doi]

126. Wang JX, Zhang XJ, Li Q, Wang K, Wang Y, Jiao JQ, Feng C, Teng S, Zhou LY, Gong Y, Zhou ZX, Liu J, Wang JL, Li PF. MicroRNA-103/107 Regulate Programmed Necrosis and

Myocardial Ischemia/Reperfusion Injury Through Targeting FADD. *Circ Res*. 2015;117:352-363. CIRCRESAHA.117.305781 [pii];10.1161/CIRCRESAHA.117.305781 [doi]

127. Zeng XC, Li L, Wen H, Bi Q. MicroRNA-128 inhibition attenuates myocardial ischemia/reperfusion injury-induced cardiomyocyte apoptosis by the targeted activation of peroxisome proliferator-activated receptor gamma. *Mol Med Rep*. 2016;14:129-136. 10.3892/mmr.2016.5208 [doi]

128. Li S, Xiao FY, Shan PR, Su L, Chen DL, Ding JY, Wang ZQ. Overexpression of microRNA-133a inhibits ischemia-reperfusion-induced cardiomyocyte apoptosis by targeting DAPK2. *J Hum Genet*. 2015;60:709-716. jhg201596 [pii];10.1038/jhg.2015.96 [doi]

129. Li J, Li Y, Jiao J, Wang J, Li Y, Qin D, Li P. Mitofusin 1 is negatively regulated by microRNA 140 in cardiomyocyte apoptosis. *Mol Cell Biol*. 2014;34:1788-1799. MCB.00774-13 [pii];10.1128/MCB.00774-13 [doi]

130. Wang Y, Ouyang M, Wang Q, Jian Z. MicroRNA-142-3p inhibits hypoxia/reoxygenationinduced apoptosis and fibrosis of cardiomyocytes by targeting high mobility group box 1. *Int J Mol Med*. 2016;38:1377-1386. 10.3892/ijmm.2016.2756 [doi]

131. Zhang X, Wang X, Zhu H, Zhu C, Wang Y, Pu WT, Jegga AG, Fan GC. Synergistic effects of the GATA-4-mediated miR-144/451 cluster in protection against simulated ischemia/reperfusion-induced cardiomyocyte death. *J Mol Cell Cardiol*. 2010;49:841-850. S0022-2828(10)00294-4 [pii];10.1016/j.yjmcc.2010.08.007 [doi]

132. Li R, Yan G, Li Q, Sun H, Hu Y, Sun J, Xu B. MicroRNA-145 protects cardiomyocytes against hydrogen peroxide (H(2)O(2))-induced apoptosis through targeting the mitochondria apoptotic pathway. *PLoS One*. 2012;7:e44907. 10.1371/journal.pone.0044907 [doi];PONE-D-12-11469 [pii]

133. Wang X, Ha T, Liu L, Zou J, Zhang X, Kalbfleisch J, Gao X, Williams D, Li C. Increased expression of microRNA-146a decreases myocardial ischaemia/reperfusion injury. *Cardiovasc Res*. 2013;97:432-442. cvs356 [pii];10.1093/cvr/cvs356 [doi]

134. Das S, Kohr M, Dunkerly-Eyring B, Lee DI, Bedja D, Kent OA, Leung AK, Henao-Mejia J, Flavell RA, Steenbergen C. Divergent Effects of miR-181 Family Members on Myocardial Function Through Protective Cytosolic and Detrimental Mitochondrial microRNA Targets. *J Am Heart Assoc*. 2017;6. JAHA.116.004694 [pii];10.1161/JAHA.116.004694 [doi]

135. Zhang Y, Huang R, Zhou W, Zhao Q, Lu Z. miR-192-5p mediates hypoxia/reoxygenation-induced apoptosis in H9c2 cardiomyocytes via targeting of FABP3. *J Biochem Mol Toxicol*. 2017;31. 10.1002/jbt.21873 [doi]

136. Hang P, Sun C, Guo J, Zhao J, Du Z. BDNF-mediates Down-regulation of MicroRNA-195 Inhibits Ischemic Cardiac Apoptosis in Rats. *Int J Biol Sci*. 2016;12:979-989. 10.7150/ijbs.15071 [doi];ijbsv12p0979 [pii]

137. Chen Z, Zhang S, Guo C, Li J, Sang W. Downregulation of miR-200c protects cardiomyocytes from hypoxia-induced apoptosis by targeting GATA-4. *Int J Mol Med*. 2017;39:1589-1596. 10.3892/ijmm.2017.2959 [doi]

138. Aurora AB, Mahmoud AI, Luo X, Johnson BA, van RE, Matsuzaki S, Humphries KM, Hill JA, Bassel-Duby R, Sadek HA, Olson EN. MicroRNA-214 protects the mouse heart from ischemic injury by controlling Ca(2)(+) overload and cell death. *J Clin Invest*. 2012;122:1222-1232. 59327 [pii];10.1172/JCI59327 [doi]

139. Qin D, Wang X, Li Y, Yang L, Wang R, Peng J, Essandoh K, Mu X, Peng T, Han Q, Yu KJ, Fan GC. MicroRNA-223-5p and -3p Cooperatively Suppress Necroptosis in Ischemic/Reperfused Hearts. *J Biol Chem*. 2016;291:20247-20259. M116.732735 [pii];10.1074/jbc.M116.732735 [doi]

140. Ren XP, Wu J, Wang X, Sartor MA, Jones K, Qian J, Nicolaou P, Pritchard TJ, Fan GC. MicroRNA-320 is involved in the regulation of cardiac ischemia/reperfusion injury by targeting heat-shock protein 20. *Circulation*. 2009;119:2357-2366. 10.1161/CIRCULATIONAHA.108.814145 [doi]

141. Meng X, Ji Y, Wan Z, Zhao B, Feng C, Zhao J, Li H, Song Y. Inhibition of miR-363 protects cardiomyocytes against hypoxia-induced apoptosis through regulation of Notch signaling. *Biomed Pharmacother*. 2017;90:509-516. S0753-3322(16)32624-5 [pii];10.1016/j.biopha.2017.03.080 [doi]

142. Fang J, Song XW, Tian J, Chen HY, Li DF, Wang JF, Ren AJ, Yuan WJ, Lin L. Overexpression of microRNA-378 attenuates ischemia-induced apoptosis by inhibiting caspase-3 expression in cardiac myocytes. *Apoptosis*. 2012;17:410-423. 10.1007/s10495-011-0683-0 [doi]

143. Xu H, Jin L, Chen Y, Li J. Downregulation of microRNA-429 protects cardiomyocytes against hypoxia-induced apoptosis by increasing Notch1 expression. *Int J Mol Med*. 2016;37:1677-1685. 10.3892/ijmm.2016.2558 [doi]

144. Xie J, Hu X, Yi C, Hu G, Zhou X, Jiang H. MicroRNA451 protects against cardiomyocyte anoxia/reoxygenation injury by inhibiting high mobility group box 1 expression. *Mol Med Rep*. 2016;13:5335-5341. 10.3892/mmr.2016.5192 [doi]

145. Wang X, Zhang X, Ren XP, Chen J, Liu H, Yang J, Medvedovic M, Hu Z, Fan GC. MicroRNA-494 targeting both proapoptotic and antiapoptotic proteins protects against ischemia/reperfusion-induced cardiac injury. *Circulation*. 2010;122:1308-1318. CIRCULATIONAHA.110.964684 [pii];10.1161/CIRCULATIONAHA.110.964684 [doi]

146. Wang JX, Jiao JQ, Li Q, Long B, Wang K, Liu JP, Li YR, Li PF. miR-499 regulates mitochondrial dynamics by targeting calcineurin and dynamin-related protein-1. *Nat Med*. 2011;17:71-78. nm.2282 [pii];10.1038/nm.2282 [doi]

147. Wu Z, Qi Y, Guo Z, Li P, Zhou D. miR-613 suppresses ischemia-reperfusion-induced cardiomyocyte apoptosis by targeting the programmed cell death 10 gene. *Biosci Trends*. 2016;10:251-257. 10.5582/bst.2016.01122 [doi]

148. Long B, Wang K, Li N, Murtaza I, Xiao JY, Fan YY, Liu CY, Li WH, Cheng Z, Li P. miR-761 regulates the mitochondrial network by targeting mitochondrial fission factor. *Free Radic Biol Med*. 2013;65:371-379. S0891-5849(13)00340-7 [pii];10.1016/j.freeradbiomed.2013.07.009 [doi]

149. Wang K, Long B, Li N, Li L, Liu CY, Dong YH, Gao JN, Zhou LY, Wang CQ, Li PF. MicroRNA-2861 regulates programmed necrosis in cardiomyocyte by impairing adenine nucleotide translocase 1 expression. *Free Radic Biol Med*. 2016;91:58-67. S0891-5849(15)01140-5 [pii];10.1016/j.freeradbiomed.2015.11.031 [doi]

150. Lu Y, Zhang Y, Shan H, Pan Z, Li X, Li B, Xu C, Zhang B, Zhang F, Dong D, Song W, Qiao G, Yang B. MicroRNA-1 downregulation by propranolol in a rat model of myocardial infarction: a new mechanism for ischaemic cardioprotection. *Cardiovasc Res*. 2009;84:434-441. cvp232 [pii];10.1093/cvr/cvp232 [doi]

151. Park KM, Teoh JP, Wang Y, Broskova Z, Bayoumi AS, Tang Y, Su H, Weintraub NL, Kim IM. Carvedilol-responsive microRNAs, miR-199a-3p and -214 protect cardiomyocytes from simulated ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2016;311:H371-H383. ajpheart.00807.2015 [pii];10.1152/ajpheart.00807.2015 [doi]

152. Shan H, Li X, Pan Z, Zhang L, Cai B, Zhang Y, Xu C, Chu W, Qiao G, Li B, Lu Y, Yang B. Tanshinone IIA protects against sudden cardiac death induced by lethal arrhythmias via repression of microRNA-1. *Br J Pharmacol*. 2009;158:1227-1235. BPH377 [pii];10.1111/j.1476-5381.2009.00377.x [doi]

153. Zhang Y, Zhang L, Chu W, Wang B, Zhang J, Zhao M, Li X, Li B, Lu Y, Yang B, Shan H. Tanshinone IIA inhibits miR-1 expression through p38 MAPK signal pathway in post-infarction rat cardiomyocytes
2. *Cell Physiol Biochem*. 2010;26:991-998. 000324012 [pii];10.1159/000324012 [doi]

154. Ye Y, Hu Z, Lin Y, Zhang C, Perez-Polo JR. Downregulation of microRNA-29 by antisense inhibitors and a PPAR-gamma agonist protects against myocardial ischaemia-reperfusion injury. *Cardiovasc Res*. 2010;87:535-544. cvq053 [pii];10.1093/cvr/cvq053 [doi]

155. Mukhopadhyay P, Mukherjee S, Ahsan K, Bagchi A, Pacher P, Das DK. Restoration of altered microRNA expression in the ischemic heart with resveratrol
1. *PLoS One*. 2010;5:e15705. 10.1371/journal.pone.0015705 [doi]

156. Mukhopadhyay P, Das S, Ahsan MK, Otani H, Das DK. Modulation of microRNA 20b with resveratrol and longevinex is linked with their potent anti-angiogenic action in the ischaemic myocardium and synergestic effects of resveratrol and gamma-tocotrienol
1. *J Cell Mol Med*. 2012;16:2504-2517. 10.1111/j.1582-4934.2011.01480.x [doi]

157. Li DF, Tian J, Guo X, Huang LM, Xu Y, Wang CC, Wang JF, Ren AJ, Yuan WJ, Lin L. Induction of microRNA-24 by HIF-1 protects against ischemic injury in rat cardiomyocytes. *Physiol Res*. 2012;61:555-565. 932270 [pii]

158. Shi H, Chen L, Wang H, Zhu S, Dong C, Webster KA, Wei J. Synergistic induction of miR-126 by hypoxia and HDAC inhibitors in cardiac myocytes. *Biochem Biophys Res Commun*. 2013;430:827-832. S0006-291X(12)02236-X [pii];10.1016/j.bbrc.2012.11.061 [doi]

159. Forini F, Kusmic C, Nicolini G, Mariani L, Zucchi R, Matteucci M, Iervasi G, Pitto L. Triiodothyronine prevents cardiac ischemia/reperfusion mitochondrial impairment and cell loss by regulating miR30a/p53 axis. *Endocrinology*. 2014;155:4581-4590. 10.1210/en.2014-1106 [doi]

160. Kang B, Hong J, Xiao J, Zhu X, Ni X, Zhang Y, He B, Wang Z. Involvement of miR-1 in the protective effect of hydrogen sulfide against cardiomyocyte apoptosis induced by ischemia/reperfusion. *Mol Biol Rep*. 2014;41:6845-6853. 10.1007/s11033-014-3570-2 [doi]

161. Kang B, Li W, Xi W, Yi Y, Ciren Y, Shen H, Zhang Y, Jiang H, Xiao J, Wang Z. Hydrogen Sulfide Protects Cardiomyocytes against Apoptosis in Ischemia/Reperfusion through MiR-1-Regulated Histone Deacetylase 4 Pathway. *Cell Physiol Biochem*. 2017;41:10-21. 000455816 [pii];10.1159/000455816 [doi]

162. Toldo S, Das A, Mezzaroma E, Chau VQ, Marchetti C, Durrant D, Samidurai A, Van Tassell BW, Yin C, Ockaili RA, Vigneshwar N, Mukhopadhyay ND, Kukreja RC, Abbate A, Salloum FN. Induction of microRNA-21 with exogenous hydrogen sulfide attenuates myocardial ischemic and inflammatory injury in mice. *Circ Cardiovasc Genet*. 2014;7:311-320. CIRCGENETICS.113.000381 [pii];10.1161/CIRCGENETICS.113.000381 [doi]

163. Bian C, Xu T, Zhu H, Pan D, Liu Y, Luo Y, Wu P, Li D. Luteolin Inhibits Ischemia/Reperfusion-Induced Myocardial Injury in Rats via Downregulation of microRNA-208b-3p. *PLoS One*. 2015;10:e0144877. 10.1371/journal.pone.0144877 [doi];PONE-D-15-26956 [pii]

164. Olson JM, Yan Y, Bai X, Ge ZD, Liang M, Kriegel AJ, Twaroski DM, Bosnjak ZJ. Up-regulation of microRNA-21 mediates isoflurane-induced protection of cardiomyocytes. *Anesthesiology*. 2015;122:795-805. 10.1097/ALN.0000000000000567 [doi]

165. Qiao S, Olson JM, Paterson M, Yan Y, Zaja I, Liu Y, Riess ML, Kersten JR, Liang M, Warltier DC, Bosnjak ZJ, Ge ZD. MicroRNA-21 Mediates Isoflurane-induced Cardioprotection against Ischemia-Reperfusion Injury via Akt/Nitric Oxide Synthase/Mitochondrial Permeability Transition Pore Pathway. *Anesthesiology*. 2015;123:786-798. 10.1097/ALN.0000000000000807 [doi]

166. Yang Q, Yang K, Li AY. Trimetazidine protects against hypoxia-reperfusion-induced cardiomyocyte apoptosis by increasing microRNA-21 expression. *Int J Clin Exp Pathol*. 2015;8:3735-3741.

167. Ma N, Bai J, Zhang W, Luo H, Zhang X, Liu D, Qiao C. Trimetazidine protects against cardiac ischemia/reperfusion injury via effects on cardiac miRNA21 expression, Akt and the Bcl2/Bax pathway. *Mol Med Rep*. 2016;14:4216-4222. 10.3892/mmr.2016.5773 [doi]

168. Han Q, Zhang HY, Zhong BL, Zhang B, Chen H. Antiapoptotic Effect of Recombinant HMGB1 A-box Protein via Regulation of microRNA-21 in Myocardial Ischemia-Reperfusion Injury Model in Rats. *DNA Cell Biol*. 2016;35:192-202. 10.1089/dna.2015.3003 [doi]

169. He SF, Zhu HJ, Han ZY, Wu H, Jin SY, Irwin MG, Zhang Y. MicroRNA-133b-5p Is Involved in Cardioprotection of Morphine Preconditioning in Rat Cardiomyocytes by Targeting Fas. *Can J Cardiol*. 2016;32:996-1007. S0828-282X(15)01544-5 [pii];10.1016/j.cjca.2015.10.019 [doi]

170. Li D, Wang J, Hou J, Fu J, Liu J, Lin R. Salvianolic acid B induced upregulation of miR-30a protects cardiac myocytes from ischemia/reperfusion injury. *BMC Complement Altern Med*. 2016;16:336. 10.1186/s12906-016-1275-x [doi];10.1186/s12906-016-1275-x [pii]

171. Hendgen-Cotta UB, Messiha D, Esfeld S, Deenen R, Rassaf T, Totzeck M. Inorganic nitrite modulates miRNA signatures in acute myocardial in vivo ischemia/reperfusion. *Free Radic Res*. 2017;51:91-102. 10.1080/10715762.2017.1282158 [doi]

Figure 1. An overview of miRNAs and lncRNAs in acute myocardial ischemia/reperfusion injury

This figure illustrates the modulation of survival signaling cascades and death pathways (apoptosis and necrosis) by miRNAs (in blue) and lncRNAs (in pink) within the cardiomyocyte following acute ischemia/reperfusion injury. Pro-death target proteins include Bcl-2-associated X protein (Bax), Bcl-2 homologous antagonist/killer (Bak), Bisindolylmaleimide (Bim), receptor-interacting serine/threonine-protein kinase 1 and 3 (RIPK1 & RIPK3, respectively). Pro-survival

proteins include B-cell lymphoma 2 (Bcl-2), Myeloid cell leukemia 1 (Mcl-1). Heat shock proteins include heat shock protein 20 (Hsp20), heat shock protein 60 (Hsp60), heat shock protein 70 (Hsp70), phosphatase and tensin homolog deleted on chromosome ten (PTEN), Phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt). Proteins involved in mitochondrial function and dynamics include dynamin-related protein-1 (Drp1), Mitofusin 1 (Mfn1), Optic atrophy 1 (Opa1), adenine nucleotide translocase (ANT). Autophagy-related proteins include autophagy-related protein 7 (Atg7), BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (Bnip3). Other proteins include Sirtuin-1 (Sirt1), proliferator activated receptor-g coactivator-1α (PGC1α),

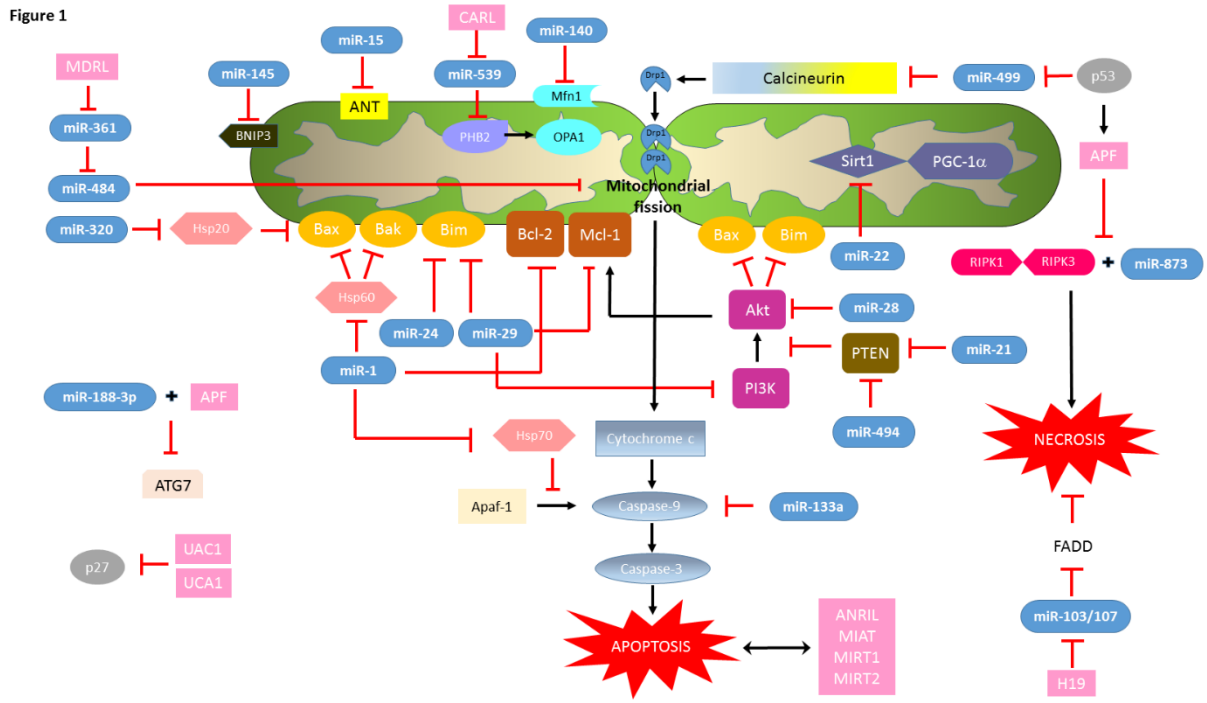

Figure 2. General mechanism of conditioning

This scheme illustrates the general mechanisms of conditioning achieved via either pre-conditioning or post-conditioning, both of which can be achieved remotely. The conditioning strategies initiate the production of protective kinases which target the mitochondria, leading to the protective effects of cardioprotection via mechanisms such as closure of the mPTP, maintenance of ATP production, tolerance towards calcium overloading and ROS production and prevention of cyt c release.

Figure 2

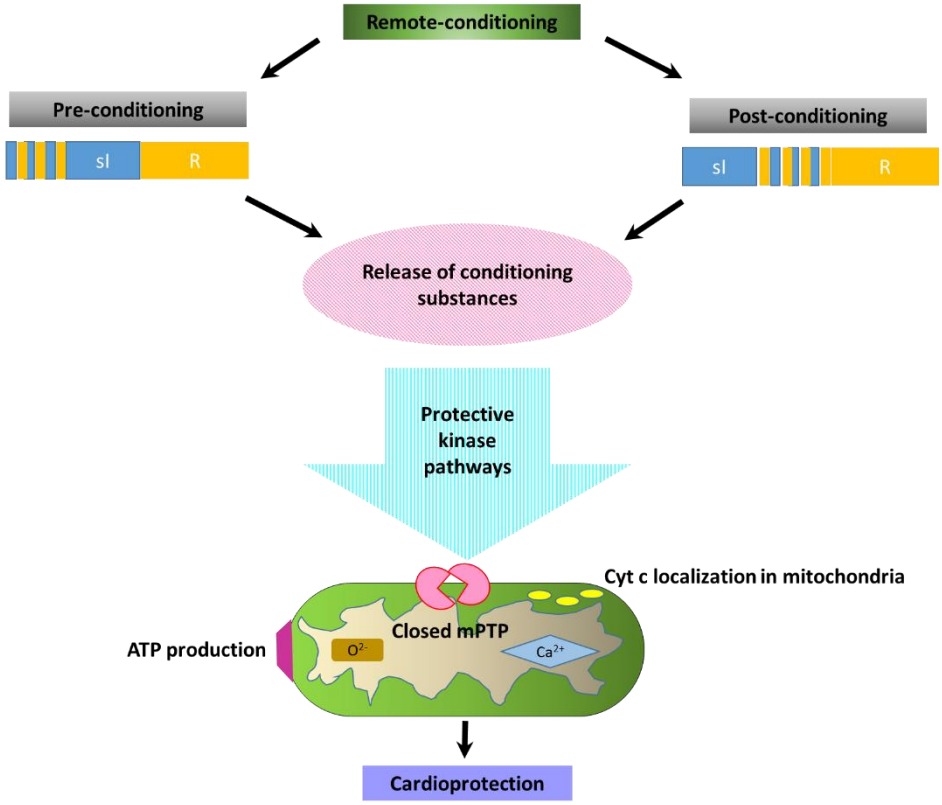

Table 1: Summary of major studies investigating miRNAs in the setting of acute myocardial ischemia-reperfusion injury

miRNA	Changes in expression with acute IRI*	Downstream targets/effects	Potential Therapeutic application	Study
miR-1	↑	Increases cell death by inhibiting Bcl-2	Inhibition of miR-1 to reduced cell death following H_2O_2 and reduced MI size following AMI	[113,114]
	↓ (human)			[115]
miR-7	N/A	Regulates mPTP by targeting VDAC1	May prevents mPTP opening at the onset of reperfusion thus reducing MI size	[116]
miR-15	↑ (pig)	Increases cell death	Inhibition of miR-15 using LNA anti-miRs reduced MI size following AMI in mice and pigs	[117]
miR-21	↓	miR-21 protects by downregulating PCDC4, AP1, PTEN and Fas ligand. Akt upstream of miR-21	Over-expression of miR-21 reduced MI size following AMI	[118-120]
miR-22	↓	miR-22 protects by downregulating Cav3 and upregulating eNOS miR-22 protects by downregulating P53, CBP-AP1, TNF-α and IL-6, and upregulating Bcl2/Bax ratio	Over-expression of miR-22 reduced MI size following AMI	[121,122]
miR-24	↓	miR-24 protects by downregulating Bim	Over-expression of miR-22 reduced MI size following AMI	[123]
miR-29	↓ (Border area human)	Increased expression of fibrosis proteins (collagens, fibrillins, and elastin) due to de-repression of miR-29	Over-expression of miR-29 to reduce cardiac fibrosis following AMI	[13]
miR-31	↑	Increased cell death miR-31 protects by upregulating PKC-ε and downregulating NFκB	Inhibition of miR-31 using antagomir reduced MI size following AMI in mice	[124]
miR-92a	↑ (pig)		Inhibition of miR-92a using LNA anti-miR reduced MI size following AMI in pigs	[125]
miR-103/107	↑	Increased cell necrosis by downregulation of FADD and activation of RIPK1/RIPK3. lncRNA upstream of miR-103/107	Inhibition of miR-103/107 using antagomir reduced MI size following AMI in mice	[126]
miR-128	N/A	Increase cell death by antagonizing Akt, PPARγ and Mcl‑1 protein	Inhibition of miR-128 using antagomir reduced MI size following AMI in mice	[127]

miR-133	↓	miR-133 protects by inhibiting DAPK2	Over-expression of miR-133 reduced MI size following AMI	[128]
	↓ (human)			[115]
miR-140	↑	Increased cell death by inhibiting Mfn1 and inducing mitochondrial fission	Inhibition of miR-140 using antagomir reduced MI size following AMI in mice	[129]
miR-142	↓	miR-142 protects by inhibiting HMGB1	Over-expression of miR-142 reduced cell death	[130]
miR-144/451	N/A	miR-144/451 protects by targeting CUGBP2–COX-2 pathway. GATA-4 upstream of miR-144/451	Over-expression of miR-144/451 reduced cell death	[131]
miR-145	↓	miR-145 protects by inhibiting BNIP3	Over-expression of miR-145 reduced cell death and protect mitochondria	[132]
miR-146	N/A	miR-146a protects by reducing caspase 3,7,8, suppressing IRAK1 and TRAF6 expression and inhibiting NFκB/ inflammatory cytokine production	Over-expression of miR-145 reduced cell death and protect mitochondria	[133]
miR-181		miR-181c induces cell death by inhibiting COX-1 and increasing mitochondrial ROS, whereas miR-181a/b protects by inhibiting PTEN		[134]
miR-192-5p	↑	Increases cell death by targeting FABP3 and upregulating Bax/Bcl2 ratio	Inhibition of miR-192-5p using antagomir reduced apoptosis	[135]
miR-195	↑	Increases cell death by inhibiting Bcl2. BDNF upstream of miR-195	Inhibition of miR-195 using antagomir reduced MI size following AMI	[136]
miR-200c	↓	miR200c protects by upregulating GATA-4 and Bcl2	Over-expression of miR-200c reduced cell death and protect mitochondria	[137]
miR-208	↑ (human)			[115]
miR-214	↑	miR-214 protects by repression of NCX1, CaMKIIδ, CypD, and BIM		[138]
miR-223	N/A	miR-223 protects by inhibiting TNFR1, DR6, NLRP3 and IkB kinase α	Over-expression of miR-223 to reduce MI size following AMI	[139]
miR-320	↓	Increases cell death	Over-expression of miR-320 to reduce MI size following AMI	[140]
miR-363	↑	Increases cell death by inhibiting Notch signaling	Inhibition of miR-429 using antagomir reduced cell death	[141]
miR-378	↓	miR-378 protects by inhibiting caspase 3	Over-expression of miR-378 reduced cell death	[142]
miR-429	↑	Increases cell death by inhibiting Notch signaling	Inhibition of miR-429 using antagomir reduced cell death	[143]
miR-451	↓	miR-451 protects by inhibiting HMGB1	Over-expression of miR-451 reduced cell death	[144]

miR-494	↓	miR-494 protects by inhibiting PTEN, ROCK1, CaMKIIδ, and activating FGFR2 and LIF	Over-expression of miR-494 reduced MI size following AMI	[145]
miR-499	↓	miR-499 protects by inhibiting calcineurin thereby preventing Drp-1 mediated mitochondrial fission	Over-expression of miR-499 reduced MI size following AMI	[146]
miR-613	↓	miR-613 protects by phosphorylating Akt and inhibiting PDCD10	Over-expression of miR-613 reduced cell death	[147]
miR-761	↓	miR-761 protects by repressing Mff and inhibiting mitochondrial fission	Over-expression of miR-761 reduced MI size following AMI	[148]
miR-2861	↑	Increases cell death by inhibiting ANT1	Inhibition of miR-2861 using antagomir reduced cell death	[149]

AMI, acute myocardial infarction; ANT1, adenine nucleotide translocase 1; AP1, activator protein 1; Bax, Bcl-2-associated X protein; Bak, Bcl-2 homologous antagonist/killer; BDNF, brain-derived neurotrophic factor; Bcl-2, B-cell lymphoma 2; Bim, Bisindolylmaleimide; BNIP3, Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3; CaMKIIδ, Ca^{2+}/calmodulin-dependent protein kinase II δ-isoform; Cav3, caveolin 3; CBP, CREB Binding Protein; DR6, death receptor-6; CypD, cyclophilin D; DAPK2, death associated protein kinase 2; Drp-1, dynamin related protein-1; eNOS, endothelial nitric oxide synthase; FABP3, Fatty Acid Binding Protein 3; FADD, Fas-associated protein with death domain; FGFR2, fibroblast growth factor receptor 2; GATA-4, GATA Binding Protein 4; HMGB1, High mobility group box 1; IL-5, interleukin 6; IRAK1, interleukin 1 Receptor Associated Kinase 1; LIF, leukemia inhibitory factor; Mcl-1, myeloid cell leukemia 1; Mff, Mitochondrial Fission Factor; MI, myocardial infarct; Mfn1, Mitofusin 1; NCX1, sodium calcium exchanger-1; COX-1, cyclooxygenase-1 ; NLRP3, NLR Family Pyrin Domain Containing 3; PDCD, Programmed cell death protein; PGC1a, Peroxisome proliferator activated receptor-g coactivator-1a; PKC-ε, protein kinase C-ε; PPARγ, proliferator activated receptor-g coactivator gamma; PTEN, phosphatase and Tensin homolog; RIPK, Receptor-interacting serine/threonine-protein kinase; ROCK1, Rho Associated Coiled-Coil Containing Protein Kinase 1; ROS, reactive oxygen species; TNFR1, tumor necrosis factor receptor 1; TRAF1, TNF Receptor Associated Factor 1.

Table 2: Summary of major studies investigating miRNAs as mediators of cardioprotection

Cardioprotective strategy or agent	Change in miRNA expression	Downstream targets/effects	Study
Heat stress (IPC mimic)	Upregulation of miR-1, miR-21 and miR-24	Reduced MI size, associated downregulation of pro-apoptotic genes (caspases 1, 2, 8 and 14, Bid, Bcl10, Cidea, Ltbr, Trp53 and Fas ligand), and upregulation of anti-apoptotic proteins (Bag3, and Prdx2)	[10]
Propanolol (cAMP–PKA activation)	Downregulation of miR-1	Less IRI arrhythmias and preservation of Cx43 and Kir2.1	[150]
Carvedilol	Upregulation of miR-199a-3p and miR-214	Less cell death and apoptosis with upregulation of Akt phosphorylation and Sox-4, and inhibition of *ddit4* and *ing4*	[151]
Tanshinone IIA (Danshen, traditional chinese medicine)	Downregulation of miR-1	Reduced MI size, less IRI arrhythmias, preservation of Cx43 and Kir2.1, and inhibition of p38 MAPK.	[152,153]
Pioglitazone and rosiglitazone (PPAR-γ agonist)	Downregulation of miR-29	Less cell death and apoptosis. Less caspase 3 and upregulation of Mcl-2	[154]
Resveratrol (a constituent of red wine)	Modulation of a number of miRNAs affected by IRI (including upregulation of miR-21)	Reduced MI size	[155,156]
Hypoxia inducible factor-1α	Upregulation of miR-24	Less cell death and apoptosis with upregulation of BCl2	[157]
γ-tocotrienol (a constituent of palm oil)	Upregulation of miR-20b	Reduced MI size, upregulation of HIF-1α and VEGF	[156]
Choline (M3-AChR agonist)	Downregulation of miR-376b-5p	Reduced MI size, upregulation of BDNF	[157]
Hypoxia and Tricostatin A (HDAC inhibitor)	Upregulation of miR-126	Less cell death and phosphorylation of Akt and Erk1/2	[158]
Triiodothyronine (T3)	Upregulation of miR-30a	Less cell death and decreased expression of p53	[159]
Hydrogen sulphide	Downregulation of miR-1	Less cell death and apoptosis with less caspase 3, upregulation of Bcl2, and preservation of HDAC4	[160,161]
			[162]

	Upregulation of miR-21	Reduce MI size, less apoptosis, less inflammation, reduced inflammasome formation	
Luteolin (a dietary flavonoid)	Downregulation of miR-208b-3p	Less cell death and apoptosis with downregulation of BAX and caspase 3, and upregulation of avian erythroblastosis virus E26 oncogene homolog 1 and Bcl2	[163]
Isoflurane	Upregulation of miR-21	Reduce MI size and downregulation of PDCD4	[164]
		Reduce MI size, increased phosphorylation of Akt, eNOS, nNOS and less MPTP opening with isoflurane but not in miR-21 KO mice	[165]
Trimetazidine	Upregulation of miR-21	Reduced MI size, less cell death and apoptosis with suppression of PTEN, phosphorylation of Akt, decreased Bax and increased Bcl2	[166,167]
Recombinant HMGB1A-box	Downregulation of miR-21	Less cell death and apoptosis with phosphorylation of Akt, decreased Bax and caspase 3 and increased Bcl2	[168]
Morphine	Upregulation of miR-133b-5p	Less cell death and reduced Fas ligand	[169]
Salvianolic acid B (Chinese medicine herb)	Upregulation of miR-30a	Less cell death and autophagy, upregulation of PI3K and phosphorylated Akt	[170]
Inorganic nitrite	Downregulation of miR-125a-5p, miR-146b, miR-339-3p, miR-433 at reperfusion		[171]

Bax, Bcl-2-associated X protein; Bak, Bcl-2 homologous antagonist/killer; BDNF, brain-derived neurotrophic factor; Bcl-2, B-cell lymphoma 2; Bim, Bisindolylmaleimide; BNIP3, Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3; Cidea, Cell death activator; Cx43, connexin 43; eNOS, endothelial nitric oxide synthase; FADD, Fas-associated protein with death domain; HDAC4, histone Deacetylase 4; Ltbr, lymphotoxin-beta receptor; MAPK, mitogen activated protein kinase; MI, myocardial infarct; MPTP, mitochondrial permeability transition pore; nNOS, neuronal nitric oxide synthase; PDCD, Programmed cell death protein; Trp53, transformation related protein 53.