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 IncRNAs 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 IncRNAs 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.\(^2\,^3\,^4\,^5\) 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 IncRNA, 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.

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. 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)\textsuperscript{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\textsuperscript{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\textsuperscript{23,35-37}; downregulation of miR-208\textsuperscript{23}; enhanced expression of miR-29b, miR-133a, and miR-146b in pig hearts\textsuperscript{38}; down-regulation of miR-1 in the rat heart\textsuperscript{21}; and downregulation of miR-1 and upregulation of miR-21 in atrial tissue harvested from patients undergoing cardiac valve surgery\textsuperscript{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\textsuperscript{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\textsuperscript{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 PDCD4 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\textsuperscript{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\textsuperscript{56}. Similarly, Hu et al\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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 \(^6^2\).

Interestingly, it has been reported that limb RIC, repeated daily for one month, prevented adverse LV remodeling following AMI in the rodent heart \(^6^3\). Yamaguchi et al \(^6^4\) 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 \(^1^0\), 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)

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 and brain, have described roles for
lncRNAs in liver and brain tissue following acute IRI. Dharap et al found that lncRNAs in brain
tissue were highly dysregulated following acute IRI in a murine model of stroke. Chen et al
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 IncRNAs in the setting of acute IRI in the heart, was by Liu et al who demonstrated marked upregulation of 5 IncRNAs (AK035396, ENSMUST00000156081, AK005401, ENSMUST00000118172 and ENSMUST00000118702), and downregulation of 5 IncRNAs (uc007prv.1, AK080112, ENSMUST00000170410, AK156124 and ENSMUST00000166777), in the infarcted murine heart at 2.5 hours of reperfusion. Changes in myocardial expression of IncRNAs 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 have shown changes in a large number of IncRNAs in infarcted tissue when compared to remote myocardium including IncRNAs 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 have recently investigated the changes in myocardial IncRNA 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 IncRNAs including RP11-64B16.4 (the extent of which was related to the ischemic time), and downregulation of 13 IncRNAs, changes which were associated with mRNA implicated in the stress and immune response to ischemia.

The role of IncRNAs as mediators of acute IRI and potential targets for cardioprotection has been recently investigated. Wang et al found that the IncRNA, 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 in 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 IncRNA (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. Finally, the IncRNA, 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.

6. MiRNAs and IncRNAs 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, 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. Several clinical studies have measured changes in circulating levels of miRNAs and IncRNAs 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 ). Although the plasma contains RNases, circulating ncRNAs especially circular IncRNAs 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, 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. demonstrated that downregulation of the KCNQ1OT1 IncRNA, protected H9C2 cardiac cells from simulated IRI, and this protective effect was mediated via the adiponectin receptor and suppression of the p38 MAPK/NF-κB signal cascade.

In another clinical study, plasma levels of the circular IncRNA, 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. demonstrated reciprocal changes in the IncRNAs, 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 IncRNAs were shown to independently predict AMI. Another clinical study has reported elevations in the IncRNA 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. Finally, in a recent clinical study, reduced plasma levels of the IncRNAs (ENST00000416860.2, ENST00000421157.1 and TCONS_00025701) were demonstrated, but the impact of these changes on MI size or subsequent remodeling was not investigated.

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. 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. 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 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 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 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 IncRNAs 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\textsuperscript{112} identified the hit compound Kenpaullone as a cardioprotective small molecule capable of increasing myocardial expression of miR-182 and protecting against acute IRI.

8. Conclusions

A number of miRNAs and more recently IncRNAs 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 antagonists 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


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]


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 synergistic effects of resveratrol and gamma-tocotrienol.


*Figure 1. An overview of miRNAs and IncRNAs 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 IncRNAs (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

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Changes in expression with acute IRI*</th>
<th>Downstream targets/effects</th>
<th>Potential Therapeutic application</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-1</td>
<td>↑</td>
<td>Increases cell death by inhibiting Bcl-2</td>
<td>Inhibition of miR-1 to reduced cell death following H\textsubscript{2}O\textsubscript{2} and reduced MI size following AMI</td>
<td>113,114</td>
</tr>
<tr>
<td></td>
<td>↓ (human)</td>
<td></td>
<td></td>
<td>115</td>
</tr>
<tr>
<td>miR-7</td>
<td>N/A</td>
<td>Regulates mPTP by targeting VDAC1</td>
<td>May prevents mPTP opening at the onset of reperfusion thus reducing MI size</td>
<td>116</td>
</tr>
<tr>
<td>miR-15</td>
<td>↑ (pig)</td>
<td>Increases cell death</td>
<td>Inhibition of miR-15 using LNA anti-miRs reduced MI size following AMI in mice and pigs</td>
<td>117</td>
</tr>
<tr>
<td>miR-21</td>
<td>↓</td>
<td>miR-21 protects by downregulating PCDC4, AP1, PTEN and Fas ligand. Akt upstream of miR-21</td>
<td>Over-expression of miR-21 reduced MI size following AMI</td>
<td>118-120</td>
</tr>
<tr>
<td>miR-22</td>
<td>↓</td>
<td>miR-22 protects by downregulating Cav3 and upregulating eNOS</td>
<td>Over-expression of miR-22 reduced MI size following AMI</td>
<td>121,122</td>
</tr>
<tr>
<td></td>
<td></td>
<td>miR-22 protects by downregulating P53, CBP-AP1, TNF-α and IL-6, and upregulating Bcl2/Bax ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-24</td>
<td>↓</td>
<td>miR-24 protects by downregulating Bim</td>
<td>Over-expression of miR-24 reduced MI size following AMI</td>
<td>123</td>
</tr>
<tr>
<td>miR-29</td>
<td>↓ (Border area human)</td>
<td>Increased expression of fibrosis proteins (collagens, fibrillins, and elastin) due to de-repression of miR-29</td>
<td>Over-expression of miR-29 to reduce cardiac fibrosis following AMI</td>
<td>13</td>
</tr>
<tr>
<td>miR-31</td>
<td>↑</td>
<td>Increased cell death miR-31 protects by upregulating PKC-ε and downregulating NFκB</td>
<td>Inhibition of miR-31 using antagomir reduced MI size following AMI in mice</td>
<td>124</td>
</tr>
<tr>
<td>miR-92a</td>
<td>↑ (pig)</td>
<td></td>
<td>Inhibition of miR-92a using LNA anti-miR reduced MI size following AMI in pigs</td>
<td>125</td>
</tr>
<tr>
<td>miR-103/107</td>
<td>↑</td>
<td>Increased cell necrosis by downregulation of FADD and activation of RIPK1/RIPK3. lncRNA upstream of miR-103/107</td>
<td>Inhibition of miR-103/107 using antagomir reduced MI size following AMI in mice</td>
<td>126</td>
</tr>
<tr>
<td>miR-128</td>
<td>N/A</td>
<td>Increase cell death by antagonizing Akt, PPARY and Mcl-1 protein</td>
<td>Inhibition of miR-128 using antagomir reduced MI size following AMI in mice</td>
<td>127</td>
</tr>
<tr>
<td>miR</td>
<td>Direction</td>
<td>Function</td>
<td>Observations</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
<td>--------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>miR-133</td>
<td>↓</td>
<td>miR-133 protects by inhibiting DAPK2</td>
<td>Over-expression of miR-133 reduced MI size following AMI</td>
<td></td>
</tr>
<tr>
<td>miR-140</td>
<td>↑</td>
<td>Increased cell death by inhibiting Mfn1 and inducing mitochondrial fission</td>
<td>Inhibition of miR-140 using antagomir reduced MI size following AMI in mice</td>
<td></td>
</tr>
<tr>
<td>miR-142</td>
<td>↓</td>
<td>miR-142 protects by inhibiting HMGB1</td>
<td>Over-expression of miR-142 reduced cell death</td>
<td></td>
</tr>
<tr>
<td>miR-144/451</td>
<td>N/A</td>
<td>miR-144/451 protects by targeting CUGBP2–COX-2 pathway. GATA-4 upstream of miR-144/451</td>
<td>Over-expression of miR-144/451 reduced cell death</td>
<td></td>
</tr>
<tr>
<td>miR-145</td>
<td>↓</td>
<td>miR-145 protects by inhibiting BNIP3</td>
<td>Over-expression of miR-145 reduced cell death and protect mitochondria</td>
<td></td>
</tr>
<tr>
<td>miR-146</td>
<td>N/A</td>
<td>miR-146a protects by reducing caspase 3,7,8, suppressing IRAK1 and TRAF6 expression and inhibiting NFκB/ inflammatory cytokine production</td>
<td>Over-expression of miR-145 reduced cell death and protect mitochondria</td>
<td></td>
</tr>
<tr>
<td>miR-181</td>
<td></td>
<td>miR-181c induces cell death by inhibiting COX-1 and increasing mitochondrial ROS, whereas miR-181a/b protects by inhibiting PTEN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-192-5p</td>
<td>↑</td>
<td>Increases cell death by targeting FABP3 and upregulating Bax/Bcl2 ratio</td>
<td>Inhibition of miR-192-5p using antagomir reduced apoptosis</td>
<td></td>
</tr>
<tr>
<td>miR-195</td>
<td>↑</td>
<td>Increases cell death by inhibiting Bcl2. BDNF upstream of miR-195</td>
<td>Inhibition of miR-195 using antagomir reduced MI size following AMI</td>
<td></td>
</tr>
<tr>
<td>miR-200c</td>
<td>↓</td>
<td>miR200c protects by upregulating GATA-4 and Bcl2</td>
<td>Over-expression of miR-200c reduced cell death and protect mitochondria</td>
<td></td>
</tr>
<tr>
<td>miR-208</td>
<td>↑</td>
<td>miR-208 protects by repression of NCX1, CaMKIIδ, CypD, and BIM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-214</td>
<td>↑ (human)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-223</td>
<td>N/A</td>
<td>miR-223 protects by inhibiting TNFR1, DR6, NLRP3 and IκB kinase α</td>
<td>Over-expression of miR-223 to reduce MI size following AMI</td>
<td></td>
</tr>
<tr>
<td>miR-320</td>
<td>↓</td>
<td>Increases cell death</td>
<td>Over-expression of miR-320 to reduce MI size following AMI</td>
<td></td>
</tr>
<tr>
<td>miR-363</td>
<td>↑</td>
<td>Increases cell death by inhibiting Notch signaling</td>
<td>Inhibition of miR-429 using antagomir reduced cell death</td>
<td></td>
</tr>
<tr>
<td>miR-378</td>
<td>↓</td>
<td>miR-378 protects by inhibiting caspase 3</td>
<td>Over-expression of miR-378 reduced cell death</td>
<td></td>
</tr>
<tr>
<td>miR-429</td>
<td>↑</td>
<td>Increases cell death by inhibiting Notch signaling</td>
<td>Inhibition of miR-429 using antagomir reduced cell death</td>
<td></td>
</tr>
<tr>
<td>miR-451</td>
<td>↓</td>
<td>miR-451 protects by inhibiting HMGB1</td>
<td>Over-expression of miR-451 reduced cell death</td>
<td></td>
</tr>
<tr>
<td>miR-494</td>
<td>↓</td>
<td>miR-494 protects by inhibiting PTEN, ROCK1, CaMKIIδ, and activating FGFR2 and LIF</td>
<td>Over-expression of miR-494 reduced MI size following AMI</td>
<td></td>
</tr>
<tr>
<td>miR-499</td>
<td>↓</td>
<td>miR-499 protects by inhibiting calcineurin thereby preventing Drp1-mediated mitochondrial fission</td>
<td>Over-expression of miR-499 reduced MI size following AMI</td>
<td></td>
</tr>
<tr>
<td>miR-613</td>
<td>↓</td>
<td>miR-613 protects by phosphorylating Akt and inhibiting PDCD10</td>
<td>Over-expression of miR-613 reduced cell death</td>
<td></td>
</tr>
<tr>
<td>miR-761</td>
<td>↓</td>
<td>miR-761 protects by repressing Mff and inhibiting mitochondrial fission</td>
<td>Over-expression of miR-761 reduced MI size following AMI</td>
<td></td>
</tr>
<tr>
<td>miR-2861</td>
<td>↑</td>
<td>Increases cell death by inhibiting ANT1</td>
<td>Inhibition of miR-2861 using antagomir reduced cell death</td>
<td></td>
</tr>
</tbody>
</table>

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; Mtn1, 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

<table>
<thead>
<tr>
<th>Cardioprotective strategy or agent</th>
<th>Change in miRNA expression</th>
<th>Downstream targets/effects</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat stress (IPC mimic)</td>
<td>Upregulation of miR-1, miR-21 and miR-24</td>
<td>Reduced MI size, associated downregulation of pro-apoptotic genes (caspases 1, 2, 8 and 14, Bid, Bcl10, Cidea, Lrtr, Trp53 and Fas ligand), and upregulation of anti-apoptotic proteins (Bag3, and Prdx2)</td>
<td>10, 157</td>
</tr>
<tr>
<td>Propanolol (cAMP–PKA activation)</td>
<td>Downregulation of miR-1</td>
<td>Less IRI arrhythmias and preservation of Cx43 and Kir2.1</td>
<td>150</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>Upregulation of miR-199a-3p and miR-214</td>
<td>Less cell death and apoptosis with upregulation of Akt phosphorylation and Sox-4, and inhibition of dodl4 and ing4</td>
<td>151</td>
</tr>
<tr>
<td>Tanshinone IIA (Danshen, traditional chinese medicine)</td>
<td>Downregulation of miR-1</td>
<td>Reduced MI size, less IRI arrhythmias, preservation of Cx43 and Kir2.1, and inhibition of p38 MAPK.</td>
<td>152,153</td>
</tr>
<tr>
<td>Pioglitazone and rosiglitazone (PPAR-γ agonist)</td>
<td>Downregulation of miR-29</td>
<td>Less cell death and apoptosis, Less caspase 3 and upregulation of Mcl-2</td>
<td>154</td>
</tr>
<tr>
<td>Resveratrol (a constituent of red wine)</td>
<td>Modulation of a number of miRNAs affected by IRI (including upregulation of miR-21)</td>
<td>Reduced MI size</td>
<td>155,156</td>
</tr>
<tr>
<td>Hypoxia inducible factor-1α</td>
<td>Upregulation of miR-24</td>
<td>Less cell death and apoptosis with upregulation of BCI2</td>
<td>157</td>
</tr>
<tr>
<td>γ-tocotrienol (a constituent of palm oil)</td>
<td>Upregulation of miR-20b</td>
<td>Reduced MI size, upregulation of HIF-1α and VEGF</td>
<td>156</td>
</tr>
<tr>
<td>Choline (M3-AChR agonist)</td>
<td>Downregulation of miR-376b-5p</td>
<td>Reduced MI size, upregulation of BDNF</td>
<td>157</td>
</tr>
<tr>
<td>Hypoxia and Tricostatin A (HDAC inhibitor)</td>
<td>Upregulation of miR-126</td>
<td>Less cell death and phosphorylation of Akt and Erk1/2</td>
<td>158</td>
</tr>
<tr>
<td>Triiodothyronine (T3)</td>
<td>Upregulation of miR-30a</td>
<td>Less cell death and decreased expression of p53</td>
<td>159</td>
</tr>
<tr>
<td>Hydrogen sulphide</td>
<td>Downregulation of miR-1</td>
<td>Less cell death and apoptosis with less caspase 3, upregulation of Bcl2, and preservation of HDAC4</td>
<td>160,161</td>
</tr>
</tbody>
</table>

1. 10. 150. 151. 152,153. 154. 155,156. 157. 158. 159. 160,161. 162
<table>
<thead>
<tr>
<th>Compound</th>
<th>miRNA Effect</th>
<th>Biological Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteolin (a dietary flavonoid)</td>
<td>Upregulation of miR-21</td>
<td>Reduce MI size, less apoptosis, less inflammation, reduced inflammasome formation</td>
</tr>
<tr>
<td></td>
<td>Downregulation of miR-208b-3p</td>
<td>Less cell death and apoptosis with downregulation of BAX and caspase 3, and upregulation of avian erythroblastosis virus E26 oncogene homolog 1 and Bcl2</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>Upregulation of miR-21</td>
<td>Reduce MI size and downregulation of PDCD4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduce MI size, increased phosphorylation of Akt, eNOS, nNOS and less MPTP opening with isoflurane but not in miR-21 KO mice</td>
</tr>
<tr>
<td>Trimetazidine</td>
<td>Upregulation of miR-21</td>
<td>Reduced MI size, less cell death and apoptosis with suppression of PTEN, phosphorylation of Akt, decreased Bax and increased Bcl2</td>
</tr>
<tr>
<td>Recombinant HMGB1A-box</td>
<td>Downregulation of miR-21</td>
<td>Less cell death and apoptosis with phosphorylation of Akt, decreased Bax and caspase 3 and increased Bcl2</td>
</tr>
<tr>
<td>Morphine</td>
<td>Upregulation of miR-133b-5p</td>
<td>Less cell death and reduced Fas ligand</td>
</tr>
<tr>
<td>Salvianolic acid B (Chinese medicine herb)</td>
<td>Upregulation of miR-30a</td>
<td>Less cell death and autophagy, upregulation of PI3K and phosphorylated Akt</td>
</tr>
<tr>
<td>Inorganic nitrite</td>
<td>Downregulation of miR-125a-5p, miR-146b, miR-339-3p, miR-433 at reperfusion</td>
<td></td>
</tr>
</tbody>
</table>

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.