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Differential expression of microRNAs in ischemic heart disease

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Abstract

Recent studies provide evidence that ischemic preconditioning (IP) and ischemia/reperfusion (IR) injury lead to altered expression of microRNAs (miRNAs) that affect the survival and recovery of cardiomyocytes. These endogenous ~22-nucleotide noncoding RNAs negatively regulate gene expression via degradation and translational inhibition of their target mRNAs. miRNAs are involved in differentiation, proliferation, electrical conduction, angiogenesis and apoptosis. These pathways can lead to physiological and pathological adaptations. This review intends to explore several facets of miRNA expression and the underlying mechanisms involved in IR injury, as well as IP as a cardioprotective strategy. In addition, we will investigate miRNA interaction with the renin–angiotensin system and the potential use of miRNAs in developing sensitive biomarkers for cardiovascular disease.

Keywords

microRNA; ischemia/reperfusion; cardiac remodeling; angiotensin II; heart

Introduction

MicroRNAs (miRNAs) are endogenous ~22-nucleotide (nt) noncoding RNAs that control diverse biological functions. These molecules promote the post-transcriptional regulation of gene expression via translational inhibition and degradation of target mRNAs [1]. The miRNA pathway to either degradation or inhibition of mRNAs depends on the degree of complementarity between the 5' seed sequence (5–7 nt) of the miRNA and the 3' untranslated region (UTR) of the target mRNA [1]. Khraiweh *et al.* have proposed an alternate pre-transcriptional regulatory pathway, which implicates DNA methylation as the epigenetic silencer of target genes [2]. This occurs through a feedback mechanism that is related to the

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Teaser: Ischemia/reperfusion injury of the heart activates cellular pathways involved in cardiac remodeling. Differentially expressed microRNAs can regulate the development of cardiac pathology and potentially provide insights into new therapeutic interventions.

Conflicts of interest

The authors declare no conflicts of interest.

ratio of miRNA to its target mRNA [2]. These findings suggest that endogenous small RNA, in a post-transcription inhibiting manner, could directly regulate the expression of multiple genes. With growing interest into new insights of gene expression regulation, recent studies have shown that miRNAs control several aspects of heart pathologies, including cardiomyopathies, cardiac remodeling, heart failure, arrhythmia and ischemia/reperfusion (IR) injury [3–8]. Friedman *et al.* estimated that more than 45 000 miRNA target sites residing within the human genes 3'UTRs are conserved [9]. Recent studies have evaluated the temporal expression of miRNAs following IR in the murine model [10], whereas others have acquired and analyzed plasma samples from patients with ST-elevated myocardial infarction (STEMI) [11]. Together, these studies suggest that miRNAs, in conjunction with troponin plasma levels, can be utilized as novel and sensitive cardiac biomarkers [12]. Further understanding of the underlying mechanisms of the miRNA pathways will provide insights into their ability to orchestrate complex cellular pathways that could be pivotal in determining the progression of ischemic heart disease. This review will attempt to clarify the role of miRNAs in cardiovascular pathology, specifically discussing the dysregulation and signaling pathway, and their implications in IR injury.

Biogenesis

The biogenesis of miRNA begins with the transcription of the primary miRNA (pri-miRNA), a 1 kb or longer transcript, by polymerase II (Figure 1). The process is compartmentalized beginning in the nucleus and reaches completion in the cytoplasm. The pre-miRNA folds onto itself forming a double-stranded hairpin structure, which is ultimately cleaved by two endonuclease RNase III enzymes: Drosha and Dicer. It is first cleaved in the nucleus by Drosha, a multi-protein complex with an essential cofactor, DGCR8 [13,14]. The product of this cleavage is the pre-miRNA (~60–70 nucleotides), with a 5' phosphate and about a 2 nt 3' overhang [15,16], which is believed to serve as a recognition site for further processing.

The pre-miRNA is then transported from the nucleus to the cytoplasm by Ran-GTP and the export receptor exportin-5 [17,18]. In the cytosol, Dicer, the cytoplasmic RNase III endonuclease, identifies and cleaves the double-stranded pre-miRNA, removing terminal base pairs and the miRNA loop, leaving a 5' phosphate and ~2 nt 3' overhang. This product is ~20 base pair double-stranded consisting of the mature miRNA and its complementary miRNA* (miRNA:miRNA*).

The RNA-induced silencing complex (RISC) is a multi-protein complex containing an essential member of the Argonaute family. The miRNA product and its complement are loaded into RISC, and the most stable strand is destined to become the miRNA [19,20] while the opposing arm is degraded. The identification of target mRNAs by RISC is guided by the miRNA itself. RISC-dependent gene downregulation is achieved by three methods: (i) mRNA cleavage; (ii) translation hindering; and (iii) mRNA degradation. The amount of complement between the target mRNA at the 3'UTR and the miRNA determines which method of gene downregulation will occur. If sufficient complement exists, the miRNA directs RISC to cleave the target mRNA. If the complement exists but is insufficient to

direct cleavage, mRNA degradation or translational repression occurs. In the classic mRNA cleavage pathway, Argonaute functions as the enzyme Slicer to cleave mRNAs.

Apart from cleavage of mRNAs based on sequence recognition, miRNAs have been shown to regulate translation of target proteins. The following studies provide strong evidence for miRNA-mediated inhibition of translation and degradation of mRNAs. Translation occurs in three steps: initiation, elongation and termination, and any of these phases can potentially be regulated. The exact mechanisms by which they are regulated are still being elucidated. Several studies support the idea that miRNA-induced repression occurs at the initiation phase [21–24].

For instance, a functional m⁷G-cap on the target seems to be essential for miRNA-induced suppression of translation, which suggests that regulation occurs at the initiation step [23,25]. A molecular target of miRNAs could be eIF4E, a translation initiation factor, which recognizes the 5' cap structure and is involved in directing the ribosome to the cap structure. Humphreys and colleagues have shown that miRNAs can impede recruitment and/or function of eIF4E [23]. Pillai *et al.* have also observed repression of translation at the initiation stage by possible interference with the cap recognition [22].

Other studies have focused on mRNAs with an internal ribosome entry site (IRES) to resolve whether the cap-recognition step of initiation is targeted by miRNAs. Translation initiated at the IRES is independent of the m⁷G-cap. Conflicting accounts exist with IRES-initiated translation. mRNAs containing IRES have been shown to be subject to translational repression [26,27], suggesting a cap- and eIF4E-independent repression. However, other studies reported that IRES elicited resistance to repression [22,25]. Alternatively, miRNA-induced repression has been shown to involve ribosomes that dissociate prematurely, thus suggesting that another method might be involved in the repression of translation [27]. Still, other studies reported repression at the elongation stage of translation [28,29].

miRNA-induced gene silencing can also be accomplished by promoting degradation of mRNAs, independent of the RISC complex. Deadenylation destabilizes mRNAs, followed by mRNA decapping and digestion. Deadenylation of the mRNA target occurs via the CAF1-CCR4-NOT1 deadenylase complex [30]. Although much work has been done understanding translation repression, many questions still remain. miRNAs can induce repression by targeting different stages of translation; however, the mechanisms are still not fully understood. A better understanding of translational repression will be essential in defining the mechanisms by which miRNAs elicit their effects and their potential role in disease.

Clinical significance of miRNA

Currently, cardiovascular disease is the leading cause of death, and ischemic heart disease (IHD) alone contributes to over 7 000 000 deaths in the USA [31]. Financially, IHD costs the USA over US\$100 billion each year [32]. IHD is an umbrella term for pathophysiology related syndromes stemming from myocardial ischemia. The most prevalent form of IHD is myocardial infarction (MI). MI causes remodeling of the heart to compensate for the loss of cardiac function. Unfortunately, factors contributing to acute compensation can collectively

cause detrimental effects in the healing process of the infarct site and potentially develop other cardiovascular syndromes, such as arrhythmia.

After MI, the heart is vulnerable to arrhythmia, rupture, congested heart failure and pericarditis [33]. IR injury is linked to temporal changes such as cardiac rupture [33]. This dynamic remodeling can lead to a pathological outcome that can continue to develop post-MI. In cardiac IR injury the interplay between multiple factors elicits various cellular signaling pathways to initiate protection, death and remodeling. Understanding the temporal development and activity of factors contributing to IR injury is crucial to be able to intervene and advance the healing process, to counter the irreversible damage caused by prolonged ischemia.

The current method of diagnosis of ischemic heart disease includes: symptoms, patient history, electrocardiogram, test for cardiac markers, cardiac stress test and coronary angiogram [34]. Therapies can range from lifestyle changes to interventional medications, such as beta-blockers, to invasive procedures such as angioplasty [35]. Although current therapies provide significant benefit, they are not without limitations. The role of miRNAs in IR injury is being substantially investigated to provide a real-time window for diagnosis of pathophysiology of damaged tissue [12,36–38]. Therefore, the evaluation of miRNAs will provide not only new insights into the pathophysiology of IR injury but also the potential of cardiac biomarkers to diagnose diseases and provide a real-time glimpse of the progression of the disease. Transfecting tissues via administration of miRNAs in viral vectors or inhibition of miRNAs is likely to emerge as an alternative and safe method to approach cardiac diseases pharmacologically, ultimately conferring short-, intermediate- and long-term protection against ischemic injuries. Therefore, the future of miRNAs as a noninvasive therapy to counteract the rapidly evolving pathology of heart disease has great potential to revolutionize the field regarding detection and treatment.

Mechanisms involved in miRNA expression in the cardiovascular system

Relatively little is known about miRNA regulation and its response to changes in the microenvironment, such as during ischemia. Over the course of IR injury there is diminished cardiac function secondary to necrotic tissue formation. This prompts the secretion of various factors including: endothelin-1 (ET-1), angiotensin II (Ang II), epinephrine (Epi) and miRNAs [11,39–41]. These miRNAs can confer protective or detrimental effects via post-transcriptional regulation of mRNAs. The downstream effects of miRNA depend heavily on their temporal expression during the development of the pathology, as well as the tissue it originates from. It is well established that the miRNA profile changes when the tissue environment deviates from its original state. Therefore, pathways involved in compensation, altered tissue function and productivity can contribute to dysregulated miRNA expression. Kulshreshtha *et al.* first reported the induction of a spectrum of miRNAs during hypoxic conditions via a hypoxia-inducible factor (HIF)-dependent mechanism [42]. Stabilization of HIF under hypoxia regulates several pathways in response to tissue damage, such as neovascularization, promotion of growth and activation of proapoptotic genes [43].

One study showed that hypoxia altered the expression profile of several miRNAs [11]. Furthermore, some of the hypoxia-regulated miRNA promoter sites recruited endogenous HIF, indicating a direct influence of hypoxia on miRNA expression [42]. Similarly, protein kinase A signaling, triggered by compensatory β -adrenoceptor [s1]activation, positively regulated miR-1 expression by promoting the transcription enhancer [serum response factor (SRF)] [44]. A change in the microenvironment, such as ischemia, alters normal cellular physiology and thus results in altered expression of miRNAs.

In cell physiology, numerous genes, proteins and factors are regulated by a feedback mechanism. The transcriptional regulation of miR-1 by myocyte enhancer factor 2 (Mef2) demonstrates one such feedback mechanism. Interestingly, Mef2, a target of miR-1 [45], has been shown to activate the transcription of miR-1-2 and miR-133a-1 via an intragenic muscle-specific enhancer [46]. Therefore, elevated miR-1 levels by Mef2 serves as a negative feedback mechanism to attenuate Mef2 [46]. However, some miRNA expressions are involved in a positive feedback mechanism. IP₃-induced calcium release (IICR) is involved in hypertrophy and arrhythmogenesis of cardiomyocytes. miR-133 targets inositol 1,4,5-trisphosphate receptor (IP₃RII), upstream of IICR, leading to antihypertrophic changes; furthermore, IICR can downregulate miR-133 expression [47]. Thus, IICR promotes hypertrophy by diminishing the expression of miR-133 and creating an indirect positive feedback that further promotes hypertrophy [47]. Similarly, miR-21 is upregulated in cardiac fibroblasts, leading to the activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase (ERK) pathway, which favors the development of fibrosis [48]. One study showed that elevated levels of Ang II during heart failure could promote Rac1 activation and lead to connective tissue growth factor (CTGF) and a lysyl-oxidase-mediated increase in miR-21 [48]. CTGF is actively promoted in wound healing and the development of fibrosis. Thus, expression of CTGF increases miR-21 expression creating a positive feedback mechanism that ultimately promotes fibrosis.

Luo and colleagues proposed a potential mechanism involving nuclear factor of activated T cells (NFAT) in the negative regulation of miR-26 transcription, leading to elevated expression of Kir2.1 [49]. However, during heart failure miR-1 is downregulated leading to increased calcineurin–NFAT activity [28]. Therefore an increase in NFAT will suppress miR-26 expression [45,49]. Together, these studies suggest that miRNAs can indirectly affect the expression of other miRNAs.

Altogether, these studies suggest a complex network of regulatory pathways that alter miRNA expression. The interplay between transcription factors and signaling molecules is altered following IR injury, which is reflective of the newly induced microenvironment. When considering the mechanism of miRNA regulation we must take into account the multiple facets that can independently or synergistically alter their expression.

Understanding the overlap of cellular pathways and miRNA expression provides several entry points for therapeutic interventions. First is to focus the treatment around the upstream regulators of miRNA expression. Second is to target the miRNA directly and third is to modulate the downstream factors of the miRNA signaling pathway leading to phenotypic alterations. However, the first and third forms of treatment strategy pose similar problems.

The upstream regulators can alter the expression of several miRNAs including undesired targets, leading to an increase in the prevalence of adverse side-effects. Whereas downstream regulation of miRNA can be more specific and minimize the side-effects; the effectiveness of this treatment will be dependent on knowing all the factors involved in order to confer the desired outcome. Therefore, unless we are able to understand the mechanism in its entirety, it will be difficult to abrogate or induce specific changes. miRNA is unique in that it can potentially target pathways leading to a desired phenotypic outcome with minimal adverse effects.

However, the use of miRNAs as a therapeutic intervention is not without limitations. They are involved in numerous pathways of cell physiology and require more studies to build a comprehensive map of the terminal effects of miRNA. Nevertheless, treatment of pathophysiological conditions using temporal regulation of miRNA is likely to become a formidable treatment in the future.

miRNA involvement in IR injury

Ischemia is characterized by insufficient delivery of blood to tissues causing tissue oxygen deprivation, exhaustion of glucose and accumulation of metabolic byproducts, including carbon dioxide, lactic acid and reactive oxygen species (ROS). It is a case of diminished oxygen supply and inability to meet the increasing demands. Low oxygen content leads to numerous responses all with the goal of relieving tissue hypoxia and preventing irreversible damage. Prolonged ischemia followed by reperfusion leads to irreversible damage, and is collectively referred to as IR injury. However, intermittent, nonpathological ischemia can lead to a cardioprotective effect known as ischemic preconditioning [33](IP).

In response to ischemia, several survival mechanisms are induced, including recruitment of the renin–angiotensin system (RAS), β -adrenergic receptor activation, peroxisome proliferator-activated receptor (PPAR) α to PPAR γ (shift from ketones to glucose as the main source of energy) [50] and physiological adaptations to maintain fluid balance and cardiac output (CO). Severe IR injury involves cell death, arrhythmia, fibrotic hypertrophy and cardiac remodeling. The outcome will depend on various parameters, including pathways, cell cycle and the miRNAs involved, as well as the severity and the duration of ischemic stress [51].

With the recent implication of miRNAs involved in IR, studies have invested in the discovery of underlying mechanisms and downstream effectors for potential therapeutic use. Although several studies reported conflicting results in the expression and correlation between miRNA and its target effect, the varying consistency in miRNA expression reported in heart disease could reflect a difference in disease models, myocardial regions sampled, time when the sample was taken and assay platforms. However, these differences also highlight the idea that miRNA involvement in disease is time-, tissue- and region-dependent. This review is focused on understanding miRNA expression, interaction with surrounding downstream signaling and overall physiology and function related to IR injury. The subsequent sections are categorized into arrhythmia, cardiac remodeling, cell survival and

death, and angiogenesis, all factors that determine the majority of cardiac outcome and prognosis of patients suffering from IR injury.

Arrhythmia

The heart consists of conducting and contracting cardiomyocytes. Cooperation between these two cell types gives rise to three intrinsic properties responsible for automated propagation of electrical conductance leading to synchronized contraction. These intrinsic properties are automaticity, electric conduction and membrane repolarization, which all allow the heart to function as a unit. Arrhythmia is an inappropriate rate or rhythm of the heart that can have dire consequences. In IR injury, early onset (<48 h) of arrhythmia is mostly due to an anatomical block in which the infarct site is unable to synchronize with the non-infarct site and acts as a physical barrier to prevent conduction between cardiomyocytes. However, later onset of arrhythmia can also occur and is more physiological in nature, where there is altered expression of factors involved in maintaining electrochemical balance. Factors contributing to arrhythmogenesis are abnormal automaticity, neurohumoral changes, structural remodeling, conduction and repolarization abnormalities, altered expression and distribution of gap-junction or a combination of these mechanisms [52].

In an early study, miR-1 expression in the ischemic zone is found significantly increased and the expression of miR-1 tends to affect heart rate and rhythm directly [53]. The way by which miR-1 mediates arrhythmogenesis is through a disturbance in the conduction and resting membrane potential of cardiomyocytes. GJA1 and KCNJ2 are confirmed targets of miR-1 [53]. GJA1 encodes the main cardiac gap junction channel connexin 43 (Cx43) and KCNJ2 encodes for the Kir2.1 channel responsible for maintaining the cardiac resting membrane potential [53]. Therefore, attenuation of Cx43 and Kir2.1 by miR-1 resulted in arrhythmogenesis. Ischemic changes brought upon by MI altered miR-1 expression and the downstream translation of GJA1 and KCNJ2, leading to an irregular heart rhythm [53]. Interestingly, the expression of miR-1 increased several fold in the first 3–6 h after onset of MI in STEMI patients and gradually decreased to basal levels by day 5 [53,54]. One of the factors contributing to the high prevalence of mortality is the early onset of arrhythmia (48 h post-MI). Thus, miR-1 increased expression and disturbance in Cx43 and Kir2.1, correlating with the induction of fatal arrhythmia.

In addition, miR-133a, which is commonly coexpressed with miR-1, also targets Cx43; whereas miR-26 can target Kir2.1 [6,49]. Similarly to miR-1, transfection of miR-133 and miR-26 mediates the spontaneous development of arrhythmia through downregulation of Cx43 and Kir2.1 [6,49]. The collective targeting of Cx43 and Kir2.1 by these three miRNAs (miR-1, -26 and -133) increases the likelihood of arrhythmia in the ischemic heart. Therefore, the induction of early onset arrhythmia is linked to a disturbance in electrical conductance owing to the newly formed conduction barrier by the infarct site and the upregulation of miR-1, -26 and -133.

However, a recent study by Curcio *et al.* provides a different perspective on miR-1 in relation to Cx43 expression and Ang II in the development of tacharrhythmia in ventricular hypertrophy [55]. Ventricular hypertrophy is a common physiological adaptation following

increased circulation levels of Ang II in response to reduced cardiac function. Ang-II-induced hypertrophy results in ventricular tachyarrhythmia due to displacement of Cx43 [56]. The mechanism by which Ang II confers this effect involves the MAPK-extracellular signal-regulated protein kinase (ERK1/2) pathway and decreased levels of miR-1 expression [55,56]. Phosphorylation of Cx43 in combination with the hypertrophy of cardiomyocytes is responsible for the dissociation of Cx43 from gap-junctions, contributing to the formation of tachyarrhythmia [55]. In addition to targeting Cx43 mRNA, miR-1 has been previously implicated in negatively influencing the activity of MAPK/ERK1/2 [57]. Thus, a hypertrophic stimulus by Ang II upregulates MAPK/ERK1/2 activity, which then phosphorylates Cx43 and leads to its displacement from the gap-junction. This process is further potentiated by the downregulation of miR-1, which typically attenuates MAPK/ERK1/2 activity. Therefore, in the case of Ang-II-induced arrhythmia, increased miR-1 expression could provide potential benefit owing to its ability to minimize phosphorylation of Cx43. Although increased miR-1 levels in the early stages of MI contribute to high mortality as a result of arrhythmogenesis, the decrease in miR-1 in later stages post-MI can also contribute to tachyarrhythmia resulting in increased morbidity.

In summary, during the early stage (first 48 h after MI) the rise in miR-1 expression targets Cx43 and the decreased expression of Cx43 contributes to arrhythmia. In the later stage of infarction a decrease in miR-1 expression [11] coincides with a reduction in cardiac function leading to increased Ang II. This decrease in miR-1 will leave Cx43 expression intact and thus will not be the source of arrhythmia, as it was in the early stages of IR injury. However, the reduced levels of miR-1 will be unable to attenuate the activity of the MAPK/ERK pathway, which leads to uninhibited activation of MAPK/ERK1/2 by Ang II. Thus, Ang-II-induced MAPK/ERK1/2 activation will lead to phosphorylation of Cx43 resulting in arrhythmia at later stages of IR injury.

Several studies have found that intracellular calcium imbalance promotes arrhythmogenesis. Protein phosphatase (PP)2A is responsible for dephosphorylating the ryanodine receptor (RyR2) leading to decreased release of calcium from the sarcoplasmic reticulum (SR). miR-1 and miR-133 target B56 α which is a regulatory subunit of PP2A [58,59]. The deactivation of PP2A leads to hyperphosphorylation of RyR2, causing an increased release of calcium during calcium-induced calcium release [58]. As the excitation-contraction cycle continues, the release of calcium will exceed the uptake by the SR leading to calcium imbalance in cardiomyocytes. The prolonged increase of calcium in the cytosol can contribute to long QT syndrome and elicit premature ventricular contraction. By targeting B56 α , miR-1 and miR-133 can indirectly cause an increase in calcium release. This will lead to an imbalance in calcium homeostasis, which increases the susceptibility of unwanted contraction and contributes to arrhythmia formation [58,59].

By contrast, a decrease in intracellular calcium content can also cause irregular heart rhythm. Unlike skeletal muscle, cardiomyocytes have two sources of calcium: endogenously from the SR and extracellularly from the L-type calcium channel. The reduction in extracellular calcium current during action potential leads to the development of fibrillations [60]. The shortened action potential duration is in response to attenuation of *CACNA1C* and *CANNB1*, which encode for the L-type calcium channel [60]. This decrease in overall

conductance of calcium will cause ‘out-of-sync’ contractions leading to arrhythmia. miR-328 is found significantly increased in atrial fibrillation patients and appears to target *CACNA1C* and *CANNB1* [60]. Therefore, calcium imbalance will increase the likelihood of arrhythmia in heart failure patients owing to IR injury. miR-328 expression in heart failure demonstrates the effects of reduced intracellular calcium by decreasing the expression of L-type calcium channels and thus increasing the susceptibility of the heart to arrhythmia.

There are several factors contributing to arrhythmogenesis and many studies implicate the involvement of miRNAs. As these studies reveal the mechanisms, targets and the interplay between miRNAs and the arrhythmogenic signaling pathways, it becomes more likely that miRNAs have an integral role in regulating the heart rate and rhythm. A recent study suggests that miR-499, which targets *KCNN3* that encodes for the small-conductance calcium-activated potassium channel 3 (SK3), is involved in the generation of arrhythmias [61]. Therefore, miRNAs regulate the physiological and anatomical outcomes of IR injury, which can induce or prevent the development of arrhythmogenesis (Figure 2). The involvement of miRNAs in a number of signaling pathways could implicate them as key regulators of cardiovascular disease.

Cardiac remodeling

Cardiac remodeling is the change in structure and physiology of the heart in order to compensate for the diminished function. Compensatory mechanisms such as the RAS and sympathetic activation are secondary factors that contribute to excessive workload for the heart. The major inducer of cardiac remodeling is acute MI. The prolonged ischemia can cause irreversible damage and formation of necrotic tissue also known as an infarction. Loss of tissue will result in a decline in cardiac function and again elicit compensatory mechanisms such as activation of the sympathetic and RAS system to maintain cardiac function. However, chronic activation of the sympathetic and RAS systems will eventually lead to cardiac remodeling via hypertrophy and fibrosis.

Pathological hypertrophy is a compensatory mechanism in response to diminished hemodynamic flow to the tissues and is paralleled by exaggerated cell growth and accelerated cell death of cardiomyocytes. This creates a vicious cycle until most cardiomyocytes are depleted, ultimately resulting in insufficient cardiac output. Fibroblasts are activated to replace necrotic tissue and reinforce the cardiac structure to maintain pressure and prevent rupture. Cardiac remodeling is an active process of IR and results in complete scar formation at the infarct site. Owing to these structural and physiological changes, disturbance of the electrical conductance is prevalent and can ultimately lead to arrhythmia and heart failure.

Hypertrophy—Various miRNAs can have anti- or pro-hypertrophic effects. The expression of pro-hypertrophic genes is dependent on a mechanism mediated by the calmodulin (CaM)/calcineurin/NFAT pathway and hypertrophic gene transcription factors: myocyte enhancer factor 2 (Mef2a) and Gata4 [45]. In the non-IR injured heart, inhibition of these transcription factors and the CaM signaling pathway minimizes their hypertrophic effects. Studies show that miR-1 and miR-133 are involved in suppressing the expression of

these hypertrophic genes [45,62]. CaM, Mef2a and Gata4 are targeted by miR-1, whereas calcineurin is targeted by miR-133, leading to an antihypertrophic effect [45,62]. However, in areas of infarct, miR-1 and miR-133 are downregulated, which leads to the transcription of hypertrophic genes. Therefore, diminished levels of miR-1 and miR-133 after IR injury favor the hypertrophy of cardiomyocytes.

Another contributor to increases in cell size is twinstin-1 (TWF1) activity [63]. TWF1 is an actin-binding protein that regulates cytoskeletal structures. The overexpression of TWF1 in neonatal cardiomyocytes results in an increase in cell size and the expression of a hypertrophic marker, thus indicating that TWF1 promotes cardiomyocyte hypertrophy. However miR-1 targets TWF1 and thus abrogates the effect of TWF1 on cell size [63]. Diminished miR-1 expression is unable to suppress TWF1 expression sufficiently, leading to the hypertrophic phenotype. It appears that early expression of miR-1 opposes hypertrophic changes and the suppression of miR-1 promotes cardiomyocyte hypertrophy.

Other factors contributing to hypertrophy are RhoA and Cdc42, a signal transduction kinase [64]. miR-133 can negatively regulate the expression of RhoA and Cdc42 by targeting their respective genes [64]. Thus, miR-133 can inhibit factors leading to hypertrophy. In addition, miR-133 targets inositol 1,4,5-trisphosphate receptor (IP₃RII), which regulates calcium release from the SR [47]. IP₃RII leads to downstream activation of IICR, which promotes pro-hypertrophic gene expression and initiates arrhythmogenesis [47]. Depression of IP₃RII activity leads to lower calcium signaling, and prevents IICR-induced hypertrophic gene expression [47]. Similar to miR-1, miR-133 has a major role in promoting antihypertrophic effects. Expression of miR-133 peaks shortly after IR injury, however several hours later the expression decreases [11]. Thus, miR-133-mediated downregulation of RhoA, Cdc42 and IP₃RII altogether suppresses hypertrophy. However a subsequent decrease in miR-133 expression after IR injury ultimately contributes to cardiomyocyte hypertrophy.

A recent study by Bang *et al.* showed the induction of hypertrophy via paracrine signaling from neighboring cardiac fibroblasts [65]. Exosomes are released containing miR-21, which targets sorbin SH3-domain-containing protein 2 (SORBS2) and PDZ and LIM domain 5 (PDLIM5) to induce hypertrophy [65]. Interestingly, this study provides two key insights to the miRNA regulation of hypertrophy. First, it suggests that miR-21 is pro-hypertrophic by targeting antihypertrophic factors such as SORBS2 and PDLIM5. Second, this study shows that miRNA-21 can be released from cardiac fibroblasts and stimulate cardiomyocytes to undergo hypertrophy. Thus, this finding suggests a cross-talk between two different cell types that can be mediated by miRNAs to induce a specific phenotypic change. Most studies identify the effect of miRNAs within the cell that secretes them; however this study highlights the potential for miRNAs to affect neighboring tissues. Although this provides evidence that miRNAs can be released by exosomes to elicit paracrine signals, more studies are required to understand whether miRNAs are secreted in the circulation to elicit effects on distant tissues.

Initially hypertrophic changes can be beneficial to the ischemic heart that has diminished cardiac output. However, chronic induction of cardiomyocyte hypertrophy can be detrimental to the terminally differentiated cardiomyocyte population, eventually leading to

cell death. Often, rapid growth will lead to the synthesis of contraction fibers, such as actin and myosin, however they will be misaligned and lead to inefficient contraction and consumption of energy. At this point, cardiomyocytes become greatly dependent on glucose as the main source of energy, which leads to ROS production. Uncontrolled hypertrophy will promote cell death. Prevention of excessive cardiac remodeling has been a mainstay goal for long-term treatment in MI patients. Current studies have revealed miRNA as a major factor contributing to cardiac hypertrophy (Figure 3). Understanding the expression and downward effects of miRNAs will increase the understanding of mechanisms involved in hypertrophy and potentially lead to new therapies.

Fibrosis—Cardiac fibrosis is a pathologic and a compensatory mechanism of the infarcted myocardium. The loss of cardiomyocytes activates the proliferation of fibroblasts, leading to the deposition of extracellular matrix (ECM) components in the myocardium. Formation of fibrotic scar tissue at the site of the infarct diminishes cardiac function and serves as a physical barrier for proper electrical conduction. The benefits of fibrosis are to fortify the infarct site, which is at a high-risk of rupture after IR injury, and to maintain pressure in the heart chambers. Unfortunately, the formation of fibrotic tissue can strengthen the myocardium wall but it is incapable of participating in electrical conduction and contraction. Therefore, for the myocardium to maintain pre-MI cardiac function, the surrounding cardiomyocytes are stimulated to undergo hypertrophy. Thus, fibrosis actively and inadvertently contributes to pathological cardiac remodeling.

CTGF is a key regulator involved in tissue wound repair and the development of fibrosis. Increased expression of CTGF induces fibroblast proliferation and increases collagen deposition in the ECM [66]. Increased expression of miR-133 opposes the effect of CTGF on fibroblast and collagen synthesis [66]. miR-133 targets genes encoding CTGF and collagen1a1 (Col1a1) causing a decrease in their expression and preventing fibrosis [67]. Other studies report a correlation between miR-1 and the reduction of profibrotic genes, CTGF and transforming growth factor beta-1 (TGFβ1) [57]. However, additional studies are needed to confirm whether CTGF and TGFβ1 are actual targets of miR-1. In general, these studies suggest that miR-133 expression is inversely related to cardiac remodeling and results in antifibrotic effects.

Cardiomyocytes comprise 30–40% of the total myocardial cell number but actually occupy approximately 75% of myocardial tissue [68]. The remaining cell population mainly consists of fibroblasts [68]. Myocardial fibroblasts predominately express miR-21 during active proliferation and synthesis of collagen [50]. miR-21 targets sprout homolog 1 (Spry1), a potent inhibitor of ERK/MAPK signaling, which promotes the secretion of fibroblast growth factor 2 (FGF2) [48,69]. Thus, repression of Spry1 removes the inhibition of ERK/MAPK, resulting in cardiac fibrosis.

Among the MI-regulated miRNAs, miR-29 depresses the expression of proteins involved in fibrosis such as collagens and elastin [70]. However, in regions adjacent to the border zone, miR-29 is significantly downregulated [70]. One study demonstrated that TGFβ depressed miR-29 expression [70]. Thus, the repression of miR-29 in the infarct border zone might lead to an increase in proteins involved in fibrosis.

Although the formation of fibrosis reinforces structural stability of the heart after IR injury, the scar tissue impairs cardiac contractility, electrical signaling and promotes hypertrophy of neighboring cells. Therefore, initially, fibrosis will benefit the heart but continued induction of fibrosis and the cross-talk between the fibrosis and hypertrophy pathways will lead to continued remodeling and exhaustion of remaining cardiomyocytes. Modulation of IR-induced miRNAs could provide an alternate method in minimizing cardiac remodeling, including hypertrophy and fibrosis, following IR injury (Figure 3).

Cardiomyocyte survival and death in IR injury

Cardiomyocyte death occurs during the initial ischemic event by necrosis and during the transition from compensatory hypertrophy to ischemic heart failure by apoptosis [71]. Tissue damage from MI is a result of prolonged ischemia exposure causing irreversible damage to cellular structure and physiology. The damage is further exacerbated by reperfusion. The surviving cardiomyocytes are stimulated to undergo hypertrophy via activation of the sympathetic and RAS systems to compensate for the diminished cardiac function. Hypertrophy of cardiomyocytes will allow the heart to regain function initially; however, in the long-term the nutrient demand of cardiomyocytes will far exceed the supply. Studies have demonstrated that ischemic preconditioning (IP) provides significant protection against prolonged ischemia. The dysregulation of miRNA expression in IR and IP_{s4} could provide a potential pathway to mediate the survival of cardiomyocytes following injury and minimize apoptosis.

Cardiomyocyte death—Cell death can arise from cellular insults, leading to necrosis, as well as programmed cell death or apoptosis. The caspase pathway and organelle dysfunction are major downstream effectors of the death signal that induces apoptosis [72]. BCL-2 (antiapoptotic) and BAX (proapoptotic) are pivotal regulators of cell survival [72]. BCL-2 and BAX are found downstream of the death signal pathway and can lead to caspase activation and mitochondria dysfunction. An IR-induced miRNA has been implicated in the cell death pathway by altering the expression of BCL-2. When BCL-2 expression is low the likelihood of cell survival is reduced. Increased expression of miR-1 has been shown to promote cell death by targeting BCL-2 [73]. In addition, protein kinase C (PKC) ϵ and heat shock protein (HSP)-60, involved in protecting against IR damage, are targeted by miR-1 [74]. Therefore, miR-1 can target BCL-2, PKC ϵ and HSP-60; and, in combination with a rise in caspase-3 activity, will confer a proapoptotic effect and lead to cell death [73,74] (Figure 2).

Pioglitazone (PIO) is a PPAR γ agonist that protects against IR injury and improves cell viability [75]. The administration of PIO is correlated with a decrease in miR-29 and reduction in apoptosis of the myocardium [75]. One study showed that miR-29 suppressed Mcl-2, an antiapoptotic relation of BCL-2; and as a result caspase-3 and BAX activities are unopposed leading to apoptosis [75]. Therefore, the inhibition of miR-29 promotes cell survival in IR injury. Thus, PIO commonly used in the treatment of diabetes mellitus type 2 could also potentially be used in the treatment of IR injury. It will be interesting to conduct a prospective study on the effects of PIO on IR injury.

The initial ischemia that leads to necrotic tissue formation following IR injury is unavoidable, but it can be minimized by the method of ischemic preconditioning. However, without therapeutic interventions, even the surviving cardiomyocytes will gradually undergo cardiac remodeling, which will inevitably lead to apoptosis. It is important to understand the underlying mechanisms in order to minimize cell death through apoptosis to maintain heart health after IR injury.

Cardiomyocyte survival—ROS, such as hydrogen peroxide (H₂O₂), are increased during reperfusion and are known to induce cell death and apoptosis. During their study, Cheng *et al.* noticed that H₂O₂ treatment increased the expression of miR-21 and decreased programmed cell death (PDCD)4 expression [76]. PDCD4 induces cell death through inhibition of activator protein 1. However, a decrease in PDCD4 minimizes cell death and maintains cell viability after induction of reperfusion injury. By specifically attenuating PDCD4 expression, miR-21 provides protection against H₂O₂-induced cell death and improved cell survival [76]. A subsequent study by Cheng *et al.* showed that miR-21 was upregulated in ischemic IP [77], and adjacent areas of infarction [78]. These studies suggest that miR-21 provides antiapoptotic protection in IP and IR injury by targeting PDCD4.

HIF-1 is stabilized upon induction of hypoxia. One of the central roles of HIF-1 is to reinstate perfusion via angiogenesis and regulate cell viability to protect the myocardium from ischemic damage. HIF-1 mediates cell survival by increasing the promoter activity of miR-24 [42,79] and miR-210 [80]. The upregulation of miR-24 provides protection against apoptosis by targeting BCL2L11 (Bim), a proapoptotic factor [81], as well as by inhibiting the translocation of BAX from the cytosol to the mitochondria [79]. The protective effect of miR-24 is determined by its expression level which is directly proportional to the duration of hypoxia [81,82]. In general, miR-24 opposes proapoptotic stimuli in IR injury.

Another player, miR-210, conveys IP protection via targeting the caspase-8-associated protein-2 [80]. In addition to HIF promoting miR-210 expression, a HIF-independent mechanism via a pathway involving p53 and protein kinase B (Akt) has also been suggested [83]. Therefore, several signaling pathways elicited by reperfusion injury can trigger an increase in miR-210, leading to cardioprotection and survival of cardiomyocytes. Previously, miR-133 has been thought to be involved in antiapoptotic properties; however mixed results are reported with its role in cell viability. Therefore, more studies are required to elucidate the role of miR-133 in apoptosis.

The endpoint of IR injury is depletion of functional cardiomyocytes, which ultimately leads to heart failure. The final stages leading to heart failure is the induction of necrosis and apoptosis. It is crucial to intervene with pharmaceutical therapies to minimize the loss of cardiomyocytes and maintain cardiac function. Therefore, the interplay between miRNAs and cell viability pathways could provide a new perspective on future therapy (Figure 2).

Angiogenesis

Following ischemia, neoangiogenesis is a crucial component involved in maintaining and recovering blood perfusion to relieve tissue hypoxia. Although adult endothelial cells remain quiescent, upon exposure to hypoxia, ischemia and tissue injury these cells can re-enter the

cell cycle to migrate and proliferate and form primary capillaries. Several miRNAs have been shown to modulate angiogenesis and can be subdivided into pro- and anti-angiogenic categories (Figure 3).

A major contributor to miRNA antiangiogenic effect in IR injury is miR-24. Several aspects including endothelial apoptosis, inhibition of tubular formation and regulation of multiple factors comprise the antiangiogenic property of miR-24 [84]. The transcription factors, GATA2 and p21-activated kinase (PAK4) are established to be involved in tube formation and angiogenesis [84]. GATA2 promotes SIRT1 and HMOX1 expression and PAK4 promotes BAD phosphorylation. Altogether, these factors elicit antiapoptosis and promotion of angiogenesis. miR-24 conveys antiangiogenic properties by targeting GATA2 and PAK4 [84]. The expression level of miR-24 is significantly upregulated in ischemic myocardium [81]. miR-24 expression is promoted by HIF [42], whereas areas bordering the infarct site have diminished levels of miR-24 [82].

HIF orchestrates various pathways involved in cardiac physiology. It can stimulate either a pro- or anti-angiogenic effect. For promotion of angiogenesis, miR-210 is the main mediator of HIF. miR-210 can stimulate the formation of capillary-like structure, endothelial cell migration [85] and target ephrin-A3, which is an inhibitor of angiogenesis [86,87]. Ephrin-A3 is essential in the remodeling of blood vessels, involving endothelial cells, pericytes and vascular smooth muscle cells. miR-210 is highly upregulated and remains at an elevated level in IP and IR injury. Therefore, miR-210 appears to be a major contributor of angiogenesis by suppressing the effect of ephrin-A3 and prompting tubulogenesis in infarct areas [85].

Coronary artery disease can lead to ischemia and diminished perfusion via thrombosis, emboli, atherosclerosis and spontaneous vasoconstriction. To relieve ischemic injury, the myocardium must reestablish perfusion. Angiogenesis is a compensatory mechanism to re-route blood supply to provide perfusion to the infarct areas. However, a clear distinction must be made between the rapid reperfusion, which leads to reperfusion injury owing to production of ROS, and the gradual reperfusion caused by angiogenesis. The induction of HIF-regulated miRNAs can procure the necessary steady perfusion of damaged myocardium and provide improved prognosis for MI patients.

Ischemic preconditioning as a cardioprotective strategy

Despite early risk management and current interventional treatments, ischemic heart disease is the number one contributor to morbidity and mortality associated with heart disease [31,88]. Therefore, there is continued interest in developing novel therapeutic targets that will potentially protect the heart against ischemia-related injuries. Interestingly, it has been shown that a short episode of nonpathological ischemia followed by reperfusion evokes an endogenous protective mechanism against prolonged ischemic injury [82]. This phenomenon is referred to as IP [89]. Unfortunately, IP itself is considered an impractical treatment owing to its innate ability to cause injury. Therefore, considerable studies have invested in understanding the fundamental nature of IP and its mechanisms that lead to cardioprotection.

Several factors have been shown to play a part in mediating IP protection including: heat shock protein factor (HSF)-1, HSP-70, HIF-1 α , Sirt1, HSP-90 and nitric oxide synthase [90–93]. Recent studies have found that miRNAs (miR-1, miR-20, miR-21, miR-24 and miR-210) are differentially regulated after preconditioning and are able to elicit protection of the heart against IR injury [42,80–82,90,94]. Protein kinase C (PKC) [87] and HIF [42,80] are considered major factors that act as mediators of IP. PKC receives signals following nonpathological ischemia to activate stress-responsive transcription factors [nuclear factor (NF)- κ B, STAT and phosphoinositide 3 kinase (PI3K)/Akt] and leads to the expression of cardioprotective genes [93]. Therefore, PKC serves as a convergence point for extracellular ischemic signaling, through which the message can be relayed to intracellular pathways for IP. HIF becomes stabilized in ischemic conditions and promotes miRNA expression, as well as directly activating pathways such as angiogenesis via vascular endothelial growth factor (VEGF). Induction of IP upregulates miR-1, miR-21 and miR-24 as well as increases eNOS, HSF-1 and HSP-70, altogether leading to reduced infarct size in the subsequent IR injury [90]. Furthermore, isolation of IP-mediated miRNAs and administering them to non-IP-conditioned hearts resulted in cardioprotection [90]. However, no specific miRNA was specifically identified as responsible for this protective effect during IP. Rather, these results suggest that it is the accumulation of miRNAs that leads to increased cell viability following IR injury. Interestingly, this study showed that miRNAs can be isolated from preconditioned tissue and the protective effect of IP-miRNAs can be collected and transferred to a nonpreconditioned heart through direct administration [90].

Previously, several mechanisms have been hypothesized to play a part in the propagation of remote ischemic conditioning [95]. A recent study utilizing the remote ischemic preconditioning (RIPC) model demonstrated an increase in the release of extracellular vesicles from the heart following preconditioning. This study suggests that these vesicles are responsible for the transmission of remote conditioning signals to elicit cardioprotection [96]. This provides a new possibility for therapeutic use of a novel vesicular mechanism in the transmission of cardioprotective signals from a preconditioned heart to another heart subjected to IR injury [96]. Although induction of miR-1, miR-21 and miR-24 leads to IP, a study by Pan *et al.* identified PKC ϵ and HSP-60 to be targets of miR-1 [74]. Furthermore, Duan *et al.* reported that miR-21 targeted the *PDCD4* gene, and significantly reduced the infarct size and apoptosis after IR injury [77,94]. Therefore, miR-21 might act as a main contributor to IP, leading to cardioprotection against IR injury. Other miRNAs including miR-133, miR-126, miR-320 and miR-92a are also correlated with IP and have been extensively reviewed by Salloum *et al.* [93] and Muller and Dhalla [95].

In the clinical setting, variant angina leading to narrowing of the coronary artery is similar to IP. However, coronary artery disease is still the main contributor of morbidity and mortality leading to myocardial infarction. Therefore, clinically evoking IP is impractical. Other studies have focused on prompting cardiac preconditioning via pharmaceutical means [97–99], preconditioning without ischemia [100–103] and exercise [104–106].

Exercise, in particular, has been widely accepted as a mode of improving overall heart health and is considered the main factor in lower Framingham scoring by targeting modifiable risk factors [102]. A study by Abete *et al.* suggested that a decrease in

norepinephrine production attenuated the effect of IP on the post-ischemic heart, whereas exercise training restored preconditioning in the senescent heart [104]. In another study, aerobic exercise has been shown to increase miR-27a and -27b, which target the angiotensin-converting enzyme (ACE), as well as decrease miR-143 that targets ACE2 in the heart [107]. Thus, exercise lowers Ang II, which is a key modulator in cardiac remodeling after IR injury. In addition, swimming, an intermittent anaerobic exercise, led to an upregulation of miRNAs including miR-21 involved in IP and activating the PI3K/Akt/mammalian target of rapamycin (mTOR) signaling pathway [105]. These studies suggest that exercise can influence the miRNA expression and induce IP-like conditioning. In the case of IP, miRNAs appear to provide protection against potentially pathological ischemic episodes. Therefore, developing an miRNA-orientated therapy could emerge as an alternative and safe method for conferring preventative, acute and chronic protection against IR injury.

Interaction between miRNAs and the RAS related to IR injury

One of the compensatory mechanisms in IR injury is the activation of the RAS. In the early phases of IR injury, a decline in cardiac function leads to an increase in the conversion of angiotensin to Ang II. This results in a significant improvement in cardiac function via changes in cardiac output (CO), contractility strength (dP/dT), ejection fraction (EF), heart rate (HR) and blood pressure (BP). Unfortunately, chronic overexpression of Ang II has also been documented to elicit cardiac remodeling, cell death and endothelial damage following IR injury [108].

Ang II primarily manifests its effect through two G-protein-coupled receptors: Ang II type 1 receptor (AT1R) and Ang II type 2 receptor (AT2R). The AT1R mainly activates growth-promoting pathways, fluid homeostasis and is also a major contributor of oxidative stress leading to endothelial dysfunction, inflammation and cardiovascular disease [109].

Activation of the AT2R provides many opposing effects such as vasodilation, hypotension, apoptosis, antihypertrophic and inhibition of the AT1R [110]. There is a great deal of complexity in the RAS pathway, including numerous factors and receptors that participate in the pathway such as ACE2, Ang(1–7) and Ang(1–12) [111,112]. However, the AT1R and AT2R are the major contributors in transduction of extracellular signals to elicit the effects of Ang II.

The AT1R is expressed in the cardiovascular system on cardiomyocytes, fibroblasts, vascular smooth muscle cells and endothelial cells. In addition to PKC activation, the AT1R is able to activate MAPK [113], epidermal growth factor receptor (EGFR) [114], insulin receptors [115] and many more. A review by Dasgupta and Zhang highlights the interaction between Ang II and cardiovascular disease [108]. Ang II has a significant role in maintaining cardiovascular health and the development of pathology. Reperfusion injury is mediated by ROS, which can contribute to the development of coronary artery disease such as atherosclerosis. Ang II potentiates oxidative stress by activating NADPH oxidase to produce ROS [116], attenuate NO production leading to severe vasoconstriction [117] and induce cardiomyocyte hypertrophy by TGFβ [118].

Although some studies implicate an indirect interaction between Ang II and miRNAs, more studies are required to establish their interaction in relation to cardiovascular disease. Ang II induces the production of H_2O_2 and, in response, miR-21 is upregulated [116]. miR-21 targets PDCD4, a promoter of apoptosis, and thus suppresses cell death and opposes the damage induced by Ang II [76]. Moreover, Ang II promotes vasoconstriction by AT1R activation and diminished production of NO. The mixture of miR-1, miR-21 and miR-24, which are all upregulated by IP, opposes cardiomyocyte damage and relieves vasoconstriction [90]. IP-miRNA expression results in elevated levels of NOS, HSF-1 and HSP-70 mRNA and protein [90,94]. In addition, Ang II induces cardiomyocyte hypertrophy and drives the transcription of fibrotic markers such as collagen, fibronectin and CTGF. miR-1 expression *in vivo* inhibits pressure-overload-induced cardiac hypertrophy and protect against adverse cardiac remodeling [57]. miR-1 reduces myocardial fibrosis by targeting fibullin-2 (Fbln2), a secreted protein implicated in extracellular matrix remodeling. Moreover, exercise can induce miR-27a and -27b expression, which results in a decrease in ACE [107]. Therefore, these studies suggest a possible crossover between miRNA pathways and Ang II in cardiovascular disease; however more studies are needed to map this interaction fully.

miRNAs as sensitive clinical biomarkers for cardiovascular disease

The importance of miRNAs acting as a regulator, adaptor and inducer of cardiovascular disease has been well established and highlights their use as a potential therapeutic target. Studies have focused on the ability of miRNAs to reflect the changes in tissues, caused by stresses. In light of these recent findings, it is possible that miRNAs could be utilized as biomarkers for disease.

Cardiac troponin T (cTnT) and troponin I (cTnI) are cardiac biomarkers that are clinically used for establishing a timely and correct diagnosis. The levels of cTnI peak approximately 3.5 h after the onset of chest pain and remain increased for 4 days in samples from acute MI patients [119]. cTnT exhibits a second and smaller peak at about day 4 following acute MI. Thus, cTnI is used for early detection and cTnT is used later to identify acute MI [119,120]. Although cTnT and cTnI play a fundamental part in the diagnosis of acute MI, many of these markers have low sensitivity and specificity.

Recent studies indicate the presence of miRNAs in the circulation of humans and animals [96]. The administration of coronary-perfusate-containing IP-induced miRNAs has been shown to provide remote protection from ischemia [90]. Investigations by Meder *et al.* assessed peripheral blood samples of patients with acute MI to quantify serum miRNA expression and observed a correlation with cardiovascular diseases [39]. These blood samples identified over a hundred miRNAs that are significantly differentially regulated in acute MI patients. The blood for miRNA and troponin T measurements was drawn several hours after the reported onset of symptoms. Of the 121 dysregulated miRNAs, 45 were found to be upregulated and 76 were downregulated in acute MI patients [39]. Among 17 of the 20 MI samples, the level of miR-1291 was found to be significantly decreased, indicating a specificity and sensitivity of 85% [39]. miR-663b was also identified as decreased in 19 of the 20 MI blood samples, with a specificity of 96% and a sensitivity of

90% [39]. Furthermore, Meder *et al.* correlated the levels of miR-145 and miR-30c with that of cTnT, suggesting that miRNAs isolated from peripheral blood could be utilized in conjunction with current biomarkers for cardiovascular diseases [39]. The degree of detection was further improved in this study by utilizing multiple miRNAs as biomarkers. Utilizing the expression profile of 20 miRNAs enhanced the diagnosis of acute MI patients with a sensitivity and specificity of 90% and 96%, respectively [39]. Altogether this indicates that miRNAs are highly useful tools in detecting cardiovascular disease with greater specificity than current biomarkers.

Other studies, including by D'Alessandra *et al.*, identified miRNAs dysregulated in cardiac remodeling following acute MI [11]. This study identified the expression of specific miRNAs dependent on time of onset of MI. Plasma samples from STEMI patients were collected several hours after the onset of MI symptoms and coronary reperfusion. MiR-1, -133a, -133b and -4995p were found to be upregulated by 15- to 140-fold, whereas miR-122 and -375 levels were reduced by 86–90% [11]. On day 5, miR-1, -133a, -133b, -499-5p and -375 returned to baseline, whereas miR-122 remained downregulated until day 30. The expression of miR-1, -133a and -133b, and cTnI reached peak levels at a similar time [11]. These results were replicated in a murine model that simulated IR injury [11]. In addition, cardiac tissues collected from infarct and border areas (3 and 6 h) in mice represented reciprocal changes in the expression [11]. This explains high miRNA levels in plasma samples owing to ischemic damage leading to the disruption of the membrane integrity of cardiomyocytes. This is further supported in a study by Gidlöf *et al.*, who observed that cardiospecific miRNAs (miR-1 and -133a) can be detected in urine samples from patients with STEMI [12]. This suggests that miRNAs can be collected in a noninvasive manner, such as in urine, for the detection of cardiovascular diseases. Altogether, recent studies indicate that miRNAs have great potential to serve as novel biomarkers of cardiovascular disease.

Concluding remarks

miRNAs, small non-coding RNAs, can influence various aspects of cell physiology by negatively regulating gene expression through the degradation and translational inhibition of their target mRNAs. In IR injury and ischemic preconditioning, miRNAs are differentially expressed. Several factors, such as HIF, ROS and the β -adrenoceptor, influence the miRNA expression profile, which leads to physiological and pathological adaptations. Current studies have investigated the underlying mechanisms of miRNAs in proliferation, differentiation, electrical conductance, angiogenesis and apoptosis. These studies have illuminated miRNAs as part of the mechanism involved in IR injury, and suggest a potential use as cardiac biomarkers and interventional therapy. miRNA studies in cardiovascular disease are constantly developing and, with gaining momentum, administration of miRNAs could become synonymous with future treatments.

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Highlights

Ischemic heart disease is a leading cause of morbidity and mortality

MicroRNAs regulate gene expression by translational inhibition and degradation of target mRNAs

Ischemic heart disease changes microRNA expression profiles

MicroRNAs provide sensitive biomarkers and novel therapeutic targets of ischemic heart disease

Figure 1.

Schematic representation of miRNA biogenesis. The process includes transcription, pre-miRNA formation, miRNA:miRNA*duplex, mature miRNA and miRNA target mRNA inhibition. This diagram outlines two potential mechanisms for miRNA/mRNA silencing: translational repression and mRNA target cleavage. A detailed discussion of post-transcriptional inhibition is included in the text.

Figure 2.

Schematic representation of miRNA and its target mRNA and proteins involved in arrhythmia, cell death and survival. Several miRNAs target mRNA and proteins, which can result in arrhythmia, cell death or cell survival.

Figure 3. Schematic representation of miRNA and its target mRNA and proteins involved in hypertrophy, fibrosis and angiogenesis. Several miRNAs target mRNA and proteins, which can lead to cardiovascular changes via hypertrophy, fibrosis and angiogenesis.