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Invited Mini Review

MicroRNAs – New Players in Cardiac Injury and Protection

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Abstract

MicroRNAs (miRNAs) have emerged as a novel class of endogenous, small, non-coding RNAs that negatively regulate gene expression via degradation or translational inhibition of their target mRNAs. Over 700 miRNAs have been identified and sequenced in humans and estimated number of miRNA genes is over 1000. Individual miRNA is functionally important as a transcription factor because it has the ability to regulate the expression of multiple genes through binding to its target with imperfect or perfect complement. In the heart, miRNAs have been involved in several clinical scenarios such as ischemia/reperfusion (I/R) injury and heart failure suggesting that regulation of their function could be used as a novel cardioprotective strategy. In particular, miRNA-1, miRNA-21, miRNA-24, miRNA-29, miRNA-92a, miRNA-126, miRNA-133, miRNA-320, miRNA-199a, miRNA-208 and miRNA-195 have been shown to be regulated after I/R injury. Because tissue miRNAs can be released into circulating blood, they also offer exciting new opportunities for developing sensitive biomarkers, including miRNA-1, miRNA-126, miR-208 and miRNA-499, for acute myocardial infarction and other cardiac diseases.

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Introduction

miRNAs constitute a large class of phylogenetically conserved single-stranded RNA molecules of 19 to 25 nucleotides that are involved in post-transcriptional gene silencing. They arise from intergenic or intragenic (both exonic and intronic) genomic regions that are transcribed as long primary RNA transcripts (pri-miRs) by RNA polymerase II (Ambros, 2004; Bartel, 2004). Primary transcripts undergo two processing steps that produce the short "mature" molecule. The approximately 70-bp precursor miRNA product is processed by the enzymes Drosha and Dicer and its partner DGCR8/Pasha to generate a mature 22-bp product that binds mRNAs in a unique manner using Watson-Crick base pairing through a conserved 6- to 8-bp "seed" sequence as well as additional contacts (Ambros, 2004). miRNAs regulate more than 30% of the protein-coding part of the human genome. Over 700 miRNAs have been identified and sequenced in humans (Friedman and Jones, 2009). The number of miRNA genes is estimated to be over 1000 in the human genome (Bentwich et al., 2005) and some are shown to play a profound role in human development and disease. An individual miRNA is capable of regulating the expression of multiple genes because it can bind to its mRNA targets with either an imperfect or perfect complement. This is an interesting characteristic because a single miRNA becomes functionally important as a transcription factor (Chen and Rajewsky, 2007). In general, their overall function is to modulate or fine-tune cellular phenotypes through repressing expression of proteins, or downregulate protein expression when needed. Therefore, miRNAs could be the pivotal regulators in normal development and physiology, as well as disease states, including cancer and cardiovascular disease (Dalmay, 2008). miRNAs participate in many cellular processes, such as apoptosis (Chan et al., 2005; Xu et al., 2007; Cimmino et al., 2005), fat metabolism (Xu et al., 2003), cell differentiation (Kwon et al., 2005; Silber et al., 2008; Tay

et al., 2008), tumorogenesis (Chang et al., 2008), cardiogenesis (Zhao et al., 2005; Chen et al., 2006; Rao et al., 2006; Zhao et al., 2007) and angiogenesis (Ji et al., 2007). This mini review primarily focuses on the role of miRNAs in relation to myocardial ischemia/reperfusion (I/R) injury and cardioprotection (Figure 1). We also discuss their potential use as biomarkers and/or therapeutic targets in myocardial infarction.

microRNAs in Myocardial Infarction

Myocardial infarction (MI) continues to be among the most frequent causes of both debilitating illness and death. Tissue damage from MI occurs as a result of the initial ischemic event-primarily determined by its duration, and then subsequent injury resulting from reperfusion. During ischemia, ATP levels drop and intracellular pH is reduced as a result of anaerobic respiration and lactic acid accumulation. This in turn results in Na/K-ATPase dysfunction and consequent intracellular sodium build-up leading to reversal of the Na/Ca antiporter and increased cytosolic as well as mitochondrial calcium levels (Ostadal et al., 1999). Although O_2 levels are restored at reperfusion, the respiratory chain complexes are in the reduced state, and a surge of reactive oxygen species (ROS) is observed particularly during the initial phase of reperfusion. The merger of all these adverse events orchestrates opening of the mitochondrial permeability transition pore (MPTP) leading to cell death through necrosis and apoptosis (Kung et al., 2011). Mitochondria also constantly undergo fusion and fission, which are necessary for the maintenance of organelle fidelity (Suen et al., 2008; Tanaka and Youle, 2008; Youle and Karbowski, 2005). Abnormal mitochondrial fission is involved in the initiation of apoptosis, which requires Drp1, a GTPase that causes scission of the mitochondrial outer membrane, resulting in fission of mitochondrial tubules into fragments (Frank et al., 2001; Tan et al., 2008).

Apoptotic signals can be mediated by calcineurin, a serine and threonine protein phosphatase that dephosphorylates pro- and anti-apoptotic factors, leading to their activation or inactivation, respectively (Wang et al., 1999). In recent years, a number of studies have shown that up or downreglation of miRNAs contribute to cardiac injury and protection by altering key signaling elements, thus making them potential therapeutic targets (Table 1). miRNA-1 is preferentially expressed in adult cardiomyocytes and skeletal muscle and is involved in cardiac development and heart disease. It is known to regulate a number of important functions including apoptosis by targeting the synthesis of HSP-60, HSP-70 and Bcl-2; arrhythmias by targeting KCNJ2, the gene encoding kir 2.1 (a subunit of the potassium ion channel) and GJA1, the gene encoding connexin 43 (a major component of the gap-junction) (Yang et al., 2007). Decreased expression of connexin-43 delays conduction, whereas decreased expression of kir 2.1 delays membrane repolarization, both of which may increase the risk of arrhythmias (Zhao et al., 2007; Yang et al., 2007; Xu et al., 2007; Tang et al., 2009). miRNA-21 is an interesting candidate for inhibition of apoptosis in the heart. It regulates programmed cell death 4 (PDCD4) and several putative cardioprotective mediators including AP-1, eNOS, HSP-70 and heat shock transcription factor-1 (HSF-1) (Dong et al., 2009; Yin et al., 2009). Upregulation of miR-21 also promotes cell survival through inhibition of PTEN (Phosphatase and Tensin Homolog Deleted on Chromosome Ten). PTEN degrades phosphatidylinositol (PI)-3,4,5-triphosphate (PIP3), which is produced by phosphoinositide 3-kinase (PI3K) and is essential for activation of the prosurvival Akt kinase pathway. miRNA-21 inhibition increased PTEN expression whereas miR-21 oligonucleotide suppressed it in cardiac fibroblasts (Roy et al., 2009). Interestingly, miRNA-21 also controlled matrix metalloproteinase-2 expression via PTEN suggesting the potential role of this miRNA in the treatment of heart failure and attenuation of inflammation. miRNA-24

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expression is down-regulated in the ischemic border zone of the LV after ischemia and suppressed cardiomyocyte apoptosis, in part by direct repression of the BH3-only domain-containing protein Bim, which positively regulates apoptosis (Qian et al., 2011) Moreover, *in vivo* expression of miRNA-24 inhibited cardiomyocyte apoptosis, attenuated infarct size, and reduced cardiac dysfunction following MI. The antiapoptotic effect on cardiomyocytes in vivo was partially mediated by Bim.

miRNA-29 family consists of 3 members (miRNA-29a, -29b, and -29c) that are transcribed from 2 bicistronic miRNA clusters. miRNA-29a and miRNA-29c may also have role in modulation of I/R (Thum et al., 2007). Its potential targets are Mcl-1 (the antiapoptotic protein of Bcl-2 family, the regulatory subunit of PI3K, p85a and cell division cycle 42 (CDC42) (Park et al., 2009). Moreover, all three members of miRNA-29 upregulate p53 levels and induce apoptosis in a p53-dependent manner. Antagomirs against miRNA-29a or miRNA-29c increased Mcl-2 expression and significantly reduced infarct size and apoptosis after I/R injury (Ye et al., 2010). Cardiomyocyte death following ischemia is followed by myocardial fibrosis, and several miRNAs have been linked to cardiac fibrosis: miRNA-29 expression is greater in cardiac fibroblasts than in cardiac-myocytes, and miRNA-29 is downregulated in the border zone of infarcted hearts, but still increases myocardial fibrosis by derepressing collagen and elastin translation (van Rooij et al., 2008). The biological function of miR-29 appears to involve fibrosis by targeting Elastin (ELN), fibrillin 1 (FBN1), collagen type I, alpha 1 and 2 (COL1A1, COL1A2) and collagen type III, α1 (COL3A1). miR-92a increased 24 h after ischemia in mice (Bonauer et al., 2009). Antagomir-92a improved LV function, reduced infarct size, attenuated apoptosis, and increased angiogenesis in the border zone. Moreover, miRNA-92a targeted Sirt1, a class III histone deacetylatase and longevity gene. Therefore, inhibiting miR-92a may

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potentially be important in improving remodeling and neovascularization after myocardial infarction. miRNA-126 is highly expressed in heart and lung tissue. It enhances the proangiogenic actions of VEGF and FGF by promoting blood vessel formation by repressing the expression of Sprouty-related protein-1 (Spred-1), an intracellular inhibitor of angiogenic signaling (Wang et al., 2008) and vascular cell adhesion molecule 1 (VCAM-1) (Harris et al., 2008). Knock-down of miRNA-126 during the embryonic development of zebrafish caused severe defects in vascular development, including collapsed blood vessels and cranial bleeding (Wang et al., 2008). Interestingly, miRNA-126 mutant mice with successful embryonic development demonstrated limited angiogenesis and decreased survival after myocardial infarction. miRNA-133 is expressed in adult cardiomyocytes and skeletal muscle. Patients who died of myocardial infarction had reduced levels of miRNA-133 in the infarcted areas of the heart (Bostjancic et al., 2010). miR-133 exerts anti-apoptotic effects by targeting caspase 9 (Xu et al., 2007). Moreover, miRNA-133 was downregulated with hypertrophy, and its upregulation reduced hypertrophy as well as correction of conduction abnormalities. miRNA-320 has been shown to be downregulated in mouse hearts after I/R injury (Ren et al., 2009). Overexpression of miRNA-320 enhanced cell death and apoptosis in cultured adult rat cardiomyocytes during simulated I/R, whereas its knockdown led to cytoprotection. Moreover, transgenic mice with cardiac-specific overexpression of miRNA-320 developed increased myocardial apoptosis and increased infarct sizes after I/R injury. The *in vivo* knockdown of miRNA-320 using an antagomir led to reduction of infarct size following I/R injury. Furthermore, miRNA-320 downregulated HSP20, a protein that protects the heart against I/R injury (fan et al., 2005), indicating HSP20 as a putative target of miRNA-320.

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miRNA-499 is present abundantly in heart under physiological conditions (Kloosterman et al., 2006). It has been recently shown that miRNA-499 is involved in inhibiting apoptosis and myocardial infarction induced by anoxia and ischemia (Wang et al., 2011) through mechanisms involving p53, calcineurin and Drp1 in executing apoptosis program in the heart. Conversely, high dose of specific antagomir of miRNA-499 induced apoptosis. These results suggest that modulation of miRNA-499 may be a novel therapeutic approach to treat apoptosis-related cardiac disease, including myocardial infarction.

Other interesting miRNAs are miRNA-208 and miRNA-195. Knockout of miRNA-208 reduced cardiomyocyte hypertrophy and fibrosis in a murine aortic banding model (Callis et al., 2009). Also, transgenic mice with cardiospecific overexpression of miRNA-195 developed a dose-dependent cardiac hypertrophy (van Rooij et al., 2006). In a recent study, Zhang et al. identified activation of miRNA-144/451 promoter by GATA-4, a critical transcription factor in the heart (Zhang et al., 2010). They observed that ectopic expression of miRNA-144 and miRNA-451 individually augmented cardiomyocyte survival, which was further improved by overexpression of miRNA-144/451, compared to control cells in response to simulated I/R. The knockdown of endogenous miRNA-144 and miRNA-451 revealed opposite effects. Using luciferase reporter assay and western blot analysis, they validated that both miRNA-144 and miRNA-451 target CUG triplet repeat-binding protein 2 (CUGBP2), a ubiquitously expressed RNA-binding protein, known to interact with COX-2 3'-UTR and inhibit its translation. Accordingly, protein levels of CUGBP2 were greatly reduced and COX-2 activity was markedly increased in miRNA-144-, miRNA-451- and miRNA-144/451-overexpressing cardiomyocytes, compared to GFP-cells. Furthermore, inhibition of COX-2 activity by either NS-398 or DUP-697

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partially offset the protective effects of the miRNA-144/451 cluster. These findings indicate that both partners of the miRNA-144/451 cluster confer partial protection against simulated I/R-induced cardiomyocyte death via targeting CUGBP2-COX-2 pathway.

miRNAs in Ischemic Preconditioning

Preconditioning (PC) is a phenomenon whereby the myocardium could be protected or "preconditioned" against prolonged ischemia by brief series of ischemia separated by bursts of reperfusion (Murry et al., 1986). The effect of PC appears in two phases: an early phase which lasts up to 2 hrs and a late phase, the effect of which persists as long as 96 hrs. The concept of PC has been of great interest to both the basic scientists and clinicians because it has helped discover numerous potential therapeutic options for cardioprotection such as adenosine, opioid agonists, phosphodieterase-5 (PDE-5) inhibitors (Kukreja et al., 2005), nitrite (Lefer, 2009), carbon monoxide (Guo et al., 2004), remote conditioning, post-conditioning (Xi et al., 2008) and more recently, miRNAs (Yin et al., 2009). We isolated miRNAs after PC of the heart using short bursts of global ischemia and reperfusion, a stimulus which also caused significant reduction of infarct size in the Langendorff's isolated perfused heart model. The pool of miRNAs extracted from the non-preconditioned and preconditioned hearts was injected into the risk zone of LV (area to be subjected to ischemia) *in situ* in another set of mice. After 48 hrs, the mice were subjected to I/R injury *in vivo*. The PC-derived miRNAs caused significantly lower infarct size (18.8 ± 2.5%) as compared to saline controls (37.5 ± 2.2%) or miRNAs prepared from non-preconditioned hearts (39.3±2.3 %). There was no difference in infarct size between the saline-injected controls versus non-preconditioned miRNA-treated hearts (Figure 2). Interestingly, there was significant up-regulation of eNOS protein (92±8.1%), heat shock

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transcription factor-1 (HSF-1) (42.7±3.0%) and HSP-70 (102.3±8.9%) 48 hrs after treatment with miRNA derived from the PC hearts. However, there was no increase in iNOS protein, which is known to increase during late PC (Takano et al., 1998). Another study showed that miRNA-21 was downregulated in the infarcted areas but upregulated in the border areas of the heart after ischemia (Dong et al., 2009). Induction of PC clearly reversed these effects and inhibited the downregulation of miRNA-21 in the infarcted areas. miR-1 has been linked to post-transcriptional repression of HSP-60 and HSP-70 in H9c2 cells (Xu et al., 2007) which is in contrast to the upregulation of HSP-70 synthesis observed in our study. The difference in models (H9c2 cells versus murine intact hearts) may explain the discrepancy in the results between the two studies. miRNA-21 is a downstream effector of Akt and partly mediates its anti-apoptotic effects through down-regulation of Fas ligand as well as caspase-8 (Sayed et al., 2010). This is important because PC also causes phosphorylation of Akt and protects the heart mainly through attenuation of apoptosis. Moreover, the inhibition of PTEN by miRNA-21 is consistent with the reported loss of PTEN activity following PC in the heart (Cai et al., 2005).

miRNAs in Cardioprotection with Heat Shock

Cells subjected to increases in temperature induce the expression of several proteins known as heat shock or stress proteins. This process enhances the cell's ability to overcome the effects of further stress. In fact, mild heat-shock has been shown to improve myocardial survival after subsequent prolonged I/R injury (Currie et al., 1993). The synthesis of HSPs (Donnelly et al., 1992), antioxidant defenses (Karmazyn et al., 1990), and enhanced mitochondrial respiration (Broderick, 2006), opening of mitochondrial K_{ATP} channel (Hoag et al., 1997)) and subsequent resistance to opening of mitochondrial permeability transition pore (He and LeMasters, 2003)

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and inhibition of apoptosis have been considered as mechanisms of heat-shock protection against I/R injury. We recently demonstrated the role of endogenous miRNAs in heat shock induced cardioprotection. We isolated the pool of miRNAs from liver and heart after heat-shock treatment in mice (42^{o}C for 15 min) and injected them into non-heat-shocked mice (Yin et al., 2008)**.** Mice treated with the pool of miRNAs extracted from heat shocked mice demonstrated improved ischemic tolerance. Infarct size was reduced significantly from 40 ± 2.7% (percentage of total risk area, mean ± S.E.M.) in the non-heat-shocked controls to 18.5 ± 3.8% in mice treated with miRNAs. Moreover, injection of chemically synthesized exogenous miRNA-21 also reduced infarct size by 64% ($P < 0.05$ versus control). The miRNA-21-induced protection was totally abolished when mice were co-treated with antagomir-21. miRNA treatment caused profound changes in several apoptotic related genes as determined by gene microarray analysis. In particular, caspase family members 1, 2, 8 and 14 were suppressed in the hearts treated with miRNAs from heat-shocked mice. Except for BNIP-3, most of the pro-apoptotic genes including Bid (BH3 interacting domain death agonist), Bcl-10 (B-cell leukemia/lymphoma 10), Cidea (cell death-inducing DNA fragmentation factor, alpha subunit-like effector A), Ltbr (lymphotoxin B receptor), Trp53 (transformation related protein 53), Fas (TNF receptor superfamily member) and Fasl (Fas ligand, TNF superfamily, member 6), were repressed. On the other hand, the anti-apoptotic genes, Bag-3 (Bcl-2-associated athanogene and Prdx2 (Peroxiredoxin 2) were increased. These findings further support the notion that manipulating endogenous miRNA profiles could be a promising therapeutic approach to combat cardiovascular disease.

miRNAs in Hypoxic Preconditioning

Similar to sub-lethal ischemia, hypoxia elicits PC-like response where the myocardium develops an adaptive phenotype leading to resistance against subsequent lethal I/R injuries

(Bolli, 2000; Meerson et al., 1973; Xi et al., 2002). This cardioprotection appears to be essentially mediated by hypoxia-inducible factor 1α (HIF-1α) (Cai et al., 2003). HIF-1 is a heterodimeric αß transcription factor that mediates tissue responses to hypoxia (Wang and Semenza, 1993). HIF-1 is essential in the transcription of numerous genes that are implicated in oxygen homeostasis, especially in response to diminished oxygen tension. Prolyl hydroxylases (PHDs) hydroxylate HIF-1 during normoxia, which allows von Hippel–Lindau to bind and ubiquitinate HIF-1, marking it for proteasomal degradation. This process is inactivated during hypoxia, thus, permitting rapid accumulation of HIF-1. Three PHD isoforms, PHD1, PHD2 and PHD3 have been identified (Ivan et al., 2002; Bruick and McKnight, 2001). PHD2 is ubiquitously expressed and exhibits the highest specific activity toward HIF-1α (Hirsila et al., 2003). Both PHD2 inhibition and hypoxia stabilize HIF-1α protein and lead to increased levels of active HIF-1α/ß heterodimers in cell nuclei (Sutter et al., 2000). HIF-1α stabilization by pharmacological inhibition of PHD2 or its silencing using a siRNA strategy induced late PC like effect via an iNOS-dependent pathway (Ockaili et al., 2005; Natarajan et al., 2006). It has been shown that HIF-1α is a predicted target of miR-199a and is rapidly induced by hypoxia (Rane et al., 2009). miR-199a interacted with the 3'-UTR of HIF-1α and inhibition of miRNA-199a increased HIF-1α expression in cardiomyocytes. Moreover, hypoxic preconditioning or miR-199a silencing upregulated iNOS and Bcl-2. Conversely, overexpression of miRNA-199a prevented upregulation of iNOS during hypoxic preconditioning. Overexpression of miRNA-199a reduced Sirt1 levels, whereas silencing miRNA-199a by antisense oligonucleotide or downregulating miRNA-199a by hypoxic preconditioning increased Sirt1 levels. miRNA-199a induced protection against I/R injury both by decreasing suppression of translation of HIF-1α

and by enhancing its stability via Sirt1-induced inhibition of PHD2. These studies suggest that miRNA-199a could be a valuable target for inducing PC.

Antagomirs as Potential Drugs

Since miRNAs have been shown to be important in human disease, approaches to interrupt miRNA function may have therapeutic utility. Small interfering double-stranded RNAs (siRNAs) engineered with certain 'drug-like' properties, such as chemical modifications for stability and cholesterol conjugation for delivery, have been shown to achieve therapeutic silencing of an endogenous gene *in vivo*. To develop a pharmacological approach for silencing miRNAs *in vivo*, Krutzfeldt et al. designed chemically modified, cholesterol-conjugated single-stranded RNA analogues complementary to miRNAs, and termed these oligonucleotides 'antagomirs' (Krutzfeldt et al., 2005). Antagomirs targeting polycistronic miRNAs (miRNA genes that are located close together and are coordinately transcribed) were shown to retain their target specificity with no effect on the expression of co-transcribed miRNAs i.e. antagomirs targeting miRNA-192 of the bicistronic cluster miR-192/194 resulted in the silencing of miRNA-192 with no effect on the expression levels of miRNA-194, and vice versa. Therefore, antagomirs have the ability to differentially silence specific miRNAs that derive from the same primary transcript. Moreover, they are valuable tools for identifying miRNA targets *in vivo* and for studying the biological role of miRNAs and miRNA-associated gene-regulatory networks in a physiological context. The therapeutic potential of antagomirs is also promising since mipomersen, an antisense oligonucleotide inhibitor of apolipoprotein B synthesis, demonstrated significant reductions in apolipoprotein B and low-density lipoprotein cholesterol in phase 1 clinical trials in healthy volunteers (Akdim et al., 2010). Mipomersen has also been tested in

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patients with familial hypercholesterolemia and showed exceptional results with the highest efficacy seen so far in this patient population, and with relatively low drop-out rates compared to other injectable drugs (Visser et al., 2010).

MicroRNAs as Biomarkers

miRNAs may serve as potential diagnostic biomarkers for cardiovascular diseases because the serum or plasma can be obtained in a less invasive manner than tissue. The endogenous circulating miRNAs have been found to be stable because of their packaging and secretion into the blood within the exosomes (Ismail et al., 2008). In the cancer field, miRNA-21 was the first miRNA suggested to be a diagnostic biomarker for diffuse large B cell lymphoma (lawrie et al., 2008). Overexpressed miRNA-21 was associated with poor survival and poor therapeutic outcome in colon adenocarcinoma (Schetter et al., 2008).

In the cardiac field, an early and correct diagnosis of acute myocardial ischemia (AMI) is critical for rapid initiation of reperfusion therapy to reduce the irreversible damage in the heart and thus enhance survival of patients. Currently, biomarkers such as creatine kinase–MB (CK-MB) isoenzymes, cardiac myoglobin, and troponins are routinely used in clinical diagnosis (de Winter et al., 1995). Among these, cardiac troponins are currently considered as the ‘gold standard’ for AMI diagnosis (Jaffe et al., 2000). Unfortunately many of these markers have reduced sensitivity, less specificity or do not allow timely diagnosis. Therefore, multiple biomarker strategy may circumvent these limitations by adding accuracy and predictive power. A recent report suggests that serum levels of cardiac-expressed miRNAs react to cardiac injury in a manner similar to cardiac enzymes. The plasma levels of miRNAs-208b and -499 have been shown to increase over 1000 fold after myocardial infarction which mirrored the levels of

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troponin T (Corsten et al., 2010). Viral myocarditis was associated with 6 to 30 fold increases while acute heart failure showed only a 2 fold increase of miRNA-499. Similarly, circulating levels of cell-free miRNA-1 were significantly increased in patients with AMI, which positively correlated with serum CK-MB levels (Cheng et al., 2010). Another clinical study showed that miRNA-1, miRNA-133a, and miRNA-208a in blood from patients with AMI were elevated compared with those from patients without AMI. Despite these encouraging results, the number of samples in the above described studies is too small to provide definite proof of the diagnostic power of microRNA signatures and their value for clinical testing of AMI patients. Therefore, future prospective trials on large patient cohorts are needed to establish miRNAs as a novel biomarker class for AMI.

Future Perspective

Similar to the first RNA revolution in the 1980s when the enzymatic activity of RNA was reported (Zaug and Cech, 1986), the discovery of miRNA represents the second RNA revolution (Kong and Han, 2005). Emerging evidence suggests that miRNAs are key regulators of cardiac diseases. Important new data suggest that miRNA-1, miRNA-133, miRNA-21, miRNA-24, miRNA-320, miRNA-29, miRNA-92a, miRNA-126, miRNA-199a, miRNA-208 and miRNA-195 appear promising and seem to play key roles in myocardial infarction, cardiac conduction, regulation of cardiac patterning, angiogenesis, cardiac hypertrophy, fibrosis and cardiac protection. Analogous to protein kinases/phosphatases, the miRNAs could work independently or in concert to achieve certain outcomes. Intervention using stable miRNA mimetics and/or antagomirs is likely to emerge as a therapeutic tool for protection against AMI. The main advances in this exciting field will be in the following areas: 1) identifying novel microRNAs

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and their targets that regulate cardiac diseases, which would help in further understanding the mechanisms of cell death during I/R injury 2) identifying the microRNAs that are changed particularly during the early phase following myocardial infarction and can be used as reliable markers for early diagnosis, improved prognosis, and treatment of ischemic heart diseases and 3) defining the best conditions for the use of miRNAs and their inhibitors for reducing I/R injury/heart failure.

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Footnotes

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Legends of Figures

Figure 1. Schematic diagram showing dynamics of microRNAs during myocardial infarction and protective strategies including ischemic preconditioning, heat shock, hypoxia and pharmacologic preconditioning, with potential utilization of synthetic miRNAs and/or antagomirs.

Figure 2. Induction of miRs by ischemic preconditioning (PC) and Effect of miR on myocardial infarct size following ischemia/reperfusion. (A) Infarct size reduction following IPC (n=6/group). (B) Gel electrophoresis image of RT-PCR products of miRNAs. (C) Average normalized results showing increase in miR-1, miR-21 and miR-24 following IPC. The results are means±SEM from 3 independent hearts. (D) Reduction of myocardial infarct size (% of risk area) following direct delivery of IPC-miRNA into the heart. *P<0.05 versus saline control and non-IPC miRNA treated hearts. (E) Uptake of miR-21 following injection in LV wall. Gel electrophoresis image of the RT-PCR for miR-21. (F) Average normalized changes in miR-21. Endogenous U1A small nuclear RNA (RNU1A) was used as control. (From Yin, Salloum and Kukreja, 2009, with permission).

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Table 1. Role of miRNA up- or downregulation in cardiac injury and protection

miRNA	Upregulation	Downregulation
1	Increased apoptosis and arrhythmias (28)	Decreased apoptosis and arrhythmias (28)
133	Increased hypertrophy (42,43)	Decreased hypertrophy (42,43)
21	Decreased apoptosis; cardioprotective(31,32,33)	Increased apoptosis (31,32,33)
24	Decreased apoptosis; cardioprotective (34)	Increased apoptosis (34)
320	Worsens I/R injury (44,45)	Increased HSP20; cardioprotective (44,45)
29	Increased apoptosis and fibrosis (35,36,37,38)	Decreased apoptosis and fibrosis; cardioprotective (35,36,37,38)
92a	Decreased angiogenesis (39)	Increased angiogenesis (39)
126	Increased angiogenesis (40,41)	Decreased angiogenesis (40,41)
199a	Increased PHD2 and decreased HIF-1α (77)	Decreased PHD2 and increased HIF-1α; cardioprotective (77)
208	Dilated cardiomyopathy (48)	Attenuates cardiomyopathy (48)
499	Decreased apoptosis (47)	Increased apoptosis (47)

Note: Numbers in parentheses indicate source(s) of reference.

Figure 1.

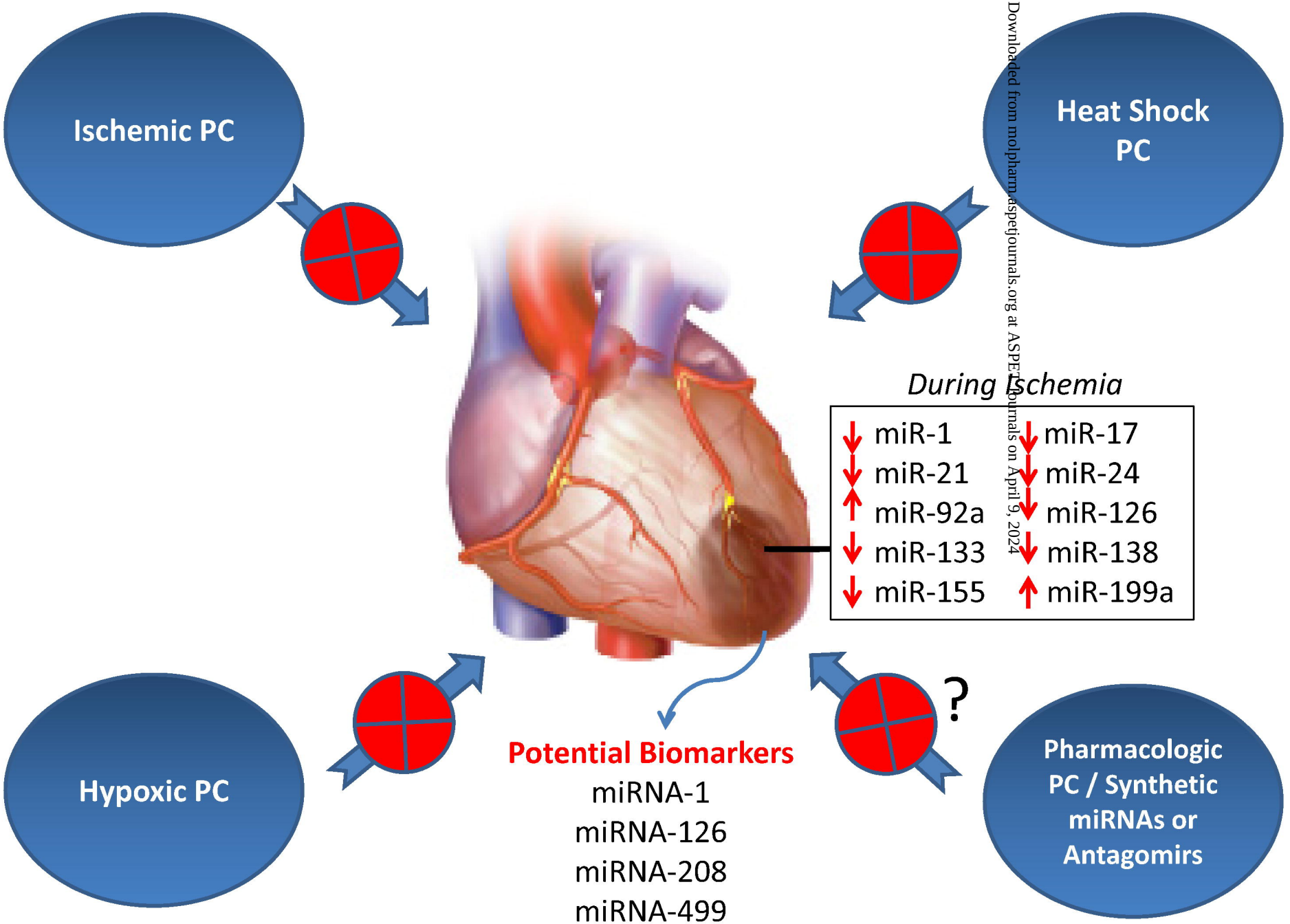

Figure 2.