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The role of microRNA in modulating myocardial ischemia-reperfusion injury

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Ye Y, Perez-Polo JR, Qian J, Birnbaum Y. The role of microRNA in modulating myocardial ischemia-reperfusion injury. *Physiol Genomics* 43: 534–542, 2011. First published October 19, 2010; doi:10.1152/physiolgenomics.00130.2010.—MicroRNAs (miRNAs) are small (~22 nt) noncoding single-stranded RNA molecules that downregulate gene expression. Studies have shown that miRNAs control diverse aspects of heart disease, including hypertrophy, remodeling, heart failure, and arrhythmia. Recently, several studies have suggested that miRNAs contribute to ischemia-reperfusion injury by altering key signaling elements, thus making them potential therapeutic targets. By altering the expression of various key elements in cell survival and apoptosis [such as phosphoinositide 3-kinase (PI3K), phosphatase and tensin homolog deleted on chromosome 10 (PTEN), Bcl-2, Mcl-1, heat shock protein (HSP)60, HSP70, HSP20, programmed cell death 4 (Pdcd4), LRRFIP1, Fas ligand (FasL), Sirt-1, etc.], miRNAs alter the response to ischemia-reperfusion injury. Studies using various in vivo, ex vivo, and in vitro models have suggested the possible involvement of miR-1, miR-21, miR-29, miR-92a, miR-133, miR-199a, and miR-320 in ischemia-reperfusion injury and/or remodeling after myocardial infarction. Thus miRNAs could be potential therapeutic targets for the treatment of heart disease. Inhibiting miRNAs by antisense strategies or pharmacological approaches is likely to emerge as an alternative and safe method for conferring short- and intermediate-term protection against ischemia-reperfusion injury.

infarct size; myocardial infarction

MICRORNAS (miRNAs) are small (~22 nt) noncoding single-stranded RNA molecules that downregulate gene expression (1, 2, 11, 39), possibly regulating up to 90% of human genes (27). miRNAs are initially transcribed as parts of longer molecules that are processed in the nucleus into hairpin RNA by the nuclear RNase III Drosha (2, 17). The pre-miRNAs are transported to the cytoplasm via exportin-5 and are processed by the ribonuclease Dicer to generate 18- to 24-nt mature miRNAs (15). Mature miRNAs bind to the 3′-untranslated region (UTR) of their mRNA targets and downregulate gene expression via degradation or translational inhibition. Studies have shown that miRNAs control diverse aspects of heart disease, including hypertrophy, remodeling, heart failure, and arrhythmia (2, 11, 33, 37, 39, 45). Recently, several studies have suggested that miRNAs contribute to ischemia-reperfusion injury by altering key signaling elements, thus making them potential therapeutic targets.

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Changes in miRNA Expression After Preconditioning Protocols or Various Ischemia-Reperfusion Injury Models

Ischemia-reperfusion affects the expression of miRNAs. Tang and colleagues (36) studied miRNA expression in mice that were exposed to 30 min of cardiac ischemia followed by 24 h of reperfusion. They found that levels of miR-1, miR-126, and miR-208 increased after ischemia-reperfusion, whereas levels of miR-21, miR-133, and miR-195 decreased. Using the same ischemia-reperfusion protocol, Ren and colleagues (30) reported downregulation of miR-320 expression and upregulation of miR-7, miR-21, miR-146b, miR-491, and miR-9651 expression in the mouse heart. However, in an ex vivo model of 45-min global ischemia and 2-h reperfusion, upregulation of miR-7, miR-21, miR-146b, miR-491, and miR-9651 was not found. They observed only downregulation of miR-320 (30). In rats, Dong and colleagues (12) found that 6 h after permanent coronary artery occlusion 38 miRNAs were differentially expressed (21 up- and 17 downregulated) in the infarcted areas and 33 miRNAs were differentially expressed (19 up- and 14 downregulated) in the border areas compared with those in the noninfarcted areas. Although most of the miRNAs had a similar expression in the noninfarcted area of the infarcted hearts compared with the sham-opened control rats, the expres-

sion of some miRNAs in the two groups was still different. Among them, miR-21, miR-27a, miR-27b, miR-30b-5p, miR-30c, miR-125a-5p, miR-126, miR-150, miR-26a, and miR-26b were upregulated; in contrast, miR-107, miR-130a, miR-145, miR-16, and miR-22 were downregulated in the noninfarcted areas of the infarcted hearts (12). Interestingly, miR-21 was downregulated in the infarcted zone 6 and 24 h after infarction; however, miR-21 levels were higher in the border zone of the infarction and in remote myocardial areas of hearts subjected to infarction (12). Ischemic preconditioning upregulated miR-21 in the noninfarcted zones and attenuated the decrease in miR-21 levels in the infarcted area (12).

In an isolated heart model, Yin and colleagues (50) showed that ischemic preconditioning upregulated miR-1, miR-21, and miR-24 expression.

In the first few hours after primary percutaneous coronary artery intervention (517 ± 309 min) for ST elevation acute myocardial infarction, plasma obtained from the patients showed a significant increase in levels of miR-1, miR-133a, miR-133b, and miR-499-5p and a decrease in miR-122 and miR-375 levels (10). Five days later miR-1, miR-133a, miR-133b, miR-499-5p, and miR-375 returned to baseline, whereas miR-122 remained lower than in control patients for 30 days (10). In mice undergoing permanent coronary artery occlusion, plasma levels of miR-499-5p were a good and sensitive marker of myocardial damage and paralleled the plasma levels of cardiac troponin I (10). As early as 15 min after coronary artery occlusion, plasma levels were 1.7-fold higher than in the control animals (10). Plasma miR-499-5p levels peaked at 24 h after coronary artery ligation. In addition, plasma levels of miR-208a also increased as early as 30 min after coronary artery ligation in the mice (10). In both the infarcted area and the border zone of the mice, myocardial levels of miR-1, miR-133a, miR-133b, and miR-499-5p decreased at 3 h and 6 h after coronary artery ligation, whereas miR-122 increased in the infarcted area (10). Tables 1–3 summarize the changes in miRNAs reported in various models of ischemia or ischemia-reperfusion injury. Table 4 summarizes the potential and proven targets of some of the miRNAs whose levels are altered by ischemia or ischemia-reperfusion injury.

miR-1

miR-1 is preferentially expressed in adult cardiomyocytes and skeletal muscle and is involved in cardiac development and in the development of heart disease. In the hearts of patients who died of myocardial infarction, the expression of miR-1 is downregulated in the infarcted areas and upregulated in the remote myocardial areas compared with the expression in healthy hearts of adults who died in accidents (5, 6).

Experimental studies have shown that coronary artery occlusion affects miR-1 expression. Yang and colleagues (45) reported that miR-1 was significantly increased in the myocardium 12 h after permanent coronary artery occlusion in rats. Furthermore, Tang and colleagues (36) showed that the increase in miR-1 levels seen in rat cardiomyocytes 24 h after a 30-min period of coronary artery occlusion correlated inversely with Bcl-2 levels 24 h after reperfusion (36).

It has been shown that oxidative stress increases miR-1 levels and subsequently decreases levels of heat shock protein (HSP)60 and HSP70 (44). Overexpression of miR-1 induces apoptosis and augments hydrogen peroxide (H_2O_2)-induced apoptosis in H9c2 cells (36, 44). In contrast, inhibiting miR-1 confers resistance of cells to H_2O_2 (36). miR-1 may induce apoptosis in H_2O_2-treated H9c2 cells by reducing the expression of Bcl-2 (which has an antioxidant effect) by interacting with the 3′-UTR of Bcl-2 (36).

miR-1 is released by cardiomyocytes after necrosis and is stable for 24 h (9). In rats, serum miR-1 levels rapidly increased after coronary artery occlusion, peaking at >200-fold over baseline levels at 6 h and gradually returning to baseline levels by 3 days (9). Serum miR-1 levels strongly correlated with myocardial infarction size in rats; both can be significantly reduced by ischemic preconditioning (9). In patients with acute myocardial infarction, serum free miR-1 correlated with the serum level of creatine kinase MB, indicating a correlation between miR-1 levels and the extent of myocardial damage (9).

In addition to Bcl-2, HSP60, and HSP70, miR-1 targets GJA1, the gene encoding connexin 43 (a major component of the gap junction), and KCNJ2, the gene encoding kir 2.1 (a subunit of the potassium ion channel) (45). 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 (35, 45).

The results of the above-mentioned studies suggest that inhibiting miR-1 may potentially ameliorate ischemia-reperfusion injury, reduce infarction size, and decrease the arrhythmogenic risk after myocardial infarction.

miR-21

miR-21, an abundant miRNA in cardiomyocytes, is upregulated in hypertrophic cardiac cells and induces cardiac hypertrophy (7, 38). The potential targets of miR-21 are tropomyosin 1, Phosphatase and Tensin Homolog Deleted on Chromosome Ten (PTEN), programmed cell death 4 (PDCD4), TAp63 isoform of the p53 family (25), heterogeneous nuclear ribonucleoprotein K (HNRPK) (25) LRRFIP1 (an NF-κB inhibitor) (18), and Fas Ligand (FasL) (32).

In the mouse heart, Roy and colleagues (31) studied the expression of 661 miRNAs in response to ischemia-reperfusion injury and found that 13 miRNAs were significantly upregulated on day 2 after infarction and on day 7 after infarction 9 were upregulated and 6 downregulated. miR-21 levels increased primarily in cardiac fibroblasts in the infarcted area but not in the noninfarcted area (31). However, they did not study changes in miR-21 levels during the period of acute ischemia

Table 1. *MicroRNAs altered by ischemia or brief ischemia-reperfusion injury*

MicroRNA	Whole Heart
	Brief ischemia-reperfusion (preconditioning)
miR-1	miR-1 ↑ (50)
miR-21	miR-21 ↑ (12, 50)
miR-24	miR-24 ↑ (50)
miR-199a	Mature miR-199a ↓ in pigs (29)
	Ischemia (<60 min)
miR-199a	Mature miR-199a ↓ in mice after 30 min and ≤6 h ischemia (29)

or early during reperfusion when most cell death occurs. Their earliest time point of study was day 2, when reparative processes are beginning. Thus their experimental design is compatible with remodeling rather than acute ischemia-reperfusion injury, and early induction of miR-21 in cardiomyocytes during ischemia or early reperfusion cannot be excluded.

PTEN degrades phosphatidylinositol(3,4,5)-P(3), which is produced by phosphoinositide 3-kinase (PI3K) and is essential for activation of the prosurvival Akt kinase pathway (16, 21). PTEN is involved in protection against ischemia-reperfusion injury (21). Inhibition of PTEN limits myocardial infarction size (16). Upregulation of miR-21 is expected to inhibit PTEN and, therefore, promote cell survival. Indeed, Roy and colleagues (31) have shown that miR-21 inhibition increases the expression of PTEN, whereas miR-21 mimic oligonucleotide suppresses PTEN in cardiac fibroblasts. In addition, they showed that miR-21 controls matrix metalloproteinase-2 (MMP-2) expression via a PTEN pathway. MMP-2 is involved in mediating ischemia-reperfusion injury and in other pathological conditions including oxidative stress, heart failure, and inflammation (42).

Cheng and colleagues (8) studied the effects of H_2O_2 on the expression of miR-21 in neonatal rat cardiomyocytes. They found that H_2O_2 increased miR-21 levels in the cells in a dose-dependent manner. High concentrations (30–200 μM) of H_2O_2 induced cell death by both apoptosis and necrosis in a dose-dependent manner, whereas low concentrations of H_2O_2 mainly induced cell apoptosis (8). Transfection with pre-miR-21 oligonucleotides in the cells decreased H_2O_2-induced cell death, whereas transfection with anti-miR-21 increased cell death, suggesting that miR-21 protects cardiomyocytes against H_2O_2-induced cell death (8). They also showed that miR-21 downregulated PDCD4 expression and that the protective effect of pre-miR-21 on cell death was inhibited by adenovirus-mediated overexpression of PDCD4 (8). PDCD4 inhibited the activity of activator protein 1 (AP-1), a key signaling molecule that affects cell death in response to extracellular stimuli (34), suggesting that AP-1 is downstream of PDCD4 and is involved in miR-21-mediated protection (8).

In contrast, Sayed and colleagues (32) reported that miR-21 levels decreases in 1- to 2-day-old rat cardiomyocytes exposed to hypoxia (by 40% within 1 h and by 75% within 12–24 h) and leads to upregulation of PTEN and Fas Ligand (FasL). They found similar results in mice after 6 h of coronary artery occlusion. During short-term exposure of cells to hypoxia (~15 min), Akt phosphorylation (resulting in Akt activation) and PTEN phosphorylation (resulting in PTEN deactivation) were enhanced and miR-21 expression was upregulated. However, during prolonged ischemia, Akt activity was downregulated, leading to decreased levels of miR-21 level with subsequent upregulation of PTEN and FasL (32). Moreover, during prolonged ischemia PTEN phosphorylation is decreased, leading to PTEN activation with subsequent deactivation of Akt (32). In a transgenic mouse model overexpressing miR-21, Sayed and colleagues (32) found that miR-21 overexpression suppressed ischemia-induced upregulation of PTEN and FasL, with a subsequent increase in Akt phosphorylation, limiting infarct size and attenuating apoptosis. Thus miR-21 activates Akt via suppression of PTEN, whereas Akt upregulates miR-21 expression, creating a positive loop.

Dong and colleagues (12) studied miRNA expression in infarcted zones, border zones, and noninfarcted zones in rats exposed to permanent coronary artery occlusion. They found that miR-21 was downregulated in the infarcted areas but upregulated in the border areas at 6 h after coronary artery occlusion. Furthermore, ischemic preconditioning inhibited the downregulation of miR-21 in the infarcted areas. Overall, expressions of 38 miRNAs were affected in the infarcted areas and 33 miRNAs in the border areas compared with those in the noninfarcted areas (12). Transfection with adenovirus expressing miR-21 reduced cell apoptosis in the border and the infarcted areas, decreased myocardial infarction size, and improved left ventricular remodeling 2 wk after infarction (12). In vitro experiments using adult rat cardiomyocytes showed that miR-21 protected against ischemia-reperfusion injury and confirmed that PDCD4 was a target of miR-21 with downstream activation of AP-1 (12). Interestingly, Dong and colleagues (12) found that miR-21 expression was also increased in the noninfarcted areas of infarcted hearts and that ischemic preconditioning increased the levels in noninfarcted areas. These findings are in accordance with the fact that preconditioning by brief occlusion of one coronary artery confers protection in myocardial areas supplied by other coronary arteries (28).

Yin and colleagues (50) reported that ischemic preconditioning upregulated miR-1, miR-21, and miR-24. Injection of these miRNAs into the left ventricular wall of mice reduced infarct size and induced expression of endothelial nitric oxide synthase (eNOS), heat shock transcription factor 1 (HSF-1), and HSP70 (50).

Taken together, these results suggest that miR-21 is involved in prosurvival signaling. miR-21 is upregulated in cardiomyocytes shortly after initiation of ischemia, resulting in suppression of PTEN and increased activation of Akt, as well as downregulation of PDCD4 with activation of AP-1. These activities enhance cell survival. Indeed, ischemic preconditioning upregulates miR-21 expression. A positive feedback loop exists between Akt and miR-21. However, with prolonged ischemia that leads to cell death, this protective mechanism is shut down and the expression of miR-21 is decreased, leading to activation of FasL, PDCD4, and PTEN and the proapoptotic pathways. When cell death occurs, miR-21 expression decreases; however, with time, increased expression of miR-21 in fibroblasts enhances their proliferation and supports the formation of the scar. It should be shown whether "preconditioning at a distance" by inducing short periods of ischemia in remote organs (3) increases myocardial miR-21 levels.

miR-29

Although changes in miR-29 expression after ischemia-reperfusion have not been described, our group has reported that miR-29a and miR-29c may potentially modulate ischemia-reperfusion injury (47). The potential targets of miR-29 are Mcl-1, a member of the antiapoptotic Bcl-2 family (22), p85a (the regulatory subunit of PI3K) (26), and Cell division cycle 42 (CDC42) (26). The miR-29 family members (miR-29a, miR-29b, and miR-29c) upregulate p53 levels and induce apoptosis in a p53-dependent manner (26). We have found that pioglitazone, a peroxisome proliferator-activated receptor (PPAR)-γ agonist used to treat diabetes mellitus, downregulated miR-29a and miR-29c in H9c2 cells. Pioglitazone has

been reported to protect against ischemia-reperfusion injury (48, 49). When miR-29 expression was downregulated by a miR-29 antisense inhibitor or by pioglitazone, H9c2 cells were protected from ischemia-reperfusion injury, as evidenced by increased cell survival and decreased caspase-3 activity. In contrast, the protective effect of pioglitazone was completely blocked by overexpression of miR-29, which promoted cell apoptosis (47). Antagomirs against miR-29a or miR-29c increased Mcl-1 expression and significantly reduced myocardial infarction size and apoptosis in mice subjected to ischemia-reperfusion (47). Thus downregulation of miR-29 leads to activation of PI3K, with subsequent activation of the prosurvival kinase Akt and the antiapoptotic mediator Mcl-1.

van Rooij and colleagues (41) have shown that members of the miR-29 family are downregulated in myocardial areas adjacent to infarction in mice and humans. The miR-29 family targets multiple mRNAs that encode proteins involved in the formation of fibrosis, including elastin and multiple collagens and fibrillins. Thus reducing miR-29 expression may enhance fibrosis by derepressing the expression of these target mRNAs. Moreover, van Rooij and colleagues (41) showed that collagen expression was reduced in fibroblasts overexpressing miR-29 and that fibrosis was induced by inhibition of miR-29.

The timing of intervention is important. Early inhibition of miR-29 just before ischemia reduced infarction size, whereas longer-term inhibition of miR-29 after infarction augmented fibrosis. Thus overexpressing miR-29 may be beneficial in the remodeling phase, whereas in the acute phase inhibition is beneficial.

miR-92a

miR-92a enhances vessel growth in various models. Bonauer and colleagues (4) reported that miR-92a levels were increased 24 h after coronary artery occlusion in mice. Furthermore, they showed that injection of antagomir-92a at 0, 2, 4, 7, and 9 days after permanent coronary artery occlusion in mice improved left ventricular function, reduced myocardial infarction size (measured 2 wk after infarction), reduced apoptosis, and increased the number of new blood vessels, especially in the border areas of infarction (4). Sirtuin 1 (Sirt1), a longevity gene, is a putative target of miR-92a (and of miR-199a as described below) (4). Overexpressing miR-92a decreased the expression of integrin subunits α5 (ITGA5) and αv, sphingosine-1-phosphate receptor 1 (S1P1), mitogen-activated kinase kinase 4 (MKK4), and eNOS (4). Thus inhibiting miR-92a may potentially reduce infarct size and improve remodeling and neovascularization after myocardial infarction.

miR-126

miR-126 is highly expressed in heart and lung tissue. miR-126 enhances the proangiogenic actions of VEGF and FGF and promotes blood vessel formation by repressing the expression of Sprouty-related protein-1 (Spred-1), an intracellular inhibitor of angiogenic signaling (43), and vascular cell adhesion molecule 1 (VCAM-1) (13). Levels of miR-126 are increased in the noninfarcted zone at 6 h after permanent coronary artery occlusion (12) and at 24 h after 30-min ischemia (36). The survival rate in miR-126-null mice after permanent coronary artery ligation is significantly reduced compared with wild-type mice, because of defective angiogenesis (43). miR-126 is present in apoptotic bodies and represses the function of regulator of G protein (heterotrimeric guanosine triphosphate-binding protein) signaling 16, an inhibitor of G protein-coupled receptor (GPCR) signaling. This enabled CXCR4, a GPCR, to trigger an autoregulatory feedback loop that increased the production of CXCL12. Activation of the CXCL12 chemokine receptor CXCR4 counteracts apoptosis and recruits progenitor cells (51). Thus currently we have evidence that miR-126 is involved mainly in the reparative phase after myocardial injury by promoting recruitment of progenitor cells and enhancing angiogenesis.

miR-133

miR-133 is expressed in adult cardiomyocytes and skeletal muscle. In patients who died of myocardial infarction, miR-133 levels are reduced in the infarcted areas of the heart (5). Overexpressing miR-133 reduced apoptosis and increased viability of H9c2 cells after exposure to H_2O_2, whereas downregulating miR-133 expression with an inhibitory oligonucle-

Table 2. *MicroRNAs altered long after reperfusion (>60 min) after ischemia (>30 min)-reperfusion*

MicroRNA	Infarcted Zone	Border Zone	Cardiomyocytes	Fibroblasts
miR-1	miR-1 ↑ at 24 h (36) miR-1 ↓ in hearts of humans who died from myocardial infarction (5, 6)			
miR-21	miR-21 ↓ at 6 h (12) and 24 h (12, 36) miR-21 ↑ at 24 h (30) miR-21 ↑ at 2 days and 7 days (31)	miR-21 ↑ at 6 h and 24 h (12)		miR-21 ↑ at 2 days and 7 days (31)
miR-122	miR-122 ↑ 3 h and 6 h after occlusion (10)			
miR-126	miR-126 ↑ at 24 h (36)			
miR-133	miR-133a and miR-133b ↓ 3 h and 6 h after occlusion (10) miR-133 ↓ at 24 h (36)	miR-133a and miR-133b ↓ 3 h and 6 h after occlusion (10)		
miR-146b	miR-146b ↑ at 24 h (30)			
miR-195	miR-195 ↓ at 24 h (36)			
miR-208	miR-208 ↑ at 24 h (36)			
miR-320	miR-320 ↓ at 24 h (30)			
miR-491	miR-491 ↑ at 24 h (30)			
miR-499-5p	miR-499-5p ↓ 3 h and 6 h after occlusion (10)	miR-499-5p ↓ 3 h and 6 h after occlusion (10)		
miR-9651	miR-9651 ↑ at 24 h (30)			

Table 3. *MicroRNAs altered by permanent coronary occlusion*

MicroRNA	Infarcted Zone	Border Zone	Noninfarcted Zone	Cardiomyocytes	Fibroblasts
miR-1	miR-1 ↓ at 3 h and 6 h (10) miR-1 ↑ at 12 h (45)	miR-1 ↓ at 3 h and 6 h (10) miR-1 ↑ at 12 h (45)			
miR-15b		miR-15b ↑ 3 days and 14 days after infarction in mice (41)	miR-15b ↑ 3 days and 14 days after infarction in mice (41)		
miR-21	miR-21 ↓ 6 h after occlusion (12, 32)	miR-21 ↑ 6 h after occlusion (12) miR-21 ↑ 3 days and 14 days after infarction in mice (41) miR-21 ↑ in border zones of human hearts explanted for transplantation (41)	miR-21 ↑ 6 h after occlusion (32) miR-21 ↑ 3 days and 14 days after infarction in mice (41)	miR-21 ↓ 1–48 h in vitro (32)	miR-21 ↓ 1–48 h in vitro (32)
miR-29		miR-29 ↓ 3 days and 14 days after infarction in mice (41) miR-29 ↓ in border zones of human hearts explanted for transplantation (41)	miR-29 ↓ 3 days but not 14 days after infarction in mice (41)		miR-29 ↑ (41)
miR-92a	miR-92a ↑ 24 h after infarction in mice (4)				
miR-126			miR-126 ↑ 6 h after infarction (12)		
miR-133	miR-133 ↓ in infarct zone of patients who died of infarction (5)				
miR-149		miR-149 ↓ 3 days and 14 days after infarction in mice (41) miR-149 ↓ in border zones of human hearts explanted for transplantation (41)	miR-149 ↑ 14 days after infarction in mice (41)		
miR-199a	Mature miR-199a ↓ in mice after 30 min and ≤6 h ischemia (29)				
miR-214		miR-214 ↑ 14 days after infarction in mice (41) miR-214 ↑ in border zones of human hearts explanted for transplantation (41)	miR-214 ↑ 3 days and 14 days after infarction in mice (41)		
miR-223		miR-223 ↑ 14 days after infarction in mice (41) miR-223 ↑ in border zones of human hearts explanted for transplantation (41)	miR-223 ↑ 3 days and 14 days after infarction in mice (41)		

Table 4. *Potential targets of microRNAs involved in ischemia-reperfusion*

MicroRNA	No. of Computational Potential Targets	Computational Potential Targets	Proven Direct Targets (luciferase assay)
miR-1	772	Bcl-2 (36)	Bcl-2 (36)
		Heat shock protein 60 (HSP60) (44)	HSP60 (44)
		HSP70 (44)	HSP70 (44)
		Caspase-9 (44)	Caspase-9 (44)
		GJA1 (45)	GJA1 (45)
		KCNJ2 (45)	KCNJ2 (45)
miR-15b	1,395	ADP-ribosylation factor-like 2 (Arl2) (24)	Arl2 (24)
miR-21	272	PDCD4, (8)	PDCD4, (8, 12)
		AP-1 (8, 12)	PTEN (31)
		PTEN (31, 32)	FasL (32)
		FasL (32)	Tap63 isoform of the p53 family (25)
		Tap63 isoform of the p53 family (25)	HNRPK (25)
		HNRPK (25)	LRRFIP1 (an NF-κB inhibitor) (18)
		LRRFIP1 (an NF-κB inhibitor) (18)	
miR-29	1,124	Mcl-1 (22, 47)	Mcl-1 (22)
		p85a (the regulatory subunit of PI3K) (26)	p85a (the regulatory subunit of PI3K) (26)
		CDC42 (a Rho family GTPase) (26)	CDC42 (a Rho family GTPase) (26)
		Collagen type Iα1 (41)	Collagen type Iα1 (41)
		Collagen type 1α2 (41)	Collagen type 1α2 (41)
		Collagen type IIIα1 (41)	Collagen type IIIα1 (41)
		Fibrillin 1 (41)	Fibrillin 1 (41)
		Elastin (41)	Elastin (41)
miR-92a	915	Integrin subunit α5 (ITGA5) (4)	Integrin subunit α5 (ITGA5) (4)
		Integrin subunit αV (ITGAV) (4)	
		Sphingosine-1-phosphate receptor 1 (S1P1) (4)	
		Mitogen-activated kinase kinase 4 (MKK4)	
		eNOS (4)	
		Sirtuin 1 (Sirt1) (4)	
miR-126	18	Sprouty-related protein-1 (Spred-1) (43, 51)	Spred-1 (43, 51)
		Vascular cell adhesion molecule 1 (VCAM-1) (13, 51)	(VCAM-1 (13, 51)
		G protein (heterotrimeric guanosine triphosphate-binding protein) signaling 16 (RGS16)	CXC chemokine 12 (CXCL12) (51)
		CXC chemokine 12 (CXCL12) (51)	
miR-133	610	Caspase 9 (44)	Caspase 9 (44)
miR-146	174	Neuregulin-1 ErbB4 (14)	Neuregulin-1 ErbB4 (14)
miR-149	427	Akt 1 (19)	Akt 1 (19)
		E2F1 (19)	E2F1 (19)
		b-My (19)	
miR-195	1395	ADP-ribosylation factor-like 2 (Arl2) (24)	ADP-ribosylation factor-like 2 (Arl2) (24)
miR-199a	397	Hif-1α (29)	Hif-1α (29)
		Sirt1 (29)	Sirt1 (29)
miR-208	139	Thyroid hormone receptor associated protein 1 (THRAP1) (40)	THRAP1 (40)
miR-214	716	PTEN (46)	PTEN (46)
miR-223	258	Glucose transporter 4 (Glut4) (20)	
miR-320	893	Hsp20 (30)	Hsp20 (30)
miR-491	266	Bcl-X_L (23)	Bcl-X_L (23)

otide promoted apoptosis in these cells and in neonatal rat ventricular cardiomyocytes (44). Computational and bioinformatic studies (44) have identified caspase-9 as a potential target of miR-133. miR-133 decreased the level and activity of caspase-9, whereas anti-miR-133 oligonucleotides abrogated this effect, suggesting that miR-133 has an antiapoptotic effect by suppressing caspase-9.

miR-199a

Rane and colleagues (29) have reported that miR-199a targets hypoxia-inducible factor-1α (Hif-1α) and Sirt1, suggesting that miR-199a is involved in early ischemic and hypoxic preconditioning. In mouse studies, they reported that mature miR-199a was undetectable in the heart as early as 30 min after coronary artery occlusion, whereas its precursor accumulated over time (29). Overexpression of miR-199a inhibited hypoxia-induced expression of proapoptotic proteins, such as caspase-3, caspase-6, caspase-9, caspase-12, FasL, apoptosis-inducing factor (AIF), and Bnip1, in neonatal rat cardiomyocytes (29).

Hif-1α, a predicted target of miR-199a, is rapidly induced by hypoxia and is involved in hypoxic preconditioning. A luciferase activity test showed that miR-199a interacted with the 3′-UTR of Hif-1α and that inhibiting miR-199a induced the expression of Hif-1α in cardiomyocytes (29). Both hypoxic preconditioning and silencing miR-199a were associated with upregulation of inducible nitric oxide synthase (iNOS) and Bcl-2, whereas overexpression of miR-199a prevented upregulation of iNOS during hypoxic preconditioning (29).

Another target of miR-199a predicted by the computational analysis is Sirt1, a class III histone deacetylatase and longevity gene (29). Overexpression of miR-199a reduced Sirt1 level, whereas silencing miR-199a by antisense oligonucleotide or by hypoxic preconditioning increased Sirt1 levels (29). Interest-

ingly, Rane and colleagues found that Sirt1 downregulated prolyl hydroxylase 2, which destabilizes Hif-1α (29). Thus downregulation of miR-199a induced protection against ischemia-reperfusion injury both by decreasing suppression of translation of Hif-1α and by enhancing its stability via Sirt1-induced inhibition of prolyl hydroxylase 2 (29).

miR-320

Studies (30) have shown that miR-320 is downregulated in mouse hearts after 30 min of ischemia and 24 h of reperfusion (in vivo) or after 45 min of no-flow global ischemia and 2 h of reperfusion (ex vivo). In vitro experiments using adult rat cardiomyocytes showed that overexpression of miR-320 increased cell death and apoptosis, whereas silencing miR-320 reduced cell death and apoptosis. Moreover, transgenic mice with cardiac-specific overexpression of miR-320 had a larger myocardial infarction size than wild-type mice after ischemia-reperfusion. However, inhibiting miR-320 with antagomir-320 significantly reduced infarction size (30). Furthermore, miR-320 downregulated HSP20, a protein that protects the heart against ischemia-reperfusion injury, indicating that HSP20 is a putative target of miR-320 (30).

Conclusion

Taken together, the results described above show complex and highly variable changes in expression of various miRNAs during and after ischemia or ischemia-reperfusion. These changes should be incorporated into the current understanding of the complex dynamic transcriptional and posttranscriptional changes that occur in numerous prosurvival and proapoptotic mediators at various cell types in response to ischemia and ischemia-reperfusion injury. It is plausible that in response to brief ischemia or short cycles of nonlethal ischemia-reperfusion insults alterations in miRNAs expression favoring survival are activated in cardiomyocytes, endothelial cells, and/or other types of cells. However, with prolonged ischemia leading to irreversible damage, the prosurvival signals are turned off and proapoptotic miRNAs changes occur. Later on, there are changes in miRNA levels that increase fibrosis in fibroblasts at the infarcted zone, in conjunction with stimulation of hypertrophic signaling pathways known to play a role in remodeling and angiogenesis at the border zone as well as the more remote myocardial zones. Thus different changes are expected to occur at different time points and in different cell types located in the infarcted zone, border zone, and remote myocardial regions. Moreover, it is plausible that different animal strains, sex, and age may affect miRNA response to ischemia or ischemia-reperfusion injury. Furthermore, we cannot exclude that different anesthetic agents may affect miRNA expression, as they have effects on preconditioning and infarct size. Indeed, the experimental data support complex and location- and time-dependent changes in miRNAs expression (see above and Tables 1–3). Moreover, ischemia-reperfusion induces alteration in the expression of several miRNAs; each one of them has numerous potential targets (Table 4). Together with alterations in the transcription and posttranscriptional modification of various key signaling pathways, changes in miRNA expression should be seen as part of a network response and do not stand alone, as depicted in most experimental studies.

It is commonly believed that acute ischemia-reperfusion injury occurs within minutes of reperfusion. However, apoptosis may continue for hours and days after reperfusion. It is very difficult to separate changes that are related to delayed ischemia-reperfusion injury from those that are related to remodeling. Some of the changes in miRNA expression described above are related to angiogenesis and hence should be classified as "remodeling"-related rather than "ischemia-reperfusion injury"-related changes.

Nevertheless, the above-mentioned data suggest that specific miRNAs are important in controlling ischemia-reperfusion injury and that miRNAs may be potential therapeutic targets for the treatment of heart disease. Per definition, preconditioning (either by ischemia or other mechanical interventions or by pharmacological agents) should be administered before the ischemia-injury insult. Utilization of preconditioning in the clinical setting may occur as short- or medium-term pretreatment before planned ischemia (elective cardiac or noncardiac surgery, percutaneous coronary intervention, etc.) or, alternatively, as long-term treatment to "protect the heart" if prolonged ischemia occurs. Because miRNAs have numerous potential effects that may affect various cell types and tissues, long-term administration therapies should be carefully assessed for potential benefits as well as adverse effects and safety. Postconditioning, on the other hand, refers to interventions that are done during ischemia or the early phase of reperfusion to minimize the reperfusion part of ischemia-reperfusion injury. In the clinical setting postconditioning can be used mainly for patients presenting with ongoing ischemia (ST elevation myocardial infarction). This mandates a simple route for administration of the drug and early onset of action. Currently, it is unclear how fast miRNA mimic or inhibitory agents can penetrate the cells and exert their transcriptional changes. If it is possible to show that such changes occur within minutes after intravenous or intracoronary administration, miRNA-based therapy should be tested as a means to limit acute ischemia-reperfusion injury ("postconditioning"). Inhibiting miRNAs by antisense strategies or pharmacological approaches is likely to emerge as an alternative and safe method for conferring short- and intermediate-term protection against ischemia-reperfusion injury (for example, before percutaneous coronary interventions or high-risk surgery). miRNA-based therapy has the potential to improve remodeling and prevent the deterioration of heart function and development of heart failure after myocardial infarction.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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