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MicroRNA Expression and Function in Cardiac Ischemic Injury

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Abstract

Ischemic heart disease (IHD) or myocardial ischemia is one of the leading causes of mortality all over the world. There is a definite need for new approaches to improve therapies and diagnostics. The pathological process leading to IHD is associated with an altered expression of genes that are important for cardiac functions. Micro-RNAs (miRNAs) have emerged as one of the central players regulating gene expression via degradation or translational inhibition of their target genes. Increasing evidence indicates that miRNAs may serve as potential diagnostic biomarkers and innovative therapeutic targets in several human diseases including cardiovascular disease. Here, we review the latest advances in the identification and validation of myocardial ischemia-related miRNAs and their target genes and discuss the roles of specific miRNAs in regulating ischemia-related cardiac injury, including apoptosis, fibrosis, arrhythmia, and angiogenesis.

Keywords

MicroRNAs; Target Genes; Gene Expression; Cardiac Ischemia/Reperfusion Injury

Introduction

There are more than six million Americans living with ischemic heart disease (IHD) or myocardial ischemia, and this number keeps increasing all the time. Myocardial ischemia develops when coronary blood supply to myocardium is reduced, either in terms of absolute flow rate (low-flow or no-flow ischemia) or relative to increased tissue demand (demand ischemia). The pathological process leading to IHD is associated with an altered expression of genes that are important for cardiac functions. MicroRNAs (miRNAs) have emerged as one of the central players in regulation of gene expression in various cell types via degradation or translational inhibition of their target genes. There is increasing evidence that differential downregulation and upregulation of selective miRNAs in the heart represents an important mechanism for control of the expression of myocardial ischemia-related genes.

MicroRNAs are a class of highly conserved, single-stranded, noncoding small RNAs [1,2]. They are produced from primary RNA transcripts (pri-miRNAs), which are transcribed from genomes by RNA polymerase II. Pri-miRNAs, generally several thousand of nucleotides long and containing the active miRNA in characteristic stem-loop structures, are cleaved in the nucleus by the complex of the RNase III enzyme Drosha and its partner DGCR8/Pasha to form

approximately 70-nucleotide pre-miRNAs [1,2]. Pre-miRNAs are transported into the cytoplasm by Exportin-5 and subsequently processed by the nuclease Dicer into the 20- to 24-nucleotide mature miRNA. After maturation, they enter the RNA interference pathway and regulate gene expression on the posttranscriptional level by inhibiting the translation of protein from mRNA or by promoting the degradation of mRNA [1,2].

miRNAs have been shown to regulate various cellular functions, including cell proliferation, migration, differentiation, and apoptosis [3–6]. Experimental studies indicate that miRNAs may serve as potential diagnostic biomarkers and innovative therapeutic targets in several human diseases, such as diabetes, immunodegenerative or neurodegenerative disorders, and cancer [7–11]. Furthermore, recent studies have begun to unveil previously unrecognized roles of miRNAs in cardiovascular disease, such as cardiac hypertrophy and heart failure [12–14]. This review discusses the latest advances in the identification and validation of cardiac ischemia-related miRNAs and their target genes and highlights the roles of specific miRNAs in regulating ischemia-related cardiac injury.

MicroRNA Expression Alteration in Cardiac Ischemic Injury

Myocardial ischemia is a result of imbalance between myocardial blood supply and oxygen demand, which is caused by a variable degree of coronary artery obstruction. Emerging evidence indicates that ischemia induces profound changes in miRNA expression in different tissues/organs. Table 1 summarizes findings of cardiac ischemia-related miRNAs with relevant target genes. It is interesting to note that expression profiles of miRNAs are quite different, depending on the location of injury and the nature of the situation even within different areas of the same ischemic heart. For example, Dong et al. observed that the expression of some miRNAs in the border area was much different from that of the infarcted area of the infarcted rat heart [15]. van Rooij et al. [16] also found that miRNA expression in the noninfarcted area of murine hearts in the late phase of acute myocardial infarction was different from that in sham-opened control hearts.

Recent studies show that some miRNAs are upregulated, and some are downregulated in hearts under pathophysiological conditions. In cardiac muscles, the most abundant miRNAs include miR-1, let-7, miR-133, miR-126-3p, miR-30c, and miR-26a [17]. miRNA-208 has been found to be purely cardiac specific [18]. In coronary arterial smooth muscle cells, the most abundant miRNAs are miR-145, let-7, miR-125b, miR-125a, miR-23, and miR-143, although miR-1 and miR-133 are also expressed in coronary arterial smooth muscles [19]. The differential tissue distributions of miRNAs suggest tissue-specific or even cell-type-specific function of these molecules.

Molecular mechanisms underlying altered miRNA expression in cardiac ischemic injury remain largely unknown. miRNAs are generated in a two-step processing pathway mediated by two major enzymes, Dicer and Drosha, which belong to the class of RNase III endonucleases [1,2]. Dicer is the only known enzyme involved in the maturation of miRNA and can decide the level of miRNA in cells [2]. Recent work suggests that Dicer may be involved in regulation of miRNAs in hearts [20]. Dicer mutant mice show misexpression of cardiac contractile proteins and profound sarcomere disarray. Microarray analysis shows that there is a dramatic reduction in mature miRNA expression in Dicer mutant hearts compared with wild-type control hearts [20]. Functional analyses indicate significantly reduced heart rates and decreased fractional shortening of Dicer mutant hearts. Consistent with the role of Dicer in animal hearts, Dicer expression was decreased in end-stage human dilated cardiomyopathy and failing hearts [20]. Although many miRNAs seem to be regulated by Dicer, more efforts still need to define the molecular mechanisms by which Dicer regulates

specific miRNAs in hearts under pathophysiological conditions including myocardial ischemia/reperfusion (I/R) injury.

Role of Specific MicroRNAs in Regulation of Ischemic Cardiomyocyte Apoptosis

Ischemia and reperfusion activate cardiac myocyte apoptosis, which is an important feature in the progression of ischemic heart disease. Accumulating evidence indicates that miRNAs are one kind of critical apoptotic regulator not only in tumor cells but also in heart cells. A recent elegant study by Yang et al. [21] has shown that the muscle-specific miR-1 level is markedly elevated in ischemic myocardium where apoptotic cell death plays an important role in the detrimental changes of the diseased heart. Coincidentally, another elegant study from the same group revealed that the muscle-specific miR-1 and miR-133 produce opposing effects on apoptosis induced by oxidative stress in rat cardiomyocytes, with miR-1 being proapoptotic and miR-133 being antiapoptotic [22]. miR-1 level was significantly increased in response to oxidative stress. Furthermore, they have identified single target site for miR-1 only, in the 3' untranslated regions of the heat shock protein 60 (HSP60) and HSP70 genes, and multiple putative target sites for miR-133 throughout the sequence of the caspase 9 gene [22]. Posttranscriptional repression of HSP60 and HSP70 by miR-1 and of caspase 9 by miR-133 contributes significantly to their opposing actions [22]. Moreover, others report that the level of miR-1 is inversely correlated with antiapoptotic Bcl-2 protein expression in cardiomyocytes of the I/R rat model [23]. Indeed, experimental data indicate that miR-1 regulates cardiomyocyte apoptosis through the posttranscriptional repression of Bcl2 [23].

miR-21 has also been implicated in cardiomyocyte apoptosis [15,24]. Experimental data have shown that miR-21 expression was significantly downregulated in infarcted areas but was upregulated in border areas in rat hearts 6 h after acute myocardial infarction (AMI) [15]. Overexpression of miR-21 via adenovirus expressing miR-21 (Ad-miR-21) decreased myocardial infarct size and the dimension of left ventricles at 24 h after AMI. In cultured cardiac myocytes, miR-21 is shown to exert a protective effect on ischemia-induced cell apoptosis that was associated with its target gene programmed cell death 4 (PDCD4) and activator protein 1 (AP-1) pathway [15]. The protective effect of miR-21 against ischemia-induced cardiac myocyte damage was further confirmed in vivo by decreased cell apoptosis in the border and infarcted areas of the infarcted hearts after treatment with Ad-miR-21 [15]. In addition, a study has shown that miR-21 is upregulated after ischemic preconditioning, along with upregulation of miR-1 and miR-24 and in this case was thought to be involved in miRNA-induced cardioprotection mediated by upregulation of eNOs, Hsp70, and HsF1 (the Hsp70 transcription factor) [24].

Recently, Ren et al. [25] demonstrate that miR-320 is involved in the regulation of I/R-induced cardiac injury and dysfunction via antithetical regulation of Hsp20. Experimental data indicate that miR-320 expression was significantly decreased in murine hearts subjected to I/R in vivo and ex vivo. Overexpression of miR-320 enhanced cardiac myocyte death and apoptosis, whereas knockdown was cytoprotective, on simulated I/R. Furthermore, transgenic mice with cardiac-specific overexpression of miR-320 revealed an increased extent of apoptosis and infarction size in the hearts on I/R in vivo and ex vivo relative to the wild-type controls. Conversely, in vivo treatment with antagomir-320 reduced infarction size relative to the administration of mutant antagomir-320 and saline controls. Hsp20 was identified as a real target for miR-320. Thus, miR-320 may constitute a new therapeutic target for ischemic heart diseases.

Role of Specific MicroRNAs in Regulation of Ischemic Cardiac Fibrosis

Cardiac fibrosis is an established morphological feature of the structural myocardial remodeling that occurs in several heart diseases, including ischemia and infarction, cardiomyopathies, and myocarditis. This feature confers an increased risk for ventricular dysfunction and arrhythmias. The molecular mechanisms of cardiac fibrosis remain poorly elucidated. A small number of studies suggest that altered expression of cardiac-specific miRNAs is associated with cardiac fibrosis during ischemia or mechanical overload. Recently, van Rooij et al. [16] demonstrate that miR-29 plays an important role in cardiac fibrosis during the repair process after AMI. MicroRNA microarrays show that miR-29 is expressed preferentially in the fibroblast population of the myocardium and is downregulated in areas surrounding infarcted areas in mouse and human hearts. Further, experimental data show that miR-29 targets a cadre of mRNAs that encode proteins involved in fibrosis, including multiple collagens, fibrillins, and elastin [16]. Downregulation of miR-29 with anti-miRs in vitro and in vivo induces the expression of collagens, whereas overexpression of miR-29 in fibroblasts reduces collagen expression. Thus, miR-29 may act as a regulator of cardiac fibrosis and represents a potential therapeutic target for tissue fibrosis.

Role of Specific MicroRNAs in Regulation of Ischemic Arrhythmia

Arrhythmias are a major cause for cardiac death after myocardial infarction. The electrical-conduction system, which is required to maintain proper heart rhythmicity, is composed of specialized muscle cells and distinct sets of ion channels. Myocardial ischemia can cause many malfunctions of this system with impaired cardiac excitability by triggering an aberrant gene expression. A small number of studies have implicated cardiac miRNAs in regulation of ischemic arrhythmia [21,26]. Recently, Yang et al. [21] have identified muscle-specific miR-1 as a cardiac arrhythmia-related miRNA in human and rat hearts after ischemia. They found that miR-1 levels are elevated in human hearts with coronary artery disease and in rat hearts after AMI [21]. Overexpression of miR-1 in normal or infarcted hearts exacerbates arrhythmogenesis, whereas elimination of *miR-1* by an antisense inhibitor in infarcted hearts relieved arrhythmogenesis [21]. *miR-1* overexpression slowed conduction and depolarized the cytoplasmic membrane through posttranscriptionally repressing potassium channel subunit gene (KCNJ2) and gap junction protein connexin 43 [21]. In addition, the homeodomain transcription factor *Irx5*, which regulates cardiac repolarization by repressing the potassium channel *KCND2*, has also been identified as a direct miR-1 target [26], further supporting a role for miR-1 in cardiac conduction. As discussed above, miR-1 showed proapoptotic effect on ischemic cardiomyocytes [21–23]. Cardiomyocyte apoptosis has been shown to trigger arrhythmias [27]. The excitability of cardiomyocytes in the progress of apoptosis is altered and abnormal to adjacent cardiomyocytes [28]. Thus, miR-1 upregulation during cardiac ischemic injury might provide a molecular link between the proapoptotic event and the development of arrhythmias, and targeting miR-1 might represent a new antiarrhythmic therapy.

Role of Specific MicroRNAs in Regulation of Ischemic Angiogenesis

Neoangiogenesis is an important recovery mechanism in rebuilding the blood supply and attenuating the progression of left ventricular dysfunction after AMI and thus represents an excellent therapeutic target for the treatment of ischemic heart disease. Some endothelial-specific miRNAs have been implicated in the regulation of various aspects of angiogenesis [29]. Experimental data have shown that miR-17~92 cluster is highly expressed in human endothelial cells and that miR-92a, a component of this cluster, controls the growth of new blood vessels (angiogenesis) [29]. Recently, miR-92a has been demonstrated to control angiogenesis and functional recovery of ischemic tissues in mouse models of limb ischemia and myocardial infarction [30]. miR-92a has been identified as an endogenous repressor of the

angiogenic program in endothelial cells. Forced overexpression of miR-92a in endothelial cells blocked angiogenesis in vitro and in vivo. In the two mouse models, systemic inhibition of miR-92a via administration of an antagomir is shown to promote blood vessel growth and functional recovery of damaged tissue. MiR-92a appears to target mRNAs corresponding to several proangiogenic proteins, including the integrin subunit $\alpha 5$. Thus, miR-92a may act as a regulator of ischemic angiogenesis and represents a potential therapeutic target of neoangiogenesis for rebuilding the blood supply in IHD [27].

Conclusions

Recent studies have provided increasing evidence that miRNAs play a significant role in cardiac ischemic injury, including apoptosis, fibrosis, arrhythmia, and angiogenesis. Nevertheless, our current knowledge about the regulation and function of specific miRNAs in ischemic heart disease is still quite limited. Future research needs to characterize more cardiac-specific miRNAs for their expression profiles and regulatory targets that are specifically associated with myocardial ischemia. Moreover, future studies need to focus on characterizing the in vivo functions of individual cardiac-specific miRNAs by the identification of their downstream target mRNAs as well as undesired side effects. Differential downregulation or upregulation of selective miRNA expression may constitute a new therapeutic approach to treat cardiovascular disease in the near future. For miRNA-based therapeutics, however, there is still a long way to go. Effective delivery of specific miRNAs to the specific targets (e.g., specific organs, tissues, or cell types) is the major challenge.

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Table 1

Cardiac ischemia-related miRNAs and target genes

MicroRNA	Biological function	Targets	Ref.
miR-1	Apoptosis	HSP60, HSP70, Bcl2	[22,23]
	Arrhythmias	KCNJ2, connexin 43, Irx5	[21,26]
miR-133	Antiapoptosis	CASP9	[22]
miR-21	Antiapoptosis	PDCD4, AP-1	[15]
	Cardioprotection	eNOS, HSP70, HSF1	[24]
miR-320	Apoptosis	HSP20	[25]
miR-29	Fibrosis	ELN, FBN1, COL1A1, COL1A2, COL3A1	[16]
miR-92a	Angiogenesis	Integrin subunit alpha 5	[30]