MicroRNAs in heart and circulation during physical exercise

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Abstract

Exercise training is beneficial to the cardiovascular system. MicroRNAs (miRNAs, miRs) are a class of conserved non-coding RNAs and play a wide-ranging role in the regulation of eukaryotic gene expression. Exercise training alters the expression levels of large amounts of miRNAs in the heart. In addition, circulating miRNAs appear to be regulated by exercise training. In this review, we will summarize recent advances in the regulation of miRNAs during physical exercise intervention in various cardiovascular diseases, including pathologic cardiac hypertrophy, myocardial fibrosis, ischemia-reperfusion injury, myocardial infarction, and heart failure. The regulatory role of circulating miRNAs after exercise training was also reviewed. In conclusion, miRNAs might be a valuable target for treatment of cardiovascular diseases and have great potential as biomarkers for assessment of physical performance.

Keywords: Cardiovascular diseases; Circulating microRNAs; Exercise; MicroRNAs

1. Introduction

Exercise can effectively reduce the risk of cardiovascular events. Although the precise molecular mechanism of the beneficial effects of exercise remains unclear, a number of studies have shown that physical exercise can restore myocardial function; improve maximal oxygen consumption (VO2max); and improve endothelial cell function, left ventricular systolic function, and diastolic function. Exercise-based cardiac rehabilitation is effective at all stages of cardiovascular disease treatment and has an important protective effect on cardiovascular disease. Therefore, exercise has been used as a non-pharmacologic supplement to support traditional treatment and prevent cardiovascular disease. In this review, we review the regulatory role of microRNAs (miRNAs, miRs) in the heart during physical exercise. Attention will be paid to understanding the current knowledge of (1) cardiac adaptations in response to exercise, (2) miRNAs in the heart, and (3) the adaptation of circulating miRNAs (c-miRNAs) to exercise. Finally, the implications and future prospects of miRNAs in heart and circulation will be discussed.

2. Cardiac adaptation in response to exercise

Exercise training can make the heart adapt to changes in morphology, structure, and function (Fig. 1). Long-term exercise training can induce cardiac physiological growth, which is characterized by increased formation of new cardiomyocytes and cardiomyocytes size. Exercised hearts in animals have been found to have a significantly increased number of new cardiomyocytes. The IGF-1/PI3K/AKT signaling pathway is key in regulating cardiac physiological growth. Insulin and insulin-like growth factor (IGF) are activators of AKT in cardiomyocytes, and IGF signaling regulates normal growth of embryonic and postnatal organs. High expression of IGF-1 was found in athletes’ myocardium accompanied by physiological cardiac hypertrophy. On binding of IGF-1 to the tyrosine kinase receptor on the cell membrane, it activates the activity of p110α catalytic subunit of PI3K and AKT1. The p110α knockout mice are lethal, and physiological cardiac hypertrophy was not induced. It has been found that injection of 5-fluorouracil inhibits cardiac cell proliferation and limits the exercised-induced cardioprotective effects from ischemia-reperfusion (I/R) injury without affecting the exercised-induced cardiac hypertrophy in vivo.

The IGF-1/PI3K/AKT signaling pathway is key in regulating cardiac physiological growth. Insulin and insulin-like growth factor (IGF) are activators of AKT in cardiomyocytes, and IGF signaling regulates normal growth of embryonic and postnatal organs. High expression of IGF-1 was found in athletes’ myocardium accompanied by physiological cardiac hypertrophy. On binding of IGF-1 to the tyrosine kinase receptor on the cell membrane, it activates the activity of p110α catalytic subunit of PI3K and AKT1. The p110α knockout mice are lethal, and physiological cardiac hypertrophy was not induced. It has been found that injection of 5-fluorouracil inhibits cardiac cell proliferation and limits the exercised-induced cardioprotective effects from ischemia-reperfusion (I/R) injury without affecting the exercised-induced cardiac hypertrophy in vivo.
hypertrophy can be induced by IGF-1 or long-term high-intensity exercise to activate p110α.12,14 Conversely, inhibition of p110α protein expression by p110α dominant negative mutation can impair myocardial normal growth and block exercise-induced physiological cardiac hypertrophy. The simultaneous loss of p85α and p85β in the myocardium also leads to a decrease in myocardial growth.15

Increased AKT1 in cardiomyocytes also leads to a decrease of the transcription factor CCAAT/enhancer binding protein β (C/EBPβ). C/EBPβ is one of the members of the bHLH family of transcription factors, and its main function is to regulate cell proliferation and transcription of differentiated genes. In the development of physiological cardiac hypertrophy, the expression of the C/EBPβ gene is down-regulated, and CITED4 is up-regulated.16 Silencing of C/EBPβ by small interfering RNA (SiRNA) can promote cardiomyocytes proliferation, confirming that physiological cardiac hypertrophy can be down-regulated by C/EBPβ and mediated by CITED4. This suggests that the induced cell proliferation caused by down-regulation of C/EBPβ expression may be mediated by the negative regulation of CITED4. In addition, C/EBPβ down-regulation in zebrafish embryos can effectively induce cardiomyocyte proliferation; a clear increased proliferation of cardiomyocytes in C/EBPβ heterozygous mice has also been observed. Lower C/EBPβ levels in mice have been found to be effective against heart dysfunction in transverse aortic constriction–induced pressure overload.16 CITED4 negatively regulates cardiomyocytes elongation caused by eccentric hypertrophy, which is highly associated with cardiac dysfunction.17 Overexpression of CITED4 activates mTORC1 pathways and promotes cardiac functional recovery from I/R injury.18 These reports suggest that C/EBPβ/CITED4 regulates the hypertrophy and proliferation of cardiomyocytes in mammalian hearts caused by physiological stimuli.

In addition to cardiac growth, endothelial nitric oxide synthases/NO is up-regulated and regulates vascular homeostasis during exercise training.19 Exercise also induces angiogenesis via increasing the expression level of vascular endothelial growth factor and promoting PI3K/AKT signaling.20,21 In addition, exercise regulates the action potential and excitation-contraction coupling (E-C coupling) through stimulation of G protein-cAMP-PKA signaling pathways and Ca2+ uptake.22
3. The benefits of miRNAs and exercise-regulated miRNAs to the heart

The miRNAs are a class of highly conserved non-coding RNAs whose mature sequences often consist of 18–25 nucleotides. MiRNAs regulate the expression of specific target genes by binding to complementary sequences in mRNA to induce degradation of mRNA or to inhibit translation of proteins.\(^{23}\) The maturation process of miRNAs is controlled by multiple post-transcriptional regulatory steps. Similar to mRNA, primary miRNAs are mainly produced by direct transcription under the action of RNA polymerase II. These primary miRNA transcripts are further cleaved by the Drosha-DGCR8 complex and folded into precursor miRNAs (pre-miRNAs) of 70–110 nucleotides.\(^{24}\) Generally, the pre-miRNA is transported from the nucleus to the cytoplasm. After being released into the cytoplasm, the pre-miRNA is cleaved by DICER into a short-chain mature miRNA. The mature miRNA is then loaded into the RISC/Argonaut complex, which recognizes and binds to and cleaves the sequence that the 3'-UTR of the mRNA specifically binds to the miRNA.\(^{25}\) Ultimately, the stability of the mRNA is decreased or the protein translation process is directly inhibited. Numerous miRNAs in the heart are altered after exercised training.\(^{26}\) The modulation of miRNAs in exercise-induced cardioprotection has received increasing attention.\(^{27,28}\) In the following sections, we will briefly summarize the role that miRNAs involved in exercise play in protecting against cardiovascular diseases (Fig. 2) and will describe the role of several miRNAs that have been examined in detail (Table 1).

3.1. MiRNAs mediate protective effects of exercise in myocardial fibrosis

Myocardial fibrosis is characterized by excessive proliferation of cardiac fibroblast and collagen deposition.\(^{29}\) The occurrence of cardiac fibrosis is closely related to various cardiovascular diseases such as myocardial infarction (MI),
induced cardiac growth. The use of cardiac-specific miR-222
HMBOX1. Inhibition of miR-222
ingly, it has been reported that miR-222 was also elevated in
both wheel running and swimming exercise models. Interest-
found that miR-222 was elevated in mice that participated in
constitutes a miR-222
MiR-222 resides in close proximity to the X chromosome and
injury.
Because of the high incidence and high mortality of I/R injury,
3.2. miRNAs mediate protective effects of exercise in I/R
injury
After partial or complete acute obstruction of the coronary
artery, the ischemic myocardium can resume normal perfusion
when it is re-established, but its tissue damage is a pathologic
process of progressive aggravation. When the ischemic tissue
restores blood perfusion, it leads to significant pathophysiological
changes in the myocardium and the local vascular network
in the reperfusion area, as well as a series of damaging changes
caused by ischemia. These changes work together to promote
further tissue damage, a phenomenon known as myocardial
I/R injury. I/R injury will greatly reduce the efficacy of myo-
cardial reperfusion. Its mechanism is currently thought to be
mainly related to the large amount of intracellular oxygen-free
radicals, calcium ion overload, inflammatory effects of white
blood cells, and high-energy phosphate compounds. Because of
the high incidence and high mortality of I/R injury, the
mechanism and treatment of such diseases have received
extensive attention. MiRNAs and exercise protection from I/R
injury have also been studied in relation to myocardial I/R
injury.
MiR-222 is the first miRNA to be fully studied in relation to
exercise and the prevention of myocardial I/R injury. MiR-222 resides in close proximity to the X chromosome and
constitutes a miR-222~221 cluster. A microarray analysis
found that miR-222 was elevated in mice that participated in
both wheel running and swimming exercise models. Interest-
ingly, it has been reported that miR-222 was also elevated in
the peripheral blood of young athletes. In addition, elevated
levels of miR-222 were found in circulating blood of 28 exercise-
trained patients with heart failure. Overexpression of
miR-222 promotes cardiomyocytes growth and proliferation
in vitro and in vivo through targeting p27, HIPK1, and HMBOX1. Inhibition of miR-222 in vivo prevented exercise-
induced cardiac growth. The use of cardiac-specific miR-222
transgenic mice to study the miR-222 in vivo effects demon-
strated that overexpression of miR-222 protects against cardiac
I/R injury. Another miRNA, miR-17-3p, was also found at
increased levels in 2 different murine exercise models. Similar-
lar to miR-222, the miR-17-3p expression level was found to be
heightened in serum from exercise-trained patients with
chronic heart failure. MiR-17-3p promotes cardiomyocyte pro-
iferation and increased cardiomyocyte size through directly
targeting TIMP3 and indirectly regulating the PTEN-AKT sig-
nal pathway. In vivo, miR-17-3p inhibition could lessen the
exercise-induced cardiac growth. Overexpression of miR-17-3p could protect against I/R injury-induced cardiac remodel-
ing. Interestingly, TIMP3 was also identified as the target
gene of miR-222 in pulmonary arterial smooth muscle cells.
However, it remains to be studied whether TIMP3 in cardio-
myocytes acts as a target gene of miR-222 as it mediates the
protective effect on the heart.

3.3. MiRNAs mediate protective effects of exercise in MI
MI is an ischemic death of the myocardium. In coronary
artery disease, the blood flow of the coronary artery is drastically
reduced, causing severe and long-lasting acute ischemia of
myocardium, eventually leading to ischemic death of the
myocardium. In the ischemic heart, mitochondrial structure
and function are damaged, oxidative phosphorylation is impaired, adenosine 5'-triphosphate (ATP) production is insuf-
ficient, and the calcium pump is dysfunctional. Therefore,
when calcium is overloaded, excessive Ca2+ in the cytoplasm
cannot be excreted outside the cell, causing the Ca2+
concentration in the cytoplasm to further increase, activating various cellular pathways and leading to myocardial cell death. It is
generally believed that the ratio of Na+ concentration inside and
outside the cell is closely related to the transport and over-
load of Ca2+. In the presence of myocardial ischemia, the
expression of the sodium/calcium exchanger, sarco(plemma)/
endoplasmic reticulum calcium ATPase (SERCA-2), and phospholamban was decreased. Aerobic intensity—controlled,
interval training—increased cardiomyocyte function after MI
was accompanied by elevated SERCA-2 protein expression
and Ca2+-sensitivity. MI leads to decreased expression of
miR-1 and increased expression of miR-214; sodium/calcium
exchanger and SERCA-2 are targeted by miR-1 and miR-214
in the remote region of myocardium. It has been reported that
exercise training can prevent the decrease of miR-1 expression
and the increase of miR-214 expression induced by MI, which
in turn promotes cardiac recovery of Ca2+ handling.

3.4. MiRNAs mediate protective effects of exercise in heart
failure
Heart failure is not an independent disease but the end stage
of heart disease development. Almost all cardiovascular dis-
ases, such as MI, cardiomyopathy, hemodynamic overload,
inflammation, and other causes of myocardial damage, will
eventually lead to heart failure. Although the significance of
exercise training for the treatment of heart failure has been
widely accepted, the mechanisms by which exercise training
plays a protective role remain poorly understood. A number of studies have shown that long-term sedentariness can aggravate heart failure, and exercise training is beneficial for patients with heart failure. This phenomenon has also been verified in animal models of heart failure. In a study of the global changes in the expression of miRNAs in transverse aortic constriction—induced heart failure among rats before and after exercise training, 56 miRNAs were found to be abnormally regulated after exercise training, of which 38 were up-regulated and 18 were down-regulated. Among them, in previous studies involving I/R injury and cardioprotection by ischemic pre- and post-conditioning miR-21, miR-144, miR-146b, miR-208b, miR-212, miR-214, and miR-335 were also found to be dysregulated, which suggests that there may be some miRNAs that play a role in multiple cardioprotective intervention mechanisms, a possibility worthy of further study and discussion. Additional analysis of the expression of cardiac stress markers during heart failure showed that exercise training did not alter the expression levels of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and α-actin. Using miRTarBase, miRWalk, and further gene-term enrichment analysis, 26 pathways were clustered in 5 major biologic modules, including in programmed cell death, transforming growth factor-β signaling, cellular metabolic process, cytokine signaling, and cell morphogenesis.

4. MiRNAs mediate exercise-induced cardiac physiological hypertrophy

The cardiac adaptations to exercise training include a set of beneficial effects on muscle metabolism, circulation system, and heart performance. Physiological cardiac hypertrophy is 1 phenotype induced by exercise training. The changes of many miRNAs are associated with physiological cardiac hypertrophy (Fig. 3). The expression of miR-1 and miR-133 is reduced in the hearts of rats after treadmill training. To evaluate the role of miRNAs in regulating the cardiac signal cascades in exercised rats, they were tested with swimming tasks. MiR-21 (target PTEN), miR-144 (target PTEN), and miR-145 (target TSC2) were up-regulated, whereas miR-124 (target PI3K) was down-regulated in the swimming training group, which was consistent with the model that exercise could induce left ventricular hypertrophy. Generally, the renin-angiotensin system of the heart increases when pathologic cardiac hypertrophy occurs, typically involving an increase in angiotensin-converting enzyme (ACE) and angiotensin II (Ang II). In exercise-induced cardiac hypertrophy, miR-27a and miR-27b (both target ACE) were significantly up-regulated, but miR-143 (target ACE2) was significantly down-regulated, which has been consistently associated with reduced expression levels of ACE and Ang II but elevated expression levels of ACE2 in animals trained with aerobic exercise. In treadmill-trained mice, the miR-223 in their hearts was up-regulated; this elevation of miR-223 could lead to physiological cardiac hypertrophy through directly targeting the 3′-UTRs of FBXW7 and Acrv2a. In addition to the expression of miR-222, miR-17-3p expression was also increased in exercise-induced cardiac hypertrophy.

5. Exercise-regulated circulating miRNAs are beneficial to the heart

In recent years, studies have shown that miRNAs can be released and exist outside the cell in a stable form to become c-miRNAs in plasma. The relative content of c-miRNAs under environmental stimulation and pathophysiological stress can be adaptively changed. It was also found that c-miRNAs have excellent stability in plasma and serum. Therefore, the potential value of c-miRNAs is indicated as possible biomarkers and physiological stress assessment indicators. In the past few years, various studies have focused on cardiovascular diseases and sought to develop sensitive and effective physiological indicators. In the field of sports training, it is also necessary to develop new biomarkers to evaluate the adaptation to exercise. Therefore, certain characteristics of miRNAs, which have shown unique value in the diagnosis and prognosis of various tumors and cardiovascular diseases, have rapidly attracted widespread attention. A summary of the changes in several c-miRNAs in response to different kinds of exercise is described in the following and shown in Table 2.

Recently, there have been several studies on the effects of acute and chronic exercise training on c-miRNAs in the blood. A population of 4631 people who successfully completed the VO2max exercise test was selected for c-miRNAs screening. Participants were assigned to one of 2 groups: those with high VO2max (HV) and those with lower VO2max (LV). Each group has 12 participants, who ranged in age from 40–45 years. Among the 720 candidate c-miRNAs, miR-210 and miR-222 were found to be significantly higher in the the LV group than in the HV group. Results also showed that miR-21 was significantly higher in the male LV group compared with males in the HV group. At the same
time, it was found that miR-21 and miR-222 were significantly positively correlated with the Finnish Type 2 Diabetes Risk Score. Additionally, miR-21 and C-reactive protein and miR-210 and serum aspartate aminotransferase were also significantly positively correlated. MiRNAs microarray analyses were further conducted to screen for differences in c-miRNAs in populations with different cardiorespiratory levels, and researchers selected miR-21, miR-210, and miR-222 for follow-up validation studies. Interestingly, these 3 miRNAs have been initially studied in the physiological functions of the cardiovascular system.

In a study investigating the effects of 1-time marathon running on c-miRNAs, it was found that the changes in miRNAs that regulate muscle function (miR-1, miR-133a, miR-499, and miR-208a), miR-126, which regulates blood vessel growth, and miR-146a, which regulates inflammatory response, were significantly increased after marathon running and returned to pre-run levels at different speeds within 24 h in 21 male runners. Consistent with those findings, a study examining changes in c-miRNAs before and after running a marathon found that miR-1, miR-133a, miR-206, and miR-499, as well as miR-208, which is abundantly expressed in muscle, increased significantly among 14 male runners immediately after the run. Except for miR-499 and miR-208b, which recovered after 24 h, other miRNAs did not recover to a normal level. Similarily, both c-miR-133 and c-miR-126 levels significantly increased after running a marathon among a population of 50 to 60 year olds.

In a study of young healthy men, after 90 days of sustained exercise training it was found that miR-146 and miR-222 levels were significantly elevated after acute exercise but were not affected by long-term training. MiR-21 and miR-221 significantly increased after acute exercise before long-term training but were not sensitive to acute exercise after long-term training, suggesting that long-term exercise training reduces the sensitivity of miR-21 and miR-221 to acute exercise. MiR-20a increased significantly after long-term training but was not sensitive to acute exercise. MiR-133a, miR-210, and miR-328 did not significantly respond to long-term exercise training and acute exercise.

Circulating miRNA levels in response to acute exercise and 3 months of long-term basketball training were also detected in the basketball athletes. Among the participants, circulating miR-221 demonstrated different behaviors after acute exercise and long-term basketball training. The miR-221 level in serum decreased after acute exercise, whereas it increased after 3 months of basketball training. MiR-208b was not sensitive to acute exercise, but it decreased after long-term basketball training. Circulating miR-21, miR-146a, and miR-210 levels only decreased after acute exercise.

Table 2
Summary of the c-miRNAs in response to exercise.

| Source | Participant | Exercise type | c-miRNAs | Regulation | Reference |
|--------|-------------|---------------|----------|------------|-----------|
| Serum  | Matched on gender 40–45 years | Marathon running | miR-21, miR-210, miR-222 | Positively correlated with the FINDRISC | 60 |
| Plasma | Male 40–50 years | Marathon running | miR-1, miR-133a, miR-499, miR-208a | (immediately after running) | 56,64 |
| Plasma | Male 56.8 ± 5.2 years | Marathon running | miR-133, miR-126 | (immediately after running) | 65 |
| Plasma | Male 19.1 ± 0.6 years | Cycling | miR-21, miR-221, miR-146, miR-222 | (acute test before sustained training) | 37 |
| Plasma | Male 19.1 ± 0.6 years | Rowing | miR-20a | (90-day training) | 37 |
| Serum  | Male 21.5 ± 4.5 years | Cycling | miR-486 | (acute exercise) | 66 |
| Serum  | Male 25.90 ± 4.95 years | Basketball | miR-221, miR-21, miR-146a, miR-210, miR-208b, miR-221 | (3-month exercise) | 68 |

Abbreviations: ASAT = aspartate aminotransferase; c-miRNAs = circulating miRNAs; CRP = C-reactive protein; FINDRISC = Finnish Type 2 Diabetes Risk Score.
6. Conclusions and future perspectives

Increasing evidence has demonstrated that miRNAs are important for cardiac protection in response to exercise training. However, the regulation mechanism of miRNAs in exercise beneficial to the heart is still relatively obscure, and only a small number of studies have investigated the details about the relationship between exercise that is beneficial to the heart and miRNA regulation.

In addition to miRNAs, the role of long non-coding RNAs and circular RNAs in exercise-induced cardioprotective effects remains unknown. Further studies should be undertaken to explore the underlying mechanisms responsible for cardiac adaptations to exercise and the regulation of non-coding RNAs.

Interestingly, in addition to playing an important role in exercise that is beneficial to the heart, miRNAs are also involved in the development or progression of some cardiovascular diseases. This suggests that studying the potential role of miRNAs in response to exercise could lead to a new drug for the treatment of cardiovascular diseases or to the discovery of new therapeutic strategies. However, differences between the human species and mouse species should be considered before therapeutic strategies are transitioned from the laboratory bench to the medical clinic.

For c-miRNAs, muscle-specific miRNAs are being studied more intensely, whereas non-muscle–specific forms are being studied less frequently. The exercise-sensitive, non-muscle–specific circulating miRNAs responsible for tissue development and physiological functions are not well understood. It is important to explore these c-miRNAs and their functions before developing them as physical performance biomarkers.

Last but not least, there has been great interest in exploring c-miRNAs in blood as potential biomarkers. The c-miRNAs may be released into blood through their association with lipid vesicles (exosomes, microvesicles, apoptotic bodies) or proteins, and they further mediate cell-to-cell communication. Lipid vesicles derived from different cells/tissues exhibit different secretion characteristics when used as a carrier of miRNAs. At the same time, different miRNAs also have different carrier selectivity when secreted into blood. The c-miRNAs can be absorbed by distant tissue cells along with blood flow, thereby regulating the metabolism of other tissues and organs and playing a role in information regulation between cells. However, whether and to what extent c-miRNAs can be released from specific tissues and exert paracrine function remains to be investigated. It is important to learn more about the role of c-miRNAs in intercellular communication.

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Authors’ contributions

LW and JX performed the literature search and drafted the manuscript; YL and GL draw the figures; JX edited and revised the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

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