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# The Role of MicroRNAs in the Cardiac Response to Exercise

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Noncoding microRNAs (miRNAs) have emerged as central regulators of cardiac biology, modulating cardiac development and the response to pathological stress in disease. Although less well developed, emerging evidence suggests miRNAs are likely also important in the heart's response to the physiological stress of exercise. Given the well-recognized cardiovascular benefits of exercise, elucidating the contribution of miRNAs to this response has the potential not only to reveal novel aspects of cardiovascular biology but also to identify new targets for therapeutic intervention that may complement those discovered through studies of diseased hearts. Here, we first provide an overview of the cardiovascular effects of exercise as well as some of the major protein signaling mechanisms contributing to these effects. We then review the evidence that both cardiac and circulating miRNAs are dynamically regulated by exercise and regulate these mechanisms and phenotypes.

## THE GROWING BURDEN OF CARDIOVASCULAR DISEASE

It is ironic that, despite enormous progress in our understanding and ability to treat many cardiovascular diseases, the burden of these conditions in both human and financial terms is increasing and expected to continue to do so. There are likely three principle drivers of this disturbing trend. First, in some ways, it reflects our success at managing acute cardiovascular conditions such as myocardial infarction (MI) and hypertensive crises. As the mortality from these conditions has plummeted over the past half-century (Ford et al. 2007), the increasing number of survivors are at continued risk for late sequelae of their conditions, such as heart failure (HF) and arrhythmia, for which our therapies remain inadequate. Second, as populations age around the world (Christensen et al. 2009), the prevalence of age-related diseases, including cardiovascular disease, increases. Finally, the burgeoning epidemic of metabolic diseases such as obesity and diabetes, substantially increase the risk of most forms of cardiovascular disease, including atherosclerotic vascular disease as well as ischemic injury and HF (Bhupathiraju and Hu 2016). In the context of this growing unmet clinical need, investigation into the mechanisms responsible for the putative cardiovascular benefits of exercise may complement ongoing studies of the mechanisms of

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disease, potentially yielding new insights and targets for intervention.

## CLINICAL BENEFITS OF EXERCISE

The intuitively appealing concept that exercise protects against the development of cardiovascular disease has long been espoused. However, it is worth acknowledging that the large majority of evidence supporting this perspective is observational in nature and thus cannot establish a causal relationship (Wei et al. 2014). Such observational studies are inherently limited by multiple factors, including selection and recall biases, unrecognized confounding, and inaccurate self-reporting of activity. On the other hand, randomized controlled trials, the gold standard of clinical evidence, are also challenged in this context by imperfect adherence to lifestyle interventions, the daunting statistical requirements for adequate power in primary prevention, and the potential inability of patients with cardiovascular disease to exercise adequately in secondary prevention or treatment trials. Nevertheless, the large majority of observational studies show a reduction in cardiovascular disease among those who exercise habitually and, consistent with this, clinical interventional trials also suggest benefits accrued to those with existing cardiovascular disease. Studies of particular note include a recent meta-epidemiological analysis suggesting that the mortality benefit resulting from exercise in patients with coronary disease or early diabetes was comparable to that seen with approved medical therapies (Naci and Ioannidis 2013). One of the largest randomized controlled trials of exercise performed to date is the HF-ACTION trial, which showed a substantial improvement in HF patients randomized to a structured exercise program in self-reported health status and moderate improvements in several clinical endpoints, including a composite of all-cause mortality and hospitalization, which achieved statistical significance when adjusted for differences in baseline patient characteristics (O'Connor et al. 2009). Thus, although the clinical evidence is imperfect, the consistent message that emerges from observational, epidemiological, and interventional trials is that exercise has substantive benefits in the prevention and management of cardiovascular disease.

## RATIONALE FOR STUDYING EXERCISE

Although studies of heart disease have yielded important insights, the pathway to therapeutic translation has been challenging, in part because recognizing deleterious changes in disease does not reliably lead to corrective interventions. We know much less about what keeps the heart healthy, and thus delineating the mechanisms by which exercise leads to its cardiovascular benefits may provide a model of the "healthy heart" and in the process lead to novel strategies for preventing or treating heart disease. How likely is it that exercise-related pathways will also mitigate the response to pathological stress and/or established cardiovascular disease? Interestingly, the large majority of pathways identified thus far as functionally important in the cardiac response to exercise also protect the heart against pathological stress (Wei et al. 2014; Roh et al. 2016). As discussed below, examples include phosphoinositide-3-kinase (PI3K) (McMullen et al. 2003; Weeks et al. 2012), Akt1 (Matsui et al. 1999, 2001; DeBosch et al. 2006), endothelial nitric oxide synthase (eNOS) (Calvert et al. 2011), peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) (Arany et al. 2006, 2008), C/EBPβ (Bostrom et al. 2010), and CITED4 (Bezzerides et al. 2016). Thus, it appears that pathways functionally important in the heart's response to exercise are enriched for pathways with the potential to mitigate disease phenotypes. Given this observation, the practical question emerges as to which of these pathways are amenable to therapeutic manipulation. MicroRNAs (miRNAs) may have some advantages as therapeutic targets. Both animal and clinical studies have shown the feasibility of targeting miRNAs in vivo, with chemically modified locked nucleic acid (LNA) inhibitors (Elmén et al. 2008; Janssen et al. 2013) and, at least in some cases, agonists (Wang et al. 2013; Yan et al. 2014). Thus, identification of miRNA pathways functionally important in the cardiac exercise re-

sponse could in principle lay a foundation for new preventive or therapeutic approaches.

## CARDIAC PHENOTYPES INDUCED BY EXERCISE

As a first step toward explaining what is known about the role of miRNAs in the cardiac response to exercise, we will briefly describe the major cardiac phenotypes induced by exercise and outline the molecular pathways thought responsible. We hope this background will provide a helpful context for the miRNA effects described later, as well as potentially highlight areas where influences by miRNAs may yet be discovered.

### Cardiac Changes in Response to Exercise

In response to exercise training, the heart undergoes adaptive changes in both structure and function, resulting in what has been termed the "athlete's heart." These changes, which involve both the cardiomyocyte and noncardiomyocyte lineages of the heart, support the heart's ability to augment its output to perfuse exercising muscle and cope with the hemodynamic stress of exercise (Fig. 1). In humans, distinct patterns of cardiac adaptation have been described in response to different types of exercise from endurance to strength training (Baggish et al. 2008; Wasfy et al. 2015).

Most characteristics of the "athlete's heart" is cardiac hypertrophy. In response to exercise training, the left ventricular (LV) mass of the human heart can increase by $\geq$20% (DeMaria et al. 1978; Utomi et al. 2013). This effect, also a hallmark of animal models of exercise training, results primarily from cardiomyocyte hypertrophy (Ellison et al. 2012; McDiarmid et al. 2016). Cardiomyocytes can grow in length thus increasing LV volume (eccentric hypertrophy), in width thus increasing cardiac wall thickness (concentric hypertrophy), or a combination. Endurance exercise presents a volume challenge to the heart typically resulting in eccentric growth, whereas resistance exercise and the associated pressure overload characteristically causes concentric hypertrophy, although the concentric nature of growth in resistance-trained athletes has been challenged by a recent meta-analysis (Utomi et al. 2013; Wilson et al. 2016). In animal models, studies have been largely confined to endurance training and we focus on this because the mechanistic pathways have been delineated in this context.

Exercise-induced hypertrophy appears to be "physiological" and largely distinct from the "pathological hypertrophy," which occurs in disease states such as chronic hypertension or HF following MI. "Pathological hypertrophy" in these states is part of an adverse remodeling process, including cardiac fibrosis, electrical remodeling, and activation of a fetal gene program (Molkentin et al. 1998; Dirkx et al. 2013). In contrast, exercise-induced "physiological hypertrophy" does not share these features, has a characteristic gene expression profile, including increased $\alpha$-to-$\beta$ myosin heavy-chain ratio, increased PGC-1$\alpha$, and nonincreased atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), and is generally associated with better clinical outcomes except potentially in extreme cases in ultra-elite athletes (La Gerche and Prior 2007; Bostrom et al. 2010; O'Keefe et al. 2012; Eijsvogels et al. 2016).

To support this increase in cardiac tissue and the heart's metabolic needs (increased ventricular work during exercise can cause LV oxygen demand to increase by sixfold), coronary blood flow, and, to a lesser extent, oxygen extraction increase. Enhanced cardiac perfusion results from both structural changes to the microvasculature (increased arteriole densities and/or diameters and capillary angiogenesis) and altered function (increased endothelium-dependent vasodilation) (White et al. 1998; Hambrecht et al. 2000; Duncker and Bache 2008).

In addition to their growth in size, cardiomyocytes change their functional characteristics in response to exercise. Both cardiomyocyte contraction and relaxation respond to exercise training. Cardiomyocyte longitudinal contraction per cycle (fractional shortening) can increase 40%–50% with exercise training, whereas the rates of relaxation and contraction can increase by 20%–40%, with the degree of change correlating positively with the intensity of the

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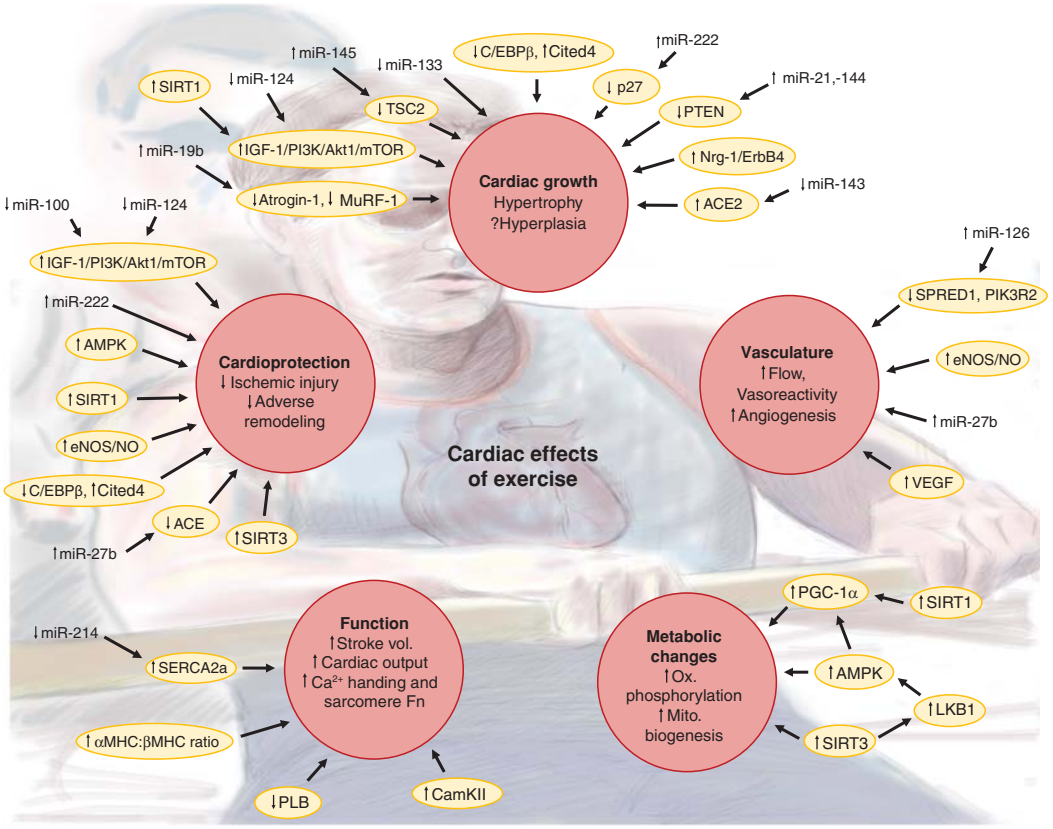


**Figure 1.** Cardiac phenotypes induced by exercise. Schematic illustrating the cardiac effects induced by chronic endurance exercise training. Red circles indicate major phenotypes or groups of phenotypes altered in response to exercise training. Yellow ovals indicate key proteins or pathways thought to contribute to the phenotype changes. Vertical arrows next to phenotypes, proteins/pathways, or microRNAs (miRNAs) indicate reported increases or decreases in response to exercise. For proteins/pathways, changes may be either in level or activity. Arrows connecting miRNAs, proteins/pathways, and phenotypes indicate regulation either demonstrated in animal models of exercise or inferred from in vitro experiments and/or in vivo experiments of related contexts. Stroke vol., Stroke volume; Sarcomere Fn, sarcomere function; Ox. phosphorylation, oxidative phosphorylation; Mito. biogenesis, mitochondrial biogenesis; ACE, angiotensin I converting enzyme; ACE2, angiotensin I converting enzyme 2; Akt1, AKT serine/threonine kinase 1; AMPK, AMP-activated kinase; CaMKII, calcium/calmodulin-dependent protein kinase II; C/EBPβ, CCAAT/enhancer binding protein β; Cited4, Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 4; eNOS, endothelial nitric oxide synthase; ErbB4, erb-b2 receptor tyrosine kinase 4; IGF-1, insulin-like growth factor 1; LKB1, serine/threonine liver kinase B1; MHC, myosin heavy chain; mTOR, mechanistic target of rapamycin; MuRF-1, muscle-specific RING finger protein 1; NO; nitric oxide; Nrg-1, neuregulin 1; p27, cyclin-dependent kinase inhibitor 1B (p27); PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; PI3K, phosphoinositide-3-kinase; PIK3R2, phosphoinositide-3-kinase regulatory subunit 2; PLB, phospholamban; PTEN, phosphatase and tensin homolog; SERCA2a, sarcoplasmic reticulum calcium ATPase 2 isoform a; SIRT1, sirtuin 1; SIRT3, sirtuin 3; SPRED1, sprouty-related EVH1 domain-containing 1; TSC2, tuberous sclerosis 2; VEGF, vascular endothelial growth factor.

exercise training (Kemi and Wisloff 2010). The maximal power output of individual cardiomyocytes is also increased (Diffee and Chung 2003). This contractile behavior of cardiomyocytes is regulated principally by $Ca^{2+}$ dynamics. Plasma membrane depolarization triggers initial cytoplasmic $Ca^{2+}$ entry through L-type calcium channels, in turn triggering $Ca^{2+}$ release from the sarcoplasmic reticulum (SR) through ryanodine receptors. The increased cytoplasmic $Ca^{2+}$ binds cardiac troponin C, producing a conformation change in the actin–tropomyosin–troponin complex, which increases actin–myosin cross-bridge formation, resulting in contraction of the myofilaments and thus the cardiomyocytes. Relaxation is subsequently mediated by reuptake of cytoplasmic $Ca^{2+}$ into the SR by the SR-$Ca^{2+}$ ATPase-2a (SERCA2a). Exercise enhances cardiomyocyte contractility by increasing sensitivity to $Ca^{2+}$ through several mechanisms, including activation of $Ca^{2+}$/calmodulin-dependent kinase II (CamKII) and isoform switching of troponins and myosin heavy chains, while promoting their relaxation by increasing SERCA2a activity through a combination of increased SERCA2a expression and phosphorylation of its inhibitor phospholamban (Wisloff et al. 2001; Kemi et al. 2007a). Exercise also mitigates the decreases in T-tubule (invaginations of the plasma membrane, which promote rapid and uniform depolarization and $Ca^{2+}$ entry) density and organization observed in HF, thus promoting excitation–contraction coupling (ECC) (Kemi et al. 2011). For a more detailed discussion of how exercise affects contractility, please see Kemi and Wisloff (2010).

Exercise not only improves the function of the healthy heart but also protects the heart and promotes recovery from injury. In animals, exercise increases resistance to and improves recovery from a wide range of cardiac insults, including MI, pressure overload, diabetic cardiomyopathy, and doxorubicin cardiotoxicity (Chicco et al. 2006; Andrews Portes et al. 2009; Stolen et al. 2009; Barboza et al. 2013). Multiple mechanisms likely contribute to the benefits of exercise in these contexts, including protection against oxidative stress, increased resistance to apoptosis, restoration of $Ca^{2+}$ handling and contractile function, restoration of cardiac energy metabolism, inhibition of fibrosis, and vascular improvements (Wisloff et al. 2002; Matsui et al. 2003; Kemi et al. 2007b; Leosco et al. 2008; Calvert et al. 2011; Vettor et al. 2014).

Recent work has also raised the possibility that exercise may promote cardiomyogenesis. The adult mammalian heart was traditionally considered a nonmitotic organ, with essentially no formation of new cardiomyocytes beyond the early postnatal period. However, multiple studies have now shown that new cardiomyocytes do form, albeit at a low rate, throughout life (Bergmann et al. 2009; Senyo et al. 2013; Ali et al. 2014). Cardiomyogenesis appears to increase after injury (Smart et al. 2011; Senyo et al. 2013) and possibly exercise. We found in mice that exercise caused increases in markers of proliferation and cell-cycle activity (PCNA expression, positivity for Ki67, pHH3 and aurora B kinase staining, and BrdU incorporation) in cardiomyocytes (Bostrom et al. 2010), whereas others have reported that exercise caused increased numbers and activation of putative cardiac progenitor and stem-cell populations (Waring et al. 2014; Xiao et al. 2014). The source of newly formed cardiomyocytes in adulthood, whether from division of preexisting cardiomyocytes or cardiac progenitor cells, remains an area of controversy.

## Major Pathways Implicated in the Cardiac Exercise Response

Several key pathways underlying the heart's response to exercise have been identified. Of these, perhaps the best established is the insulin-like growth factor 1 (IGF-1)/PI3K/Akt/mTOR signaling axis. Exercise induces increased IGF-1 production in the heart (as well as other tissues), leading to increased autocrine and paracrine signaling (Neri Serneri et al. 2001; Frystyk 2010), which stimulates physiological cardiac hypertrophy (Reiss et al. 1996; McMullen et al. 2004). Cardiac-specific IGF-1 receptor (IGF-1R) knockout mice show that IGF-1R signaling is necessary for exercise-induced hypertrophy (Kim et al. 2008). The PI3K family of enzymes appear to be the immediate downstream mediators of these signals: Genetic manipulations of

the 110α PI3K isoform show it is necessary and sufficient for exercise-induced physiological cardiac hypertrophy but not pathological hypertrophy (Shioi et al. 2000; McMullen et al. 2004; Weeks et al. 2012). Similarly, Akt1 appears to be the PI3K effector that is both necessary and sufficient for physiological hypertrophy while actually inhibiting pathological hypertrophy induced by pressure overload (DeBosch et al. 2006). Conversely, levels of the phosphatase and tensin homolog (PTEN), which inhibits PI3K/Akt signaling, decrease in the heart in response to exercise (Ma et al. 2013; Pons et al. 2013). We found that increased Akt1 expression in cardiomyocytes causes a decrease in expression of the transcription factor C/EBPβ, and mice heterozygous for C/EBPβ deletion recapitulate many of the cardiac phenotypes seen with exercise and are resistant to pressure-overload-induced cardiac dysfunction (Bostrom et al. 2010). C/EBPβ appears to act through repressing expression of the protein CITED4 (Bostrom et al. 2010), and mice with inducible cardiac-specific overexpression of CITED4 develop cardiac hypertrophy reminiscent of the "athlete's heart" as well as improved recovery from cardiac ischemia-reperfusion injury (IRI), effects that appear to be the result of increased mTORC1 activity (Bezzerides et al. 2016). Exercise also appears to increase mTORC1 activity by causing a decrease in expression of its inhibitor TSC2 (Ma et al. 2013) and by increasing phosphorylation of mTOR (Kemi et al. 2008). In addition to its effect on cardiac hypertrophy, Akt signaling also contributes to the resistance of cardiomyocytes to apoptosis on injury (Matsui et al. 1999, 2001).

Other exercise-activated pathways also contribute to increasing the resistance of the heart to injury. Neuregulin-1 (NRG-1), which is induced by exercise, binds to ErbB receptors on cardiomyocytes and has cardioprotective effects against IRI via a PI3K/Akt-dependent mechanism (Fang et al. 2010; Cai et al. 2016). NRG-1 has also been reported to stimulate cardiomyocyte proliferation and to induce angiogenesis. These effects have led to clinical trials evaluating the use of recombinant NRG-1 fragments for HF treatment (Bersell et al. 2009; Odiete et al. 2012). eNOS activity increases in response to exercise, as a result of both increased circulating catecholamines and increased phosphorylation by Akt and AMP-activated kinase (AMPK) (Zhang et al. 2009; Calvert et al. 2011) and appears important in protection against ischemic injury and oxidative stress. Exercise also induces expression of the deacetylases, Sirt1 and Sirt3, which regulate Akt, AMPK, and PGG-1α among other pathways and protect against cardiac IRI as well as oxidative stress, with Sirt3 also protecting against pathological cardiac hypertrophy and fibrosis (Ferrara et al. 2008; Sundaresan et al. 2009; Hsu et al. 2010; Pillai et al. 2010; Lai et al. 2014).

## miRNAs IN THE CARDIAC EXERCISE RESPONSE

miRNAs are small (~22 nucleotides) noncoding RNAs that modulate gene expression by RNA silencing and posttranscriptional repression (Bartel 2004). miRNAs play pivotal roles in cardiovascular development and disease (Zhao et al. 2005; van Rooij et al. 2006; Small and Olson 2011; Da Costa Martins and De Windt 2012; Wang and Yang 2012) and thus investigating their roles in exercise-induced cardiac phenotypes is logical. Multiple studies document miRNAs in the heart or circulation that are differentially regulated in response to exercise and, in some instances, functional contributions can be reasonably inferred from alterations in their known targets. However, efforts to assess the functional roles of cardiac or circulating miRNAs in exercise-induced cardiac phenotypes are relatively recent (Fernandes et al. 2011; Soci et al. 2011; Liu et al. 2015).

## miRNAs DYNAMICALLY REGULATED BY EXERCISE

### Cardiac miRNAs

Many miRNAs are altered in hearts after exercise or the manipulation of physiological hypertrophy pathways (Fig. 2A,B; Table 1). Care and colleagues found that miR-1 and -133 are down-regulated in rat hearts by treadmill train-

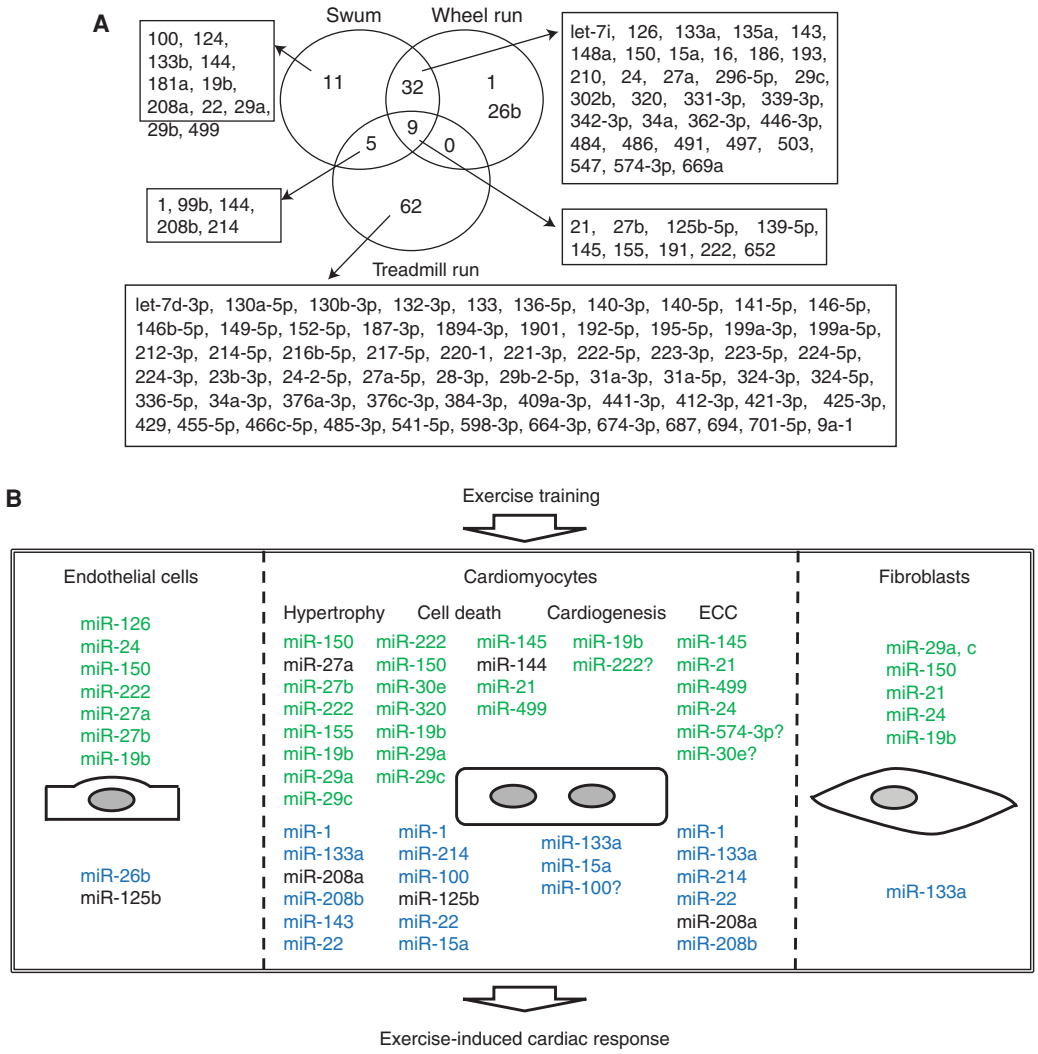


**Figure 2.** MicroRNAs (miRNAs) dynamically regulated in the heart by exercise training. (*A*) 120 miRNAs are clustered according to their reported expression altered by different exercise training in the heart. (*B*) miRNAs marked in green or blue have been reported to be up-regulated or down-regulated after exercise, respectively. The responses of miRNAs marked in black have been reported to vary depending on the health of the animals and/or the timing and exercise model studied. ECC, Excitation–contraction coupling.

ing as well as in transgenic mice with cardiac overexpression of Akt1 (Care et al. 2007), which as noted above is necessary and sufficient for physiological hypertrophy. Lin et al. profiled mouse hearts with genetic manipulation of PI3K(p110α) at baseline and after ischemic stress. They found that miR-222, -34a, and -210 were down-regulated in PI3K-induced physiological hypertrophy and up-regulated in dominant-negative PI3K transgenic hearts (Lin et al. 2010). Ma and colleagues studied miRNAs in rats subjected to 8 weeks of swim training. Cardiac expression of miR-21, -144, and -145 increased while miR-124 decreased (Ma et al. 2013). Correspondingly, in exercised hearts, PTEN (targeted by miR-21 and miR-144) and TSC2 (targeted by miR-145) were decreased while PI3K(110α) (targeted by miR-124) was

**Table 1.** miRNAs altered in exercise and their putative functional effects and targets

| miRNA | Putative targets | Functional effects | References (PMID) |
|---|---|---|---|
| miR-1 | PKCε, HSP60, BCL2, GATA4, MEF2A, NCX1 | Antihypertrophic, increased CM death, ECC | PMID: 19506341, PMID: 23226300, PMID: 21447748, PMID: 17468766, PMID: 26646371 |
| miR-100 | IGF-1R | Increased CM death | PMID: 25793527, PMID: 26191255 |
| miR-125b-5p | p53, Bak1, TRAF6 | Reduced CM death, profibrosis | PMID: 25863248, PMID: 26408546, PMID: 24576954 |
| miR-126 | Spred1, PIK3R2 | Increased angiogenesis | PMID: 22330028, PMID: 25863248 |
| miR-133a | RhoA, DAPK2, Cdc42, Nelf-A/WHSC2 | Antihypertrophic, ECC, antifibrotic, anticardiomyogenesis | PMID: 17468766, PMID: 21447748 |
| miR-143 | ACE2 | Prohypertrophic | PMID: 21709209, PMID: 24751578 |
| miR-144 | Rac1, PTEN | Inhibited CM death | PMID: 23812090, PMID: 26408546 |
| miR-145 | TSC2, Bnip3, CaMKIIδ | Inhibited CM death, ECC, promoting autophagy | PMID: 23812090, PMID: 26408546, PMID: 23028672, PMID: 23702479, PMID: 26432843 |
| miR-150 | SRF, Egr2, p2x7r, c-myb, | Inhibited CM death, ECC, antifibrosis, antihypertrophic | PMID: 24751578, PMID: 25863248, PMID: 25639779, PMID: 25824147, PMID: 27184887 |
| miR-155 | SOCS-1, Jarid2 | Increased CM death, antihypertophy | PMID: 25863248, PMID: 26408546, PMID: 24657879 |
| miR-15a | CHEK1 | Increased CM death, anticardiogenesis | PMID: 25863248, PMID: 22052914, PMID: 24043355 |
| miR-181a | MAPK1, TNF-α, GAT4 | Inflammation injury | PMID: 25793527 |
| miR-19b | Bim, TNF-α, PTEN, MuRF, atrogin-1, CTGF, TSP-1 | Prohypertrophy, inhibited CM death, cardiogenesis, antifibrosis, antiangiogenesis | PMID: 25793527, PMID: 26918829, PMID: 24117217, PMID: 21501375 |
| miR-208a | THARAP1, myostatin, GATA4, | Prohypertrophic, ECC | PMID: 27503950, PMID: 25793527, PMID: 19726871, PMID: 17379774 |
| miR-208b | THARAP1, myostatin, | Prohypertrophic, ECC | PMID: 27503950 |
| miR-21 | PTEN, PDCD4, FasL, Spry1, | Inhibited CM death, profibrosis, proangiogenesis | PMID: 23812090, PMID: 19043405, PMID: 19041309 |
| miR-214 | SERCA2a, Ncx1, BIM, CypD, CaMKIIδ, XBP1 | Inhibited CM death, ECC, antiangiogenesis | PMID: 22426211, PMID: 25822872, PMID: 26646371, |

*Continued*

**Table 1.** *Continued*

| miRNA | Putative targets | Functional effects | References (PMID) |
|---|---|---|---|
| | | | PMID: 26408546, PMID: 25656649 |
| miR-22 | CBP, CDK16, Sirt1, Sp1, PTEN, PURB, Hdac4 | Prohypertrophic, inhibiting CM death, ECC | PMID: 25793527, PMID: 22570371 |
| miR-222 | p27, HIPK1/2, Hmbox1 | Increased CM proliferation, prohypertrophy, inhibited CM death, antiangiogenesis | PMID: 25863248, PMID: 26408546 |
| miR-24 | Bim, BCL2L11, Bax, GATA2, PAK4, Furin | Inhibited CM death, ECC, antiangiogenesis, antifibrosis | PMID: 25863248, PMID: 26408546, PMID: 21383058, PMID: 21788589, PMID: 22260784 |
| miR-26b | GATA4, IGF | Antiangiogenic, increased CM death, antihypertrophic | PMID: 24751578 |
| miR-27a | ACE, SEMA6A | Prohypertrophic, proangiogenesis | PMID: 21709209, PMID: 24751578, PMID: 26408546, PMID: 25863248, PMID: 22184411 |
| miR-27b | ACE, SEMA6A | Prohypertrophic, proangiogenesis | PMID: 21709209, PMID: 26408546, PMID: 25863248, PMID: 22184411 |
| miR-29a | COL1A1, COL1A2, COL3A1, FBN1, ELN, Mcl-2 | Antifibrotic, increased CM death, prohypertrophy | PMID: 21447748, PMID: 24642957, PMID: 18723672, PMID: 17108080 |
| miR-29c | COL1A1, COL1A2, COL3A1, FBN1, ELN, Mcl-2 | Antifibrotic, increased CM death, prohypertrophy | PMID: 21447748, PMID: 24642957, PMID: 18723672, PMID: 17108080 |
| miR-30e | ?CaMKIIδ, ?Egfr1, ?Bcl2 | ?ECC, ?apoptosis | PMID: 25793527 |
| miR-320 | HSP20 | Increased CM death | PMID: 19380620, PMID: 25863248 |
| miR-499 | CaN, Drp1 | Inhibited CM death, ECC | PMID: 26387191, PMID: 21186368 |
| miR-574-3p | ATP2A2 | ECC | PMID: 25863248 |

MicroRNAs (miRNAs) implicated in the cardiac response to exercise. miRNAs reported as dynamically regulated by exercise and potentially contributing functionally to the phenotypes associated with exercise are listed, along with their putative targets and functional effects, as well as supporting citations.

CM, Cardiomyocyte; ECC, excitation–contraction coupling; PTEN, phosphatase and tensin homolog; IGF-1R, insulin-like growth factor receptor.



increased (Ma et al. 2013). These data suggest at least some of the miRNAs altered by exercise modulate PI3K/AKT/mTOR signaling, a key regulator of the cardiac response to exercise.

Soci and colleagues profiled 349 cardiac miRNAs after 10 weeks of exercise and found 87 differentially expressed compared with sedentary controls, including 48 up-regulated and 39 down-regulated miRNAs (Soci et al. 2011). miR-1, -133a, and -133b, all previously shown to be involved in pathological and stress-responsive cardiac hypertrophy, decreased after exercise

training (Care et al. 2007; Soci et al. 2011). Conversely, miR-29c was increased in exercised hearts compared with sedentary controls (Soci et al. 2011). In exercised rats, miR-27a and -27b were reported to increase while miR-143 decreased, together with reciprocal changes in expression of their angiotensin-converting enzyme targets, ACE and ACE2, respectively, suggesting specific miRNAs targeting renin–angiotensin system genes are dynamically modulated by exercise training and may contribute, at least in part, to the progression of cardiac hypertrophy (Fernandes et al. 2011). Using a microarray to assess >1000 murine miRNAs in exercise-induced LV hypertrophy, Martinelli et al. (2014) identified 35 miRNAs differentially expressed after 1 week of exercise relative to sedentary controls and 25 miRNAs differentially expressed after 5 weeks of training, documenting temporal regulation of cardiac miRNAs during exercise training. We profiled miRNAs in hearts of mice subjected to 3 weeks of either an intense swimming protocol or voluntary wheel running. We found 124 miRNAs that were differentially expressed in hearts from wheel-run mice and 55 differentially expressed in hearts from swum mice in comparison to sedentary controls (Liu et al. 2015). It is worth noting that forced swim training may cause a stress response not present in voluntary wheel running. Among these, 16 miRNAs, including miR-222, were independently validated as concordantly regulated in both models (Liu et al. 2015). In a recent study, Ramasamy et al. (2015) sequenced RNA from the hearts of rats swum for 8 weeks and sedentary controls and found that >80% of the 201 detected miRNAs were differentially regulated. Among the 128 miRNAs with read counts of >1000, 95 miRNAs were altered >1.5-fold with an adjusted false discovery rate (FDR) <0.1 (Ramasamy et al. 2015). miRNAs changed >2.5-fold were miR-208a, -19b, -133b, and -30e, which were all up-regulated, and miR-99b, -100, -191a, -22, and -181a, which were all down-regulated. Gene ontology and pathway mapping suggested that these miRNAs are associated with cardiac hypertrophy and apoptosis (Ramasamy et al. 2015).

Some studies have examined the effects of exercise on miRNA expression in the context of cardiac pathologies such as HF. Souza et al. (2015) examined cardiac miRNAs in controls and failing animals subjected to exercise training or sedentary. Only 11 miRNAs were specifically altered by exercise in the failing hearts, whereas 23 miRNAs were concordantly altered in both sedentary and exercised HF hearts (Souza et al. 2015). Of note, miR-222 was up-regulated in both HF groups (Souza et al. 2015). Other miRNAs, including miR-1, -29, -126, and -214, have also been reported to change in both healthy and diseased hearts after exercise (da Silva et al. 2012; Melo et al. 2014, 2015a,b; Zhao 2015). Given limited access to tissue, there are no clinical studies to date examining human cardiac miRNA alterations after exercise.

## Circulating miRNAs

Extracellular miRNAs, packaged in lipid microparticles such as exosomes, microvesicles, and apoptotic bodies or associated with protein complexes like RNA-binding proteins (argonaute 2, nucleophosmin 1) and high-density lipoproteins (HDLs), are widely present in body fluids such as plasma (Valadi et al. 2007; Wang et al. 2010; Arroyo et al. 2011; Turchinovich et al. 2011). Such circulating miRNAs have attracted interest as potential biomarkers for a variety of diseases (Mitchell et al. 2008; Akat et al. 2014; Galimberti et al. 2014). Recent studies have also suggested that circulating miRNAs may mediate intercellular communication (Valadi et al. 2007; Zernecke et al. 2009; Hergenreider et al. 2012). Baggish and colleagues examined the response of eight human plasma miRNAs to acute exhaustive exercise and sustained aerobic exercise training in healthy collegiate athletes. They found that plasma levels of miR-146a and -222 increased after acute exercise both before and after sustained training, whereas miR-21 and -221 were up-regulated only by acute exercise before sustained training, and miR-20a increased after sustained training but not acute exercise (Baggish et al. 2011). Strong correlations were seen between changes in both resting miR-20a and peak exercise levels of miR-146a and $V_{O2max}$ (Baggish et al. 2011). We found that serum miR-222 also increased in

HF patients after acute exercise (Liu et al. 2015). It is interesting to note that some of the miRNAs altered in human peripheral blood after exercise are altered in the hearts of animals after exercise training. Taken together, these data suggest a parallel that may reflect conservation of exercise-responsive miRNA pathways.

Among muscle-enriched miRNAs (miR-1, -133a, -133b, -206, -208b, and -486), only miR-486 was altered (decreased) in response to acute and chronic cycling exercise in young men (Aoi et al. 2013). Conversely, Gomes et al. (2014) found that circulating miR-1, -133, and -206 levels increased after a half-marathon. Mooren et al. (2014) also showed that plasma levels of miR-1, -133, -206, -499, and -206 increased after running a marathon. Intriguingly, alterations of miR-1, -133a, and -206 correlated not with cardiac injury markers but aerobic performance (Mooren et al. 2014). Sawada et al. (2013) reported that miR-149* increased, whereas miR-146a and -221 decreased in human serum 3 days after acute resistance exercise. Changes in circulating miRNAs levels appear to depend on the type of exercise, as Wardle et al. (2015) found that elite endurance athletes had higher plasma miR-222 levels than sedentary controls while elite strength athletes had lower levels, and Uhlemann et al. (2014) found that circulating miR-126 increased in response to endurance but not resistance training. Studies of alterations in plasma or serum miRNA in response to exercise have thus far been confined in humans. Although the observation that some circulating miRNAs altered in exercise coincide with miRNAs documented in animal models to be dynamically regulated or functionally important in the heart is encouraging, the tissue source(s) and role(s) of circulating miRNAs induced by exercise have yet to be defined. Moreover, their clinical value as biomarkers reflecting fitness or cardiometabolic risk remains to be established.

## FUNCTIONAL CONTRIBUTIONS OF miRNAs TO EXERCISE PHENOTYPES

### Cardiac Hypertrophy

As noted above, cardiac hypertrophy can be divided into pathological and physiological hypertrophy. miRNAs modulating pathological cardiac hypertrophy have been well studied and include both antagonists and agonists (Ucar et al. 2012; Yang et al. 2013; Bang et al. 2014; for detailed reviews, see Da Costa Martins and De Windt 2012; Wang and Yang 2012). In contrast, fewer studies have directly examined the functional role of miRNAs in physiological cardiac hypertrophy (Fernandes et al. 2011; Soci et al. 2011; Liu et al. 2015). However, multiple studies have identified cardiac miRNAs differentially regulated in exercise, which were known to regulate pathological hypertrophy, including miRNAs in Table 1 (Care et al. 2007; Fernandes et al. 2011; Soci et al. 2011; Ma et al. 2013; Martinelli et al. 2014; Liu et al. 2015). Thus, although the functional contributions of most have not been directly assessed in exercise-induced hypertrophy, these miRNAs may also modulate physiological hypertrophy. However, because the pathways mediating pathological and physiological hypertrophy are often distinct, the directionality of their influence may not be readily extrapolated from studies of pathological hypertrophy. For example, cardiac Akt1 is activated in both physiological and pathological hypertrophy models but appears necessary for the former and actually inhibitory of the latter (DeBosch et al. 2006), and thus plays a beneficial role in both settings. Similarly, our unpublished data suggest that miR-222, which is up-regulated in both physiological and pathological hypertrophy, is necessary for physiological growth but may inhibit pathological growth, again playing a positive role in both settings.

Care et al. (2007) found that cardiac miR-133 decreased in trained rats and in patients with heart disease. In vivo inhibition of miR-133 induced marked and sustained cardiac hypertrophy, suggesting that miR-133 acts as an inhibitor of cardiac hypertrophy (Care et al. 2007). Soci et al. (2011) also found decreased expression of cardiac miR-133a and miR-133b in rats after 10 weeks of swimming. In contrast, others found increased miR-133b in the hearts of rats after 8 weeks of swimming (Ramasamy et al. 2015). Whether this discrepancy is caused by differences in the exercise protocols or measurement methodologies is not clear.

We found that cardiac miR-222 was up-regulated both by swimming and voluntary wheel running in mice (Liu et al. 2015). miR-222 expression in neonatal cardiomyocytes in vitro produced cellular proliferation and hypertrophy with a gene expression pattern characteristic of physiological growth. In contrast, miR-222 inhibition reduced cardiomyocyte size and proliferation in neonatal cardiomyocytes (Liu et al. 2015). In vivo, neither miR-222 inhibition nor overexpression altered cardiac or cardiomyocyte size. However, miR-222 inhibition completely blocked the increase of cardiomyocyte and heart size induced by 3 weeks of intensive exercise as well as reduced the markers of cell proliferation induced by exercise (Liu et al. 2015). Mechanistically, HMBOX1, p27, HIPK1, and HIPK2 were implicated as direct targets contributing to miR-222's effects in cardiomyocytes (Liu et al. 2015). Taken together, these data suggest that miR-222 is necessary for exercise-induced cardiac growth. Exercise induction of cardiac miR-19b has also been reported by others to induce cardiomyocyte hypertrophy by targeting atrogin-1 and MuRF-1, as well as enhancing calcineurin/NFAT signaling (Song et al. 2014; Ramasamy et al. 2015).

## Cardiomyocyte Hyperplasia

Short-term physiological changes in heart size are mostly the result of corresponding changes in cardiomyocyte size. However, work from our group (Bostrom et al. 2010; Liu et al. 2015) and others (Waring et al. 2014; Xiao et al. 2014) has raised the possibility that exercise may also induce cardiomyocyte hyperplasia or cardiomyogenesis, perhaps in parallel to the neurogenesis well documented in animals after exercise (van Praag et al. 1999, 2005). miR-222 expression increased proliferation of neonatal cardiomyocytes in vitro and increased markers of proliferation in adult cardiomyocytes in vivo after ischemic injury (Liu et al. 2015). Conversely, an LNA anti-miR inhibitor specific to miR-222 blocked the increase in cardiomyocyte proliferation markers seen after exercise in control animals (Liu et al. 2015). These studies intriguingly raise the possibility that exercise is an inductive physiological cue regulating cardiomyogenesis in the adult heart, and that miR-222 may be part of the pathway regulating this response. Intriguingly, Li et al. (2016) recently reported that knockdown of endogenous miR-199 up-regulates its target PGC-1α and causes physiological cardiac and cardiomyocyte hypertrophy as well as an increase in markers of cardiomyocyte proliferation. However, it should be emphasized that the precise degree to which new cardiomyocytes are formed, survive, and contribute functionally to the benefits of exercise in animal models, let alone humans, remains unclear.

Although little is known about other miRNAs regulating cardiomyogenesis after exercise, other studies have suggested that miRNAs may regulate cardiomyogenesis in other settings. Eulalio et al. (2012) examined the ability of human miRNA mimics to induce proliferation of rat neonatal cardiomyocytes in vitro, and then examined the effects of the most promising candidates in adult rat cardiomyocytes in vitro and in a murine model of ischemic injury in vivo. Interestingly, they found that miR-590 and -199a promoted cell-cycle reentry of adult cardiomyocytes in vitro and markers of cardiomyocyte proliferation after ischemic injury in vivo (Eulalio et al. 2012). In addition, Chen et al. (2013) showed that the mir-17-92 cluster (specifically miR-19) is necessary and sufficient to induce markers of cardiomyocyte proliferation in adult hearts. Again, it is difficult to precisely document the degree of cardiomyogenesis and correlate this with functional benefits in vivo, leaving open the possibility that these benefits accrue from other functional effects, such as inhibition of cardiomyocyte death, which often appears to coincide with drivers of growth and proliferation. Nevertheless, it appears that identification of miRNAs that induce cardiomyocyte proliferation in vitro and/or in vivo may enrich miRNAs with beneficial effects on cardiac function and repair in vivo (Eulalio et al. 2012; Li et al. 2016).

## Excitation–Contraction Coupling

Exercise training decreases miR-1, -22, -133, -208, and -214 and increases miR-24, -145,

-499, and -574-3p in healthy hearts, although it appears to restore miR-1 and -214 and increase miR-145 in HF (Care et al. 2007; Cha et al. 2013; Liu et al. 2015; Melo et al. 2015a,b; Ramasamy et al. 2015; Souza et al. 2015; Zhao 2015). Intriguingly, these miRNAs have been implicated in regulating ECC, suggesting that miRNAs contribute to exercise-induced ECC remodeling. For example, Cha et al. (2013) showed that miR-145 suppressed reactive oxygen species (ROS)-induced $Ca^{2+}$ overload and related signaling by targeting CaMKIIδ, and thereby protected against ROS-induced cardiomyocyte apoptosis. Resistance training decreased the expression of miR-214 contributing to up-regulation of its target, SERCA2a, which enhances SR $Ca^{2+}$-uptake accelerating cardiomyocyte relaxation and loading the SR with $Ca^{2+}$, thereby improving peak $Ca^{2+}$ release and contractility (Melo et al. 2015a). Melo et al. (2015b) also found that swimming increased miR-1 (targeting NCX1) and decreased miR-214 (targeting NCX1 and SERCA2a) regulating $Ca^{2+}$ handling after MI.

## Cardioprotection from Ischemic Injury

Exercise protects against acute myocardial ischemic injury in animals and humans (Powers et al. 2002, 2008; Lennon et al. 2004; Calvert et al. 2011). Many of the miRNAs identified as altered in exercised hearts have documented roles regulating apoptosis or necroptosis, and therefore could provide mechanistic links between exercise and cardioprotection (Fig. 2B) (Cheng et al. 2009; Dong et al. 2009; Lin et al. 2010; Wang et al. 2011; Ma et al. 2013; Martinelli et al. 2014; Liu et al. 2015; Ramasamy et al. 2015; Zhao 2015; Qin et al. 2016). However, functional effects in cardiomyocytes in vitro or in vivo have only been investigated in a minority of cases and only rarely has cardioprotection against ischemic injury been investigated. Aurora and colleagues reported that miR-214 protects the mouse heart from ischemic injury by mitigating $Ca^{2+}$ overload and cell death (Aurora et al. 2012). Ramasamy et al. (2015) found that physiological hypertrophy increases cardiac miR-19b, which others have shown down-regulates proapoptotic BIM, thereby protecting cardiomyocytes against ER-stress-induced apoptosis (Song et al. 2014). Whether exercise-induced miR-19b contributes to acute cardioprotection in vivo has not been reported but seems probable. Other intriguing candidates include miR-100, which is down-regulated by exercise (Ramasamy et al. 2015), which in vitro can protect cardiomyocytes against $H_2O_2$-induced apoptosis (Chen et al. 2015).

However, not all miRNAs with antiapoptotic effects will have an acute cardioprotective effect after IRI in vivo. We found that miR-222 is necessary and sufficient to reduce cardiomyocyte apoptosis in vitro, and transgenic miR-222 overexpression in vivo reduces cardiomyocyte apoptosis late after IRI (Liu et al. 2015). However, the initial infarct size measured at 24 hours was not altered by miR-222 overexpression. Cardiac-specific overexpression of miR-222 in mice did improve cardiac function, in association with increased markers of cardiomyocyte proliferation as well as reduced cardiomyocyte apoptosis and fibrosis "late" after IRI. Thus, although miR-222 does not appear to contribute to the acute cardioprotective effects of exercise, it does substantially reduce late adverse remodeling after ischemic injury. Presumably the same may be true of other miRNA candidates with prosurvival effects in cardiomyocytes and thus the possible contribution of miRNAs to the acute cardioprotective effects of exercise remains unclear.

## Cardiac Fibrosis

Cardiac fibrosis occurs in many cardiac pathologies and is not generally associated with exercise. Cardiac fibrosis can have important clinical implications by increasing myocardial stiffness and diastolic dysfunction, as well as contributing to arrhythmia by interfering with homogeneous electrical propagation. Exercise appears to attenuate cardiac fibrosis (Wright et al. 2014; Ma et al. 2015) and multiple miRNAs may contribute in this context. van Rooij et al. (2006) showed that the miR-29 family (miR-29a, b, c) can act as negative regulators of car-

diac fibrosis after MI. In related studies, Soci et al. (2011) showed that increased miR-29a and c correlates with decreased COL1A1 and COL3A1 in the exercised heart, suggesting that miR-29 may regulate fibrosis in both physiological and pathological states. We also found that miR-29c was increased in two distinct exercise models (Liu et al. 2015). Consistently, Souza and Melo and colleagues found that the decrease in miR-29 (a and c) seen in failing rat hearts was attenuated by aerobic exercise training, resulting in decreased collagen expression (Melo et al. 2014; Souza et al. 2015) and suggesting that exercise-induced miR-29 could help mitigate fibrosis and thereby improve ventricular function. We found that overexpression of miR-222, which is also upregulated in two distinct exercise models, inhibited cardiac fibrosis by three- to fourfold after IRI (Liu et al. 2015). Similarly, multiple other miRNAs that have been reported to regulate cardiac fibrosis in disease states are altered by exercise (Fig. 2B), raising the possibility that they contribute to the antifibrotic effects of exercise.

### Angiogenesis

An increase in neovascular formation or angiogenesis is associated with endurance exercise. Using endothelial-cell-specific deletion of Dicer, Suarez et al. (2008) showed that endothelial miRNAs are essential for postnatal angiogenesis in many contexts. Although a Dicer role in exercise-induced cardiac angiogenesis was not directly examined, it seems likely. Endothelial miR-126 was shown to be involved in endothelial homeostasis and angiogenesis (Fish et al. 2008; Wang et al. 2008). Intriguingly, da Silva et al. (2012) reported that swim training increased expression of cardiac miR-126, which was positively correlated with the increase in LV capillary–fiber ratio. Taken together, these results suggest that exercise-induced miR-126 may contribute to angiogenesis in the heart. Conversely, exercise decreased expression of miR-26b (Martinelli et al. 2014), which has well-documented antiangiogenic effects (Icli et al. 2013).

## SUMMARY AND CONCLUSION

Growing evidence suggests that both circulating and cardiac miRNAs are dynamically regulated by exercise in animal models and humans. Although the functional effects of these miRNAs have been examined, several have documented roles in mediating the cardiac phenotypes associated with exercise. In other cases, functional contributions have been plausibly inferred from demonstrated effects in other settings. It appears that many miRNAs that are functionally important in the heart's response to exercise also mitigate its response to pathological stress and disease. These preliminary studies provide cause for optimism that ongoing investigations of these pathways may not only provide novel biological insights but could also lay a foundation for new therapeutic approaches to preventing or treating a range of cardiovascular diseases.

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