Chapter 6
Formation of New Cardiomyocytes in Exercise

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Abstract Heart failure is a life-threatening disorder associated with the loss of cardiomyocytes. The heart has some endogenous although limited regenerative capacity, thus enhancing cardiac regeneration or stimulating endogenous repair mechanism after cardiac injury is of great interest. The benefits of exercise in heart diseases have been recognized for centuries. Besides the promotion of a favorable cardiac function, exercise is also associated with new cardiomyocytes formation. Exercise may lead to cardiomyocytes renewal from pre-existing cardiomyocytes proliferation or cardiac stem/progenitor cells differentiation. A deep understanding of exercise-induced formation of new cardiomyocytes will enable us to develop novel therapeutics for heart diseases.

Keywords Exercise • Cardiomyocytes • Proliferation • Stem cells • Progenitor cells

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1 Introduction

Heart failure caused by ischemic cardiac diseases is a leading cause of death worldwide [1, 2]. After the onset of coronary artery occlusion, cardiomyocytes undergo apoptosis and necrosis [3]. Myocardial infarction can wipe out one billion myocytes in a few hours [4]. The cardiomyocyte loss during ischemia is also accompanied by severe inflammatory response and local fibroblast activation [5]. As adult mammalian heart has limited potential to regenerate, the self-repair mechanism in ischemic myocardium is largely associated with collagen-rich scar formation [6, 7], which may progressively lead to cardiac fibrosis and eventually develop into ventricular remodeling and heart failure [8, 9]. However, on the other hand, the heart is unable to compensate for cardiomyocyte loss occurring in myocardial ischemia and heart failure. Thus, enhancing cardiac endogenous regenerative capacity might offer novel strategies for heart failure treatment.

Exercise-induced cardiac growth has beneficial effects in the prevention and treatment of cardiac diseases [10–12]. Several studies have reported that exercise might lead to new cardiomyocytes formation by activating resident cardiac stem cells (CSCs) and progenitor cells (CPCs). Exercise has also been associated with enhanced endogenous regenerative capacity by promoting proliferation of preexisting cardiomyocytes. This chapter will summarize recent findings on exercise-induced formation of new cardiomyocytes and the molecular basis of new cardiomyocytes formation in exercise, which may provide novel therapeutics for heart diseases.

2 Limited Cardiac Regenerative Capacity

The heart has long been recognized as a postmitotic non-regenerating organ [13, 14]. Cardiomyocytes possess the proliferative capacity during fetal life but exit the cell cycle soon after birth in mammals [15]. It has been speculated that the changes to cardiomyocytes during this time period, including conversion of glycolysis to fatty acid metabolism, increase in cell size, and reduction of proliferative capacity, were an evolutionary advance [16–19]. Adult cardiomyocytes have very complex and well developed cytoskeleton, among which hundreds of sarcomeres are responsible for generating sufficient myocyte contractility in mammals [19]. Furthermore, adult mammalian cardiomyocytes are often multinucleated and polyploid, which might prevent mitosis division. Based on these concepts, the adult mammalian heart has long been considered as having no potential to regenerate and cardiomyocytes were only presumed to undergo hypertrophy, senescence, and death after myocardial infarction [20]. However, low rate of apoptosis exists in normal adult heart and is enhanced during ageing [21]. In this regard, cardiomyocyte renewal is speculated to be necessary to compensate for apoptosis-associated cardiomyocytes loss in order to balance the volume and function of heart.

To date, increasing evidence has confirmed that the adult mammalian heart had a certain degree of self-renewal [22–25]. Different strategies were used to measure cardiomyocyte turnover. The 4-OH-tamoxifen-induced labeling of pre-existing cardiomyocytes with green fluorescent protein (GFP) was utilized in double-transgenic MerCreMer-ZEG mice [26]. This genetic fate-mapping strategy showed that the percentage of GFP-positive cardiomyocytes remained unchanged during 1 year of normal ageing, while significantly declined after experimental myocardial infarction or pressure overload [26]. The "dilution" of GFP-positive cardiomyocytes indicates that stem or progenitor cells may refresh adult cardiomyocytes after injury [26]. However, scientists speculated that human may have different requirement for cardiomyocyte renewal due to their much longer life-span than rodents. Based on the high atmospheric level of carbon-14 generated by nuclear bomb tests during the Cold War, convincing evidence was provided for human cardiomyocyte renewal [27]. Through examination of the integration of carbon-14 into DNA of myocardial cells, investigators demonstrated that about 1% of cardiomyocytes were renewed annually at the age of 25, which gradually declined to 0.45% at the age of 75 [27]. Overall, nearly 50% of cardiomyocytes would be renewed during a normal human life span, though whether the new cardiomyocytes were derived from pre-existing cardiomyocytes or cardiac stem cells was unclear [27]. More recently, the multi-isotope imaging mass spectrometry (MIMS) was utilized to study cardiomyocyte turnover, which identified pre-existing cardiomyocytes as the dominant source of cardiomyocyte replacement during normal ageing [28].

3 Potential Cellular Sources of New Cardiomyocytes in the Adult Heart

The concept of very low rate of cardiomyocytes turnover in the adult mammalian heart has generated a broad focus on finding the potential cellular sources of new cardiomyocytes. Evidence has indicated that newly-formed cardiomyocytes may derive from CSCs/CPCs or pre-existing cardiomyocytes [28, 29] [30].

3.1 CSCs and CPCs

The activation and differentiation of stem cells and progenitor cells is essential to regulate tissue homeostasis in most human organs. CSCs, a group of undifferentiated cells which have the ability to self-renew, are originally characterized by cell surface marker c-kit [31]. In general, stem cells settle in niches which constitute the microenvironment to keep their undifferentiated state [32–34]. Once activated, CSCs divide symmetrically or asymmetrically to generate cells committed to new CSCs and differentiate into cardiac cell lineages [35]. Accompanying with further

investigation of CSCs, several additional and distinct CSC classes have been detected such as Sca-1 positive cells, Islet-1 positive cells, side population-Abcg2 positive cells, and progenitors generating cardiospheres [36–40]. The c-kit positive CSCs are multipotent to give rise to cardiac myocytes, smooth muscle cells, and endothelial cells [41]. However, the multipotentiality of Sca-1 positive or Islet-1 positive cells is an open issue to be addressed [42–45]. Compared with CSCs, CPCs are a group of immature but tissue-specific cells that can proliferate and develop into one of the main cardiac cell lineages (myocytes, vascular smooth cells, or endothelial cells) [46]. However, it is difficult to discriminate between CSCs and CPCs, as they may represent different developmental stages of the same cell population and specific markers for CSCs and CPCs are still lacking [47].

Several studies have reported the critical roles of CSCs and CPCs in the turnover of cardiac myocytes during normal life-span [48, 49]. The activation and differentiation of CSCs and CPCs to myocytes has also been shown in ischemic injury and pressure overload [50, 51]. However, other studies have indicated that CSCs and CPCs could not be effectively activated to promote endogenous tissue repair upon myocardial injury [52]. The benefits of CSCs and CPCs might also be due to a paracrine effect [43]. Thus, the relative contribution of resident stem cells to newly-formed cardiomyocytes during ageing or in response to ischemic injury are still debated. To develop novel strategies to enhance the stem cell-derived cardiac myocyte renewal will be of great interest.

3.2 *Pre-existing Mature Cardiomyocytes*

Although cardiac regeneration has been studied for a long time, little progress has been made in characterizing the mechanisms of mature cardiomyocyte proliferation. Cardiomyocytes undergo DNA synthesis and nuclear mitosis without cytokinesis, which makes a substantial proportion of cardiomyocytes binucleated and withdraw from the cell cycle [53, 54]. It has been proved that cardiomyocyte DNA synthesis activity and cell cycle activity were markedly decreased after birth, however, postnatal proliferation of cardiomyocytes does exist and has been documented in humans and rodents [55].

Investigators have used different methods to determine cardiomyocytes turnover. The 3H–thymidine, a material involved in DNA synthesis, was injected to MHC-nLAC mice to mark the newly generated myocytes, showing a very low rate of myocytes turnover less than 1% per year [25, 56]. The use of genetic fate-mapping with stable isotope labeling and multi-isotope imaging mass spectrometry (MIMS) demonstrated that the origin of newly-formed myocytes mainly derived from division of pre-existing cardiomyocytes both in normal mammalian myocardial homeostasis and after myocardial injury [28]. The turnover rate of cardiomyocytes is approximately 1% per year in adult mice. As the ^{15}N tagging cardiomyocytes were predominantly GFP positive, the cellular origins of new cardiomyocytes were associated with proliferation of pre-existing myocytes instead of cardiac progenitor cells

[28]. Furthermore, the low level of cardiomyocytes proliferation under normal circumstances could be increased in the border zone after myocardial injury [28]. Based on the "mosaic analysis with double markers" mouse model, it was also proved that the differentiated α-myosin heavy chain (α-MHC) expressing cardiomyocytes were the cellular source of postnatal cardiomyogenesis, though cardiomyocyte division is very limited during ageing and even after ischemic cardiac injury [57]. Indeed, a deep understanding of the mechanisms limiting adult cardiomyocyte proliferation may raise the hope of promoting new cardiomyocyte formation after myocardial injury.

4 Exercise Activates Resident Cardiac Stem Cells

Under physiological myocardial ageing, cardiomyocytes undergo telomerase shortening and apoptosis. CSCs and CPCs were supposed, to some extent, to be activated and differentiated to replace the dying cardiomyocytes, and thus maintain the myocardial homeostasis and cardiac function [58]. Equally important, a promotion of endogenous stem cell activation has been proved to protect the heart from cardiac remodeling and dysfunction after myocardial injury [50]. Increasing evidence has shown that exercise was an efficient physiological stimulus to activate and mobilize different types of stem cells, such as cardiac stem cells, skeletal muscle satellite cells, and endothelial progenitor cells [59].

The cardiomyocytes adaptations to exercise result in cardiac growth through both cardiomyocytes hypertrophy and hyperplasia, the former refers to the increase in cell size and the latter refers to the increase in cell number [60]. The potential roles of c-kit positive CSCs were the first being identified in exercise-induced cardiac growth [61]. It was demonstrated that the number of c-kit positive CSCs was significantly increased after intensity-controlled exercise in rats [61]. Interestingly, approximately 80% of the c-kit positive CSCs were either Nkx2.5 positive or Ets-1 positive, indicating that these CSCs were already committed to myocyte or endothelial cell lineage, which probably contributed to the balance between myogenesis and angiogenesis [61]. Exercise also increased the myocardial expression levels of growth factors, such as insulin-like growth factor (IGF-1), transforming growth factor-β1 (TGF-β1), bone morphogenetic protein-10 (BMP-10), neuregulin-1 (NRG-1), and periostin (POSTN), among which IGF-1 and NRG-1 promoted CSC proliferation while BMP-10 and TGF-β1 stimulated CSC differentiation [61]. In addition to c-kit positive stem cells, the Sca-1 positive progenitor cells were also found to be increased in the left ventricle and outflow tract of mice swimming for 3 weeks, accompanied with an upregulation of IGF-1 and hepatocyte growth factor (HGF) [62].

Base on the studies above, exercise-induced activation of resident CSCs and CPCs is presumed to be a physiologic repair or compensation mechanism involved in the cardioprotective response to exercise. However, the mechanisms of stem cell activation and their relative contribution to cardiomyogenesis after cardiac injury need further investigation.

5 Exercise Induces Proliferation of Pre-existing Cardiomyocytes

Increasing data have shown that endurance exercise can induce a proliferative response of adult cardiomyocytes, which is associated with cardioprotective effects. The limited proliferative capacity of cardiomyocytes was proved to be enhanced with endurance swimming [63]. Exercise leads to a reduction in C/EBPβ expression and an increase in CITED4 expression, which is sufficient to promote both hypertrophy and proliferation of primary neonatal rat cardiomyocytes *in vitro* [63] [64]. C/EBPβ knockdown mice develop physiological cardiac hypertrophy and cardiomyocyte proliferation, and are also resistant to pressure overload [63]. However, forced cardiac expression of CITED4 produces physiological cardiac hypertrophy without increasing cardiomyocyte proliferation in adult hearts [64].

The role of microRNAs (miRNAs, miRs), a large group of small non-coding RNAs, in exercise-induced cardiac growth has been extensively studied, and some of them were documented to contribute to exercise-induced cardiomyocyte proliferation. Based on microarrays and qRT-PCRs, miR-222 is found to be significantly upregulated in the heart after swimming and voluntary wheel-running exercise [65]. Importantly, miR-222 promotes both hypertrophy and proliferation of neonatal rat cardiomyocytes *in vitro*, and is necessary for exercise-induced cardiomyocyte hypertrophy and proliferation in adult mice *in vivo* [65]. Additionally, miR-17-3p, a member of miR-17-92 cluster, is identified as a critical regulator of exercise-induced cardiac growth. miR-17-3p contributes to cardiomyocyte hypertrophy and proliferation [66]. Interestingly, overexpression of miR-222 and miR-17-3p are both able to protect the heart from cardiac remodeling and heart failure after ischemia-reperfusion injury [65, 66].

6 Potential Role of Exercise-Induced Cardiomyocyte Renewal in Treating Cardiac Diseases

Exercise-induced cardiac growth is a physiological adaptive response associated with myocyte hypertrophy and renewal and angiogenesis as well [67–69]. Clinical studies have proved the cardioprotective effects of exercise, which is now becoming an effective non-invasive adjuvant therapy for many cardiac diseases [70–72]. Exercise not only reduces cardiac risk factors [73–75], but also significantly reduces cardiovascular events [76, 77]. A study recruiting more than 1000 patients has documented that the more participants exercise, the less they will suffer cardiovascular death [78]. Experts recommend that regular physical activity to patients with heart failure is associated with better functional capacity, lower hospital admissions, and reduced all-cause mortality [79]. Although the cardiovascular benefits of exercise have been well established [80], the relative contribution of exercise-induced cardiomyocyte renewal in it is largely unclear.

After myocardial infarction or pressure overload, a large number of cardiomyocytes undergo apoptosis and necrosis, leading to progressive cardiac remodeling and eventual heart failure. Exercise-induced downregulation of C/EBPβ and subsequent upregulation of CITED4 induces neonatal rat cardiomyocyte proliferation *in vitro* [63]. Interestingly, knockdown of C/EBPβ induces physiological cardiac hypertrophy as well as cardiomyocyte proliferation, and also protects against pathological cardiac remodeling after pressure overload *in vivo* [63]. Besides that, forced expression of miR-222 or miR-17-3p, although not sufficient to recapitulate exercise-induced cardiac growth, has been found to promote neonatal rat cardiomyocyte proliferation *in vitro* and prevent cardiac remodeling and dysfunction after cardiac ischemia-reperfusion injury *in vivo* [65, 66]. These studies suggest that exercise-induced physiological cardiac growth and the contributors may provide novel therapeutic targets for cardiac diseases. However, direct evidence is still lacking for the contribution of exercise-induced cardiomyocyte renewal to cardiac regeneration and repair.

Recently, the intraperitoneal injection of 5-Fluorouracil (5-FU) is performed in mice subjected to swimming exercise and ischemia-reperfusion injury to investigate the role of cardiomyocyte proliferation in exercise-induced cardiac growth and exercise-associated protection against ischemia-reperfusion injury [81]. 5-FU is used to attenuate cell proliferation. Interestingly, although 5-FU significantly reduces exercise-induced cardiomyocyte proliferation, cardiomyocyte hypertrophy still develops, indicating that cardiac cell proliferation is not required for exercise induced cardiac physiological hypertrophy. However, the protective effect of exercise against cardiac ischemia-reperfusion injury is totally abolished with 5-FU, suggesting that cardiac cell proliferation is required for the benefits of exercise [81]. Noteworthy, as 5-FU is not specific to inhibit cardiomyocyte proliferation, the loss of benefits of exercise might also be associated with other cell types, such as resident stem and progenitor cells, endothelial cells, and circulating endothelial progenitor cells [81]. It is highly needed to block cardiomyocytes proliferation specifically to investigate the role of cardiomyocytes proliferation in exercise induced cardiac growth and cardiac protective effects.

7 Challenges in Studying Exercise-Induced Cardiomyocytes Renewal

For decades, the dogma was that cardiomyocytes were terminally differentiated cells and the adult mammalian heart was a non-regenerative organ. The capacity of cardiomyocyte renewal in adult heart has not been assessed until recently. With the development of methodology, the notion of cardiomyocytes renewal has been generally accepted by the public. Two main cellular sources for newly formed cardiomyocytes have been recognized including CSCs/CPCs and pre-existing cardiomyocytes [82]. However, the slow self-renewal rate is unable to replace the huge loss of cardiomyocytes after myocardial injury [83]. CSCs and CPCs based

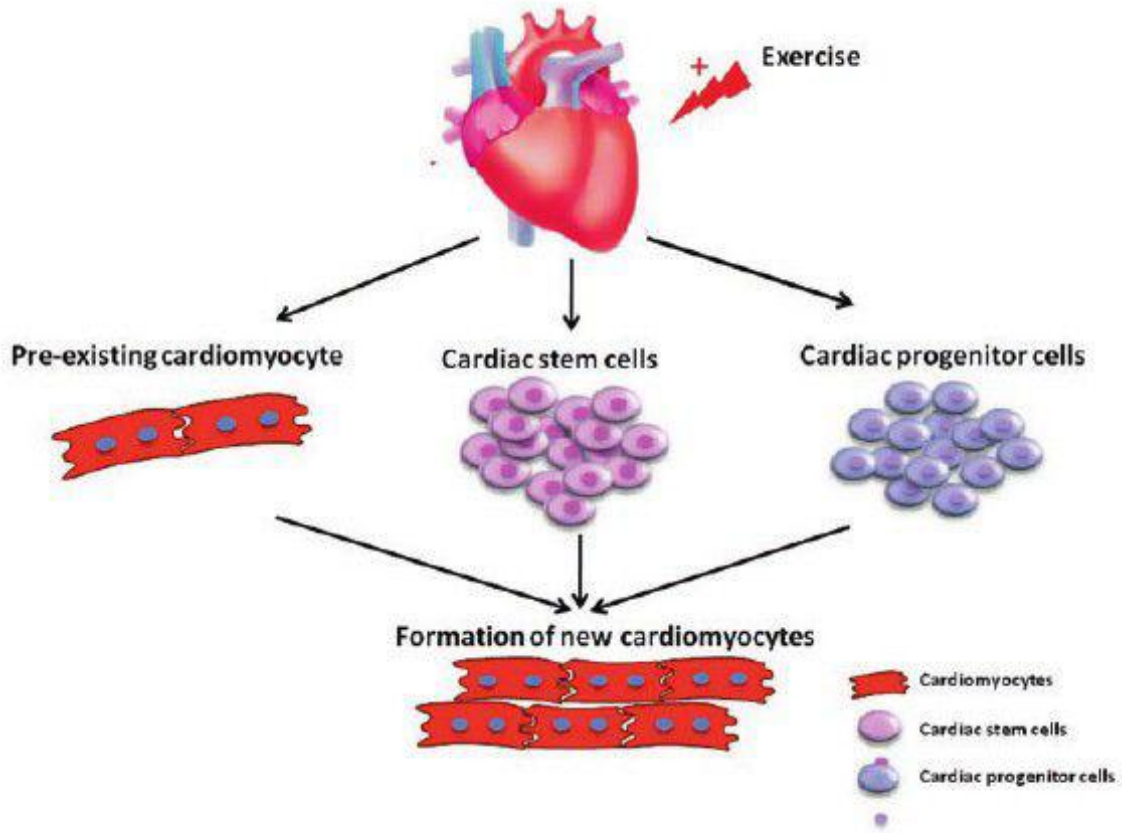

Fig. 6.1 Exercise induces new cardiomyocytes formation through activating cardiac resident stem/progenitor cells or increasing pre-existing cardiomyocytes proliferation

strategies have been largely investigated in the treatment of myocardial ischemia and other cardiac diseases. However, low survival and less attachment of these stem cells after injected into the body may greatly influence the effectiveness of stem cell therapy. Therefore, stimulating endogenous cardiomyocytes proliferation might be an alternative strategy.

Exercise has multiple systemic beneficial effects, including the heart. Recently, exercise has also been demonstrated to promote myocardium self-renewal through activating resident stem and progenitor cells and increasing pre-existing cardiomyocytes proliferation (Fig. 6.1). Although the relative contribution of exercise-induced cardiomyocytes renewal to cardiac repair after myocardial ischemic injury is far from clear, some evidence has been provided that cardiac cell proliferation is necessary for mediating the beneficial effect of exercise against ischemia-reperfusion injury [81].

Finally, the use of exercise as a therapeutic strategy to stimulate endogenous myocardial regeneration may be influenced by multiple variation factors, including patient population, exercise intensity, type, and duration [84]. In such conditions, experts need to define the patient population that benefits mostly from physical therapy, elaborate a personalized exercise program, and establish an effective evaluation method. Importantly, this network will provide the basis for exercise as a useful tool to promote cardiomyocytes proliferation and repair in patients.

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