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Endogenous Cardiac Stem Cell Therapy for Ischemic Heart Failure

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Abstract

Cardiovascular disease remains the number one cause of morbidity and mortality in the world. With great advances in medical and interventional therapies, patients who suffer from acute myocardial infarction have a longer life expectancy than before, but gradually develop chronic heart failure in their later life due to irreversible loss of cardiomyocytes. So far, heart transplantation is the only therapeutic option for advanced heart failure. However, the shortage of donor organs largely limits its role as the gold standard therapy. In the past decades, stem cell-based regenerative medicine has been proposed as a promising approach for the treatment of heart failure based on numerous animal studies. A variety of potential stem cell types, including skeletal myoblasts and bone marrow-derived stem cells, have been investigated in clinical trials for cardiac repair and regeneration, but have shown mixed results in heart functional improvement or life-threatening disadvantages such as ventricular arrhythmia. On the other hand, due to the advantages of autologous origin and cardiac-committed lineage, cardiac stem cell therapy has emerged as a promising cell-based strategy for treatment of HF. Thus, this review discusses the current therapies for heart failure and further focuses on stem cell therapy using different endogenous cardiac stem cells, purified by stem cell surface markers (e.g., c-kit or Sca-1) or derived from explants via the formation of cardiospheres. In addition, the potential effect of patient age on cell-based therapy for heart disease is discussed.

Keywords: Heart failure; Cardiac stem cell; Cardiosphere-derived cells; Cell transplantation

Introduction

Cardiovascular disease is a leading cause of death worldwide and becomes increasingly prevalent in the elderly population [1]. Irrespective of the aetiology, most cardiovascular diseases eventually lead to heart failure (HF), which is progressive and irreversible. Clinically, therapeutic options available for patients in severe HF are limited. Thus, there is an urgent need for the development of a novel approach to treatment of advanced HF.

In the last decade, a variety of stem cell types, including skeletal myoblasts, bone marrow-derived stem cells (e.g., bone marrow mononuclear cells), circulating progenitor cells and mesenchymal stem cells, have been utilized in the treatment of patients with AMI or chronic ischemic cardiomyopathy in clinical trials [2-5]. Unfortunately, previous clinical studies have presented mixed results and none of these cell types has been confirmed as the best candidate for cardiovascular disease therapy in clinical trials conducted to date [3-6].

With increasing evidence, endogenous cardiac stem cells (CSCs) represent an attractive and promising cell candidate for cardiac repair and regeneration due to their autologous origin, cardiac-committed fate, and ability to develop into three major myocardial lineages [7]. Hence, CSC therapy has emerged as a promising cell-based strategy for treatment of HF. Over the past few years, transplantation of CSCs has been shown to modulate the remodeling process, regenerate the damaged myocardium and improve heart function in animal models of myocardial infarction [8]. Recently, two phase I clinical studies, SCIPIO and CADUCEUS, using c-kit+ CSCs and cardiosphere-derived cells (CDCs), respectively, confirmed early short-term safety and therapeutic efficacy (improvement in EF, reduced infarct size or increased viable myocardium) in patients with ischemic heart failure [9,10]. Although great advances have been seen in this field, many relevant questions remain unanswered such as the optimal cell dosage for treatment, best timing for cell transplantation and effect of patient age on cell-based therapy. In this review, we focus on current therapies of heart failure and recent research into resident cardiac stem cell subpopulations, including the relationship between patient age and regenerative capability of endogenous cells, thereby providing further insights into cardiac stem cell-based therapy as a potential strategy for heart failure treatment.

Heart Failure

According to the World Health Organization (WHO), cardiovascular diseases (CVDs) are the major cause of death globally, leading to an estimated 17.3 million deaths in 2008 [1]. Furthermore, despite modern advances in therapy and management, the number of annual deaths due to CVDs worldwide continues to increase; by 2030, it is expected that nearly 23.6 million people will die from heart diseases including HF [11,12].

The majority of cardiovascular disease is composed of cardiac diseases which can be broadly divided into either ischemic (e.g., coronary artery disease and myocardial infarction) or non-ischemic heart disease (e.g., valvular heart disease and hereditary cardiomyopathy). Regardless of the underlying cause, however, HF is the final common stage of many diseases associated with the heart [13]. Based on recent statistics, more than 900,000 people are living with HF in the UK, which represents about 5% of medical hospitalizations [14]. Approximately 5.8 million people are affected with HF in the USA and over 23 million worldwide [15]. Under medical treatment, 20-30% of HF patients die in the first year of diagnosis and 45-60% after 5 years,

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respectively [11,16]. Thus, HF has become a major public health issue in terms of high mortality rate and enormous healthcare expenditure [17].

Current Therapies for Heart Failure

Medical therapy

Standard pharmacological agents for HF include diuretics, angiotensin-converting-enzyme inhibitors (ACEIs), beta-blockers, angiotensin-receptor blockers (ARBs), and aldosterone antagonists [18]. The actions of these drugs are mainly through the modification of ventricular remodeling and the systemic responses (i.e., sympathetic and renin-angiotensin-aldosterone systems) [18]. Diuretics are useful in the management of fluid retention to relieve symptoms such as dyspnea [19,20]. As the first-line therapy, ACEIs have been shown to improve symptoms, reduce ventricular size and increase the ejection fraction (EF) modestly [19,20]. Like ACEIs, beta-blockers are also in the list of first-line drugs in patients with HF, which can increase the EF and relieve symptoms if tolerated. ARBs are similar in action to ACEIs in patients with chronic HF [21,22]. The use of the aldosterone antagonist (spironolactone) showed further reduction in symptoms, hospitalization and mortality in severe HF patients receiving a diuretic, an ACEI and a beta-blocker [23]. Other drugs, such as hydralazine/isosorbide dinitrate and digoxin, can be prescribed to ameliorate symptoms and improve quality of life depending on patients' needs [18].

Interventional therapy

Interventional therapy, which is less invasive than surgery, includes percutaneous transluminal coronary angioplasty (PTCA), implantable cardioverter-defibrillator (ICD) and biventricular cardiac pacing (cardiac resynchronization therapy; CRT), which could benefit patients with HF under certain circumstances. Elective PTCA improves symptoms and heart function in patients with ischemic HF and viable myocardium by coronary revascularization with or without the use of stents. ICDs were shown to reduce mortality in HF patients with a high risk of sudden cardiac death in a systemic review of randomized controlled trials [24]. Moreover, as many as one third of patients with severe HF develop intra-ventricular conduction delays, which are associated with dyssynchronized contraction of the left ventricle, resulting in inefficient pumping work [16-18]. Based on clinical randomized trials, CRT was shown to reduce symptoms, improve heart function and increase survival rate in selected patients, when added to optimal medical therapy [25-28]. Importantly, CRT did not lead to a reduction in mortality rate in patients with a relatively low risk of death [18]. As a result, the use of CRT is recommended in subjects with severe HF, an EF less than 35%, sinus rhythm and a wide QRS complex (>120 msec) [19,20].

Surgical therapy

Coronary artery bypass graft (CABG) is an effective treatment in patients with chronic ischemic cardiomyopathy, still suffering from angina or reversible myocardial ischemia, and leads to better outcomes than medical therapy [29]. The gold standard therapy for end-stage HF remains heart transplantation, which improves patients' symptoms (95% symptom-free rate) and extends their life span with about 90% 1-year survival and 60% 10-year survival [30,31]. Nevertheless, patients receiving heart transplantation require lifelong immunosuppression and face the possibilities of severe post-operative complications, such as primary graft failure (PGF), and transplant vasculopathy [32]. Furthermore, only 5,000 heart transplants are carried out annually in more than 300 countries and, unfortunately, 10% of terminal HF patients on the waiting list for transplantation die every year because of limited organ supply and long waiting times [33,34]. Its impact is therefore epidemiologically trivial in light of a global population in need [35]. Mechanical circulatory support (MCS) with the left ventricular assist device (LVAD) has been used as bridge-to-transplant (BTT) or bridge-to-recovery (BTR) therapies over the past decade [31]. In addition, because of its efficacy in BTT and BTR and with the development of newer LVADs with continuous-flow pumps, the long-term use for end-stage HF patients who are ineligible for transplantation (i.e., destination therapy; DT) is becoming more prevalent [36-38]. LVAD resulted in 1-year survival of nearly 80% and improvements in symptoms and quality of life in patients with advanced or end-stage HF [39]. However, the use of LVAD still causes around 5-10% peri-operative mortality and is linked to frequent short- and long-term complications such as infection, bleeding and device failure [39].

Limitations of current therapy for heart failure

Pathophysiologically, HF is characterized by an irreversible loss of cardiac myocytes and residual fibrotic scar tissue, which results in progressive deterioration of cardiac function [2]. Over the past decades, great advances in pharmaceuticals, device technology and surgery have alleviated symptoms, improved quality of life and reduced mortality in patients with cardiovascular disease [18]. However, apart from transplantation, the treatments available to date are unable to reverse the state of HF or prevent the progression to end-stage HF as the lost functional myocardium is not replaced by these approaches. Unfortunately, furthermore, the number of available donor organs [37] significantly restricts the definitive therapy - cardiac transplantation -. Accordingly, it is hoped to develop a novel therapeutic method that can efficiently repair and regenerate the damaged myocardium, eventually structurally and functionally restoring the heart. Recently, cell-based cardiac repair and regeneration with cell transplantation has emerged as a promising strategy that aims to replace cardiomyocyte loss after myocardial injury.

Endogenous cardiac stem cells

Traditionally, the heart has been considered a terminally differentiated, post-mitotic organ without the capability of regenerating itself. However, this view has recently been questioned by the discovery of resident cardiac stem/progenitor cells in the heart of several species including mouse [40,41], rat [42,43], dog [44], pig [45,46] and human [47,48]. Furthermore, a study by Hsieh et al. using a genetic fate-mapping approach, demonstrated that CSCs replenished adult mammalian cardiomyocytes lost after injury due to myocardial infarction or pressure overload [49]. These different subpopulations of resident cardiac stem cells have been identified and classified based on their properties and various surface markers such as c-kit and Sca-1.

Types of Cardiac Stem Cells

Cardiac c-kit+ stem cells

C-kit (CD117), the tyrosine kinase receptor for the stem cell factor, was initially reported to be expressed on the surface of hematopoietic stem cells [50]. In 2003, Beltrami and colleagues described for the first time the discovery of a subpopulation of Lin- and c-kit+ CSCs in the rat heart, which are clonogenic, multipotent and capable of self-renewal [42]. Some cells of this population were found to co-express cardiac specific transcription factors such as Gata4, Gata5, Nkx2.5 and MEF2C, suggesting that they were at the early stage of differentiation committed to myocardial lineages. These c-kit+ cells showed remarkable potential

to differentiate into all cardiac lineages and regenerated the damaged myocardium in a rat model of MI [42]. Subsequently, Bearzi et al. developed methods for isolation and expansion of c-kit+ human CSCs (hCSCs) from small myocardial specimens. When injected into immunocompromised rats and mice, these cells differentiated into cardiomyocytes and improved the LV performance of infarcted hearts [51]. Some studies have shown that c-kit+ cells are triggered and regenerate new cardiomyocytes in response to pathological lesions [52,53]. In addition, endogenous c-kit+ cells can be activated to promote myocardial repair through the mediation of insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF) [46,54]. Endogenous Lin- c-kit+ cells are very rare within the myocardium (on average, 1 in every 104 myocytes), which makes it imperative to isolate and expand c-kit+ cells for a certain period to generate clinically relevant numbers [42]. Nevertheless, it was reported that c-kit+ CSCs can be expanded through growth in culture beyond the population doubling limit of somatic cells (> 40) and long-term *in vitro* culture up-regulated Gata4 expression, resulting in enhanced cardiomyogenic differentiation [55]. Moreover, intracoronary transplantation of c-kit+ CSCs has been shown to reverse adverse remodeling, improve heart function (EF) and stimulate endogenous cardiac stem cells in infarcted rat hearts [56].

Because of the inspiring evidence in pre-clinical animal studies, the first phase I human clinical trial using endogenous c-kit+ stem cells in patients with ischemic heart disease has been initiated. The SCIPIO trial by Bolli et al. was designed to examine the safety and efficacy of intracoronary delivery of autologous CSCs, comprising expanded c-kit-expressing cells from right atrial appendages, in patients with ischemic cardiomyopathy. The initial results, published in the November 2011 issue of Lancet, are encouraging, confirming the safety and feasibility, and providing the evidence which shows intracoronary infusion of autologous c-kit+ CSCs leads to a significant improvement in LV systolic function and a substantial reduction in scar size at one year follow up [9].

Cardiac sca-1+ stem cells

Sca-1 (stem cell antigen-1), a member of the Ly-6 family, was first described as one of the cell surface antigens expressed on hematopoietic stem cells [57]. Multipotent stem cells derived from bone marrow and skeletal muscles express Sca-1 [58-60]. Thereafter, several groups identified various heterogeneous subpopulations of Sca-1+ cells based on different subsets of markers co-expressed with Sca-1 [61]. In 2003, Oh et al. were the first to isolate Sca-1+/CD31+ stem cells from the adult mouse heart, which were negative for blood cell lineage markers, c-kit, flt-1, flk-1, CD34, and CD45, but expressed cardiac transcription factors such as Gata4, MEF2C and TEF-1. These cells could differentiate into cardiomyocytes with expression of cardiac-specific genes (Nkx2.5, cTnI, and MHC) upon stimulation with the demethylation agent 5-azacytidine [62]. In addition, Matsuura et al. reported that a population of Sca-1+, c-kit+, CD34+ and CD45+ cells gave rise to spontaneous beating cardiomyocytes and the differentiated cells showed expression of cardiac transcription factors and contractile proteins when treated with oxytocin [63]. Transplantation of Sca-1+/CD31- cells resulted in improved LVEF following MI by cardiomyocyte regeneration and myocardial neovascularisation through paracrine effects, suggesting an *in vivo* therapeutic potential [64].

Accumulating evidence in many studies has suggested that cardiac Sca-1+ stem cells from mouse heart are self-renewing, clonogenic, and multipotent, and have the potential to differentiate into cardiomyocytes both *in vitro* and *in vivo* [62,63,65,66]. However, the human equivalent of the murine Sca-1 surface marker has not yet been identified. Smits et al. isolated and expanded a population of cardiac-derived Sca-1-like cells (human cardiomyocyte progenitor cells) from fetal and adult human hearts by clonal expansion or MACS isolation using the antibody targeted at mouse Sca-1. Furthermore, they demonstrated that these cells could be differentiated into beating cardiomyocytes with high efficiency (80-90%) after treatment with 5-azacytidine and vitamin C/transforming growth factor-β in a chronological order [67]. However, these cells are yet to be studied in a clinical trial.

Cardiac side population cells

The side population (SP) cells, characterized by their ability to efflux Hoechst 33342 (a DNA-binding dye) via the transporter, ATP-binding cassette sub-family G member 2 (ABCG2; CDw338), have been identified in several adult tissues such as bone marrow and skeletal muscle [68]. Hierlihy et al. were the first to report the existence of a cardiac SP cell population with stem cell-like activity and the potential of cardiomyogenic differentiation in the postnatal murine myocardium [69]. It has been shown that adult cardiac side population cells are heterogeneous in nature, consisting of distinct subpopulations of cells expressing c-kit, Sca-1, CD31, CD34, VE-cadherin, mesenchymal progenitors, vascular endothelial cells and cardiomyogenic precursors [70]. Several studies have reported that cardiac side population cells found in rodents are able to give rise to three major cardiac lineage cardiomyocytes, endothelial cells and smooth muscle cells *in vitro* [71-73]. *In vivo*, it was shown that these cells homed to the damaged myocardium and differentiated into three cardiac lineages when infused into adult rats [73]. Furthermore, Liang et al. demonstrated that a subset of cardiac SP cells (Sca-1+/CD31-) migrated to the injured site and gave rise to cardiomyocytes or endothelial cells through the SDF-1/CXCR4 system in a murine model of myocardial ischemia [74]. Cardiac SP cells have shown the potential for the commitment of cardiovascular lineages both *in vitro* and *in vivo*, however, more research is required to investigate their therapeutic effects on cardiac function upon transplantation into a myocardial ischemic model.

Cardiospheres and cardiosphere-derived cells

Messina et al. described a method to culture cardiac stem cells via the formation of multicellular clusters, termed cardiospheres, from mouse heart explants and human ventricular biopsies. These cardiospheres were clonogenic and expressed c-kit, Sca-1, and CD31, CD34, and Flk-1 based on immunophenotypic and flow cytometric analyses [40]. Mouse cardiospheres spontaneously contracted after their generation, but human cardiospheres were seen to beat only after coculture with rat cardiomyocytes, nevertheless, both indicated that these CSC populations have the potential to differentiate into cardiomyocytes. Furthermore, murine cardiospheres were shown to differentiate into cardiomyocytes with contractility as well as vascular cells when transplanted into the ischemic heart of immunodeficient mice [40].

Subsequently, Smith et al. modified the protocol described by Messina's group to substantially expand cardiosphere-derived cells (CDCs) *in vitro* and showed myocardial regeneration and functional improvement when these cells were injected into the infarcted mouse heart [48]. In contrast to other populations of CSCs, cardiospheres and CDCs have been reported to contain a mixed population consisting of c-kit+ and Sca-1+ cardiac progenitor cells, and cells expressing CD90 (cardiac mesenchymal-related) and CD31/CD34 (endothelial progenitor-related) markers [40,48,75,76]. Intracoronary delivery

of autologous CDCs led to the formation of new tissue, reduction in infarct size and improvement of haemodynamics in a pig model of ischemic cardiomyopathy [45].

Based on most experimental studies, however, it should be highlighted that the number of newly formed cardiac myocytes from transplanted stem cells is too small to be proportional to the improvement observed in heart function [56]. This phenomenon could be attributable to the combination of poor cell engraftment and low cardiomyogenic potential *in vivo* following the introduction of stem cells into the injured myocardium [77]. Alternatively, the paracrine hypothesis is now widely believed to play a major role in the beneficial action of transplanted stem cells *via* the secretion of various cytokines and growth factors, such as VEGF, HGF and IGF-1 [78-80]. It has been reported that in human CDC populations, unselected mixed CDCs significantly improved heart function in comparison to c-kit-sorted CSCs, as determined by ejection fraction, when implanted into infarcted immunodeficient mice [81]. Furthermore, Chimenti et al. characterized potential actions of paracrine factors in human CDC transplantation and concluded that the contribution of the indirect effect rivals or exceeds that of direct myocardial regeneration [79]. In addition, Li et al. reported that human CDCs exhibited relatively high production of various growth factors, including angiopoietin-2, bFGF, HGF, SDF-1, IGF-1, and VEGF, and resulted in superior improvement of cardiac function compared with BM-MSCs, BM-MNCs and adipose tissue-derived MSCs [80].

Collectively, it is possible that the cardiac progenitor cells could readily engraft, differentiate and function when transplanted into the injured myocardium in the presence of cardiac mesenchymal stem cells and endothelial progenitor cells via synergistic paracrine effects [76,82]. In short, so far, cardiospheres and CDCs have been isolated and used for treatment of ischemic heart disease in various animal models, including mouse [40,75], rat [43,83], and pig [45,84], and shown evidence of new cardiomyocyte formation or beneficial effects on cardiac function.

After the accumulation of promising results, the CADUCEUS trial, led by Marban et al. aimed to investigate the effects of autologous CDC transplantation via the intracoronary route in patients with a recent MI and ischemic left ventricular dysfunction. The results, published in Lancet early in 2012, were that intracoronary infusion of autologous CDC contributed to significant increases in viable myocardium, regional contractility and regional systolic wall thickening despite no significant change in LVEF, which might be explained by the fact that EF at baseline was only moderately impaired (39%), leaving little room for improvement by 6 months [10]. Because of the positive findings, further research with longer follow-up and larger, phase 2 studies are required to confirm the true and persistent clinical benefits.

Islet-1+ cardiovascular progenitors

Laugwitz et al. reported the identification of a subpopulation of cardiovascular progenitor cells in postnatal mouse, rat and human myocardium, which express an embryonic marker of LIM-homeodomain transcription factor Islet-1 (Isl1) [85]. During cardiac development, Islet1+ progenitor cells, derived from the second heart field, contribute to the right ventricle, outflow tract and partial atria [86]. The number of these cells is very low at postnatal ages with between 500 and 600 cells per heart [87]. As demonstrated by Itzhaaki-Alfia et al., cultured cells derived from right atrium, obtained from patients by surgery or endomyocardial biopsy, produced higher percentages of islet-1+ cell population (7%) compared with left atrium, right ventricle and left ventricle (varying from 1% to 2.8%) [88]. Islet-1+ progenitors are negative for c-kit, Sca-1 and CD31, but co-express the cardiac specific transcription factors Nkx2.5 and Gata4; importantly they have the potential to differentiate into smooth muscle cells, endothelial cells and fully functional cardiomyocytes [85,89]. Although Islet-1+ progenitor cells may represent an attractive cell source for cardiac repair and regeneration, previous studies showed that the Islet-1+ progenitor population rapidly declines shortly after birth, which may limit their clinical application in the adult patients [7,87,90,]. Interestingly, there are several recent studies showing that the number of islet-1+ cells in rat and mouse hearts remained steady from neonatal life up to adulthood, albeit at a very low level [91-93]. It is believed that further research could provide more insights into whether this type of stem cells is a possible source for future cardiac therapy.

Epicardium-derived stem cells

Another source of endogenous resident cardiac progenitor cells with regenerative potential for the adult heart is the epicardium, with several groups reporting the discovery of epicardium-derived myocardial and vascular progenitors in embryonic mouse and adult human heart [94-98]. During heart development, a subset of epicardial cells, known as epicardium-derived cells (EPDCs), delaminate from the epicardium and subepicardium and migrate into the myocardium through a process of epithelial-to-mesenchymal transition (EMT) prior to differentiation into specialized cells [99]. EPDCs are multipotent in both embryonic and adult hearts, capable of giving rise to adventitial fibroblasts, coronary smooth muscle cells, endothelial cells and cardiomyocytes [7,61,100].

Adult human EPDCs were found to reduce remodelling and increase ejection fraction when transplanted into an immunodeficient mouse model of myocardial infarction [101]. Moreover, Smart et al. reported that the activation of quiescent EPDCs in the adult mouse heart can be enhanced using a naturally occurring protein called thymosin beta 4 (Tβ4; a small actin-binding protein that activates integrin-linked kinase). This stimulating factor releases the EPDCs from a dormant state and restores their progenitor cell potential with differentiation into cardiomyocytes after their migration to the damaged site of the heart [94,102]. Although the induction of cardiomyocyte differentiation by Tβ4 is not efficient at present, this strategy also provides another prospective means of stem cell-based cardiac therapy through in situ activation of resident cardiac progenitor cells by specific factors without additional complications of isolation and expansion ex vivo and possible problems like low retention and engraftment, relative to cell transplantation [7,103].

Effect of age on isolation and function of cardiac stem cells

It has been proposed that there is a general decline in the number and/or function of stem cells with increased age in various stem cell types [104]. Hill et al. demonstrated an inverse correlation between the number of circulating endothelial progenitor cells and age [105]. Additionally, it has been suggested that increasing age resulted in a reduction of acquired cell number and angiogenic potential in adipose tissue-derived progenitor cells [106]. Likewise, some studies have shown a link between age and decreased self-renewing ability in endothelial progenitor cells [107], c-kit positive cells in the testis and epididymis of rats [108] and neural stem cells [109]. A study by Scheubel et al. also reported that aging inhibits endothelial progenitor cell mobilization in patients undergoing CABG [110]. Moreover, other researchers have suggested that aging is involved in mediating intrinsic characteristics of stem cells, such as cell growth, proliferation, differentiation, and senescence [77,111-114].

Clearly, epidemiological studies show that heart diseases are more prevalent among the elderly population [115]. From a clinical point of view, chronological aging is an inevitable fact and may link to the biological state of tissue-specific stem cells and the disease phenotype of patients [116, 117]. These issues potentially interfere with cell acquisition in terms of clinically relevant quantity and therapeutic potential. When considering the applications of autologous CSCs, therefore, it is important to understand the impacts of age on CSC isolation, expansion and regenerative potential. Using CDCs derived from mice, our lab found that the numbers of CDCs were significantly reduced from the older mice relative to the younger animals (aged from 1.5 to 24 months). In addition, the amount of cardiac stem cells, which expressed c-kit or Sca-1, was persistently down-regulated in CDC populations with increasing age (unpublished data). This is consistent with a human CDC study by Mishra et al., which confirmed that c-kit expression and CDC proliferation declined with advancing age ranging from neonates to teenagers [118]. Furthermore, the same group published another paper demonstrating that when injected into infarcted myocardium, neonatal-derived CDCs had a significantly higher regenerative potential compared with adult-derived CDCs [119]. Reduction of proliferative capacity and degeneration in differentiation potential were reported to be related to the shortening of telomeres and telomerase deficiency in aged haematopoietic stem cells [113,120,121] and Torella et al. found that the percentage of c-kit$^+$ cells showing evidence of senescence expression (i.e., p16^{INK4a}), shortened telomere length and apoptosis was elevated in older wild-type mice [122].

In fact, aging is extremely complex and involves multiple mechanisms at various levels (i.e., molecular, cellular, organic and organismal) [123]. Although the exact interactions between senescence-related signalling pathways remain to be ascertained, theories have been proposed to explain the aging process, including theories of somatic mutation, mitochondrial DNA (mtDNA) mutation and telomere loss [123]. Furthermore, a variety of intrinsic and extrinsic systems are involved in the regulation of stem cell number and biological performance along with aging, such as cell-to-ECM, telomere-telomerase, growth factor-receptor, and ROS-antioxidant defence systems [122,124]. For instance, the IGF-1/IGF-1 receptor (IGF-1R) system preserves the pool of endogenous CSCs through enhancing telomerase activity and delaying senescence by activating the PI3K-Akt pathway, indicating that IGF-1 may protect CSCs against adverse aging effects [122]. It has been shown that the IGF-1/IGF-1R axis exists in cardiac stem cells in very old animals, but the IGF-1 synthesis and the IGF-1R expression are found to decrease in aging human CSCs [122,125,126]. In this regard, preconditioning of cultured resident CSCs in old age by over-expressing telomerase or up-regulation of favourable growth factor signalling may improve the regenerative capabilities of survival, growth and differentiation *in vivo* following transplantation [104,124,127]. Furthermore, in our group, hypoxic culture as a preconditioning treatment not only significantly increased cell yield but also enhanced telomerase levels and secretion of paracrine factors (e.g., VEGF and EPO) in rat CDCs (Tan et al. in review). In short, further understanding of the interactions between age and CSC characteristics has significant clinical implications to enhance the therapeutic ability of adult-derived CSCs in the use of stem cells for cardiac therapy [119].

Finally, it should be noted that CSCs can be cultured from failing human hearts, but pathological processes may influence the endogenous CSC pool function, as shown by Cesselli et al. [128]. In our lab, we found that CDCs could be produced from mice aged from 1.5 to 24 months despite adverse effect of age on cell biological properties (unpublished). Likewise, Itzhaaki-Alfia and colleagues demonstrated that human c-kit+ CSCs can be isolated and expanded from the right atrium of most patients aged 50 to 75 years undergoing heart surgery, such as CABG, valve replacement and heart transplantation [88]. Furthermore, D'Amario et al. reported that c-kit+ CSCs isolated from the diseased myocardium (RV septum apex or LV apical region) in patients with advanced heart failure were functionally competent, as measured by telomere length and telomerase level, all indicating that autologous CSC therapy is realistic and can therefore be considered for use in the treatment of patients with severe heart failure [129].

Conclusions

In summary, it is nearly 10 years since the identification of endogenous cardiac stem cells. Based on numerous experimental studies and recent clinical trials, there has been growing evidence showing that cardiac stem cells can be grown as autologous cells for cardiac therapy, especially c-kit$^+$ CSCs and CDCs, as demonstrated by feasibility, safety and functional improvement. Importantly, regardless of age or disease, cardiac stem cells have been isolated and expanded from the diseased heart from most patients [128,88,130]. This means that cell transplantation with autologous CSCs is a viable treatment in routine clinical practice. On the other hand, it is worthy of mention that among the various cardiac stem cells, CDCs comprise mixed subpopulations and can give rise to major cardiac lineages. In addition, CDCs are able to secrete a variety of cytokines and growth factors in support of paracrine effects. In the future, more basic research and large well-designed clinical studies are needed to further our understanding of underlying mechanisms of functional improvement and improve cell culture methods for optimal quality and quantity of adult-derived CSCs. With rapid progress in stem cell biology, in spite of huge challenges, the approach using cardiac stem cell-based therapy looks promising for treatment of patients with ischemic heart failure.

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Conflicts of Interest

The authors declare no potential conflicts of interest.

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