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Cardiac Stem Cells and their Regenerative Role on Myocardial Infarction

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Abstract

Cardiovascular diseases, especially myocardial infarction (MI) are the leading cause of death all over the world. Current treatment strategies of myocardial infarction include drug regimens, percutaneous coronary revascularisation and coronary artery bypass grafting (CABG). Despite the considerable contribution of the interventions mentioned above, the long term benefits remain unsatisfactory. Existing treatments fail to regenerate myocardium that has gone through necrosis or apoptosis. So seeking for new approaches will not only minimize patient suffering but also significantly reduce the cost and resource burdens on a healthcare system that is already stretched to the limit. Recently, transplantation of cardiac stem cells has become a hot topic on regenerative medicine. Mammalian heart has been traditionally regarded as a terminally differentiated organ with no potential for regeneration. However, studies over the past decade have suggested the existence of cardiac stem cells (CSCs) that reside in the heart itself both in normal and pathological states. These cells are self-renewing, clonogenic, and multipotent, i.e, they are capable of differentiating into myocytes, vascular smooth muscle cells, and endothelial cells in appropriate conditions. This review mainly discusses the current research on CSCs, various subpopulations of CSCs and their repair mechanisms in MI.

Keywords: Cardiac stem cells; Myocardial infarction; Myocardial regeneration; Stem cell therapy

Abbreviations:

CSCs: Cardiac Stem Cells; MI: Myocardial Infarction; CABG: Coronary Artery Bypass Grafting; ESCs: Embryonic Stem Cells; BMSCs: Bone Marrow Derived Stem Cells; CDC: Cardiosphere Derived CSCs; SP: Side Population; MCSCs: Myogenic CSCs; VCSCs: Vasculogenic CSCs; IGF-1: Insulin-like Growth Factor-1; HGF: Hepatocyte Growth Factor; SDF-1: Stromal Cell-Derived Factor-1; VEGF: Vascular Endothelial Growth Factor; BFGF: Basic Fibroblast Growth Factor; LVEF: Left Ventricular Ejection Fraction; NF: Nano Fiber

Introduction

Different stem cell populations have been intensively studied in the last decade as a potential source of new cardiomyocytes to ameliorate the injured myocardium, compensate for the loss of ventricular mass and contractility and eventually restore cardiac functions. An array of cell types has been explored in this respect, including embryonic stem cells (ESCs), skeletal myoblasts, bone marrow derived stem cells (BMSCs), and more recently cardiac stem cells (CSCs) [1]. Although ESCs have an exceptional capacity for proliferation and differentiation, the clinical application of ESCs is limited by their pluripotent nature, teratomas potential and ethical concern. Skeletal myoblasts are not suitable cells, because transplanted myoblasts are unable to turn into cardiomyocytes and they are also associated with life-threatening arrhythmias. However, the BMSCs do not become functional cardiomyocytes *in vivo*, instead they exert their benefits mainly through indirect paracrine mechanisms [2,3]. So it is pressing to find a logical cell source for cell transplantation after MI, which can differentiate into cardiomyocytes directly and effectively. More importantly, overcome ethical, immunological, and safety issues.

In 1994, Soonpaa's group delivered ESCs isolated from transgenic mice directly into the infarcted myocardium of syngeneic hosts, 2 months later, the results suggested that grafted cells could attenuate the development of scar formation and prevent cardiac failure secondary to myocardial infarction [4]. Then mitosis was observed in small amounts of cardiomyocytes both in the peri-infarct zone and normal myocardial tissues, providing that some cardiomyocytes are self-renewal even in pathological condition [5]. The adult heart is composed predominantly of postmitotic cells, but it is not a terminally differentiated organ. It contains replicating myocytes responsible for myocardial regeneration.

The year of 2003, Beltrami first isolated and expanded so-called CSCs capable of committing to the myogenic lineage and reversing cardiac dysfunction in the infarcted heart [6]. The next year, Messina successfully identified and characterized a kind of self-renewal cells from the adult human and murine heart. These cells are clonogenic, express stem and endothelial progenitor cell antigens/markers, and appear to have the properties of adult cardiac stem cells [7]. Other studies reported similar results that CSCs possess the fundamental properties of stem cells and they differentiate into or give rise to all three major cardiac lineages [8-10]. All this compelling evidence has demonstrated the existence of multipotent CSCs in adult heart.

Subpopulations of CSCs

Multipotent cardiac stem cells are relatively abundant, accessible, and autologous compared to other cell source, which makes them the most attractive and suitable cell type for the treatment of myocardial

injury. Different subpopulations of CSCs have been identified according to their properties and surface markers. These distinct CSC populations include c-kit+ CSCs, cardiosphere-derived CSCs (CDC), Sca-1+ CSCs, side population (SP) CSCs, Islet-1+ CSCs.

C-kit+ CSCs

One of the most well-known stem cells is the c-kit+ CSCs. Quantitative data in the animal and human heart have demonstrated that there is one CSC per~30,000–40,000 myocardial cells [11]. CSCs exist in small clusters with a highest density in the atria and the ventricular apex, especially the right atrial appendage [12]. These cells are characterized by expression of c-kit and absence of CD45, additionally, they are blood lineage negative and express transcription factors associated with early cardiac development, such as GATA-4, Nkx2.5, and Mef2C [6,13,14]. Moreover, they are the most extensively studied cell source with the ability to form all three cardiac lineages in vitro, which have been applied into clinical trials.

Cardiosphere-derived CSCs

Another type of CSC that has been exploited in cardiac regeneration therapy is the CDC. The cells yielded from mild enzymatic digestion of the tissue specimens are small, round, phase-bright and will form spheroid aggregates in suspension culture, thus are named as Cardiosphere-derived CSCs (CDCs) [7]. CDCs are composed of proliferating c-kit positive cells primarily in their core and differentiating cells expressing cardiac and endothelial cell markers on their periphery [15,16]. Abundant evidence suggests that CDCs have the ability of self-renewal, clonogenicity, and differentiation into cardiomyocytes and endothelial cells [7,17]. A head-to-head comparison of CDCs, bone marrow-derived mesenchymal stem cells, adipose tissue-derived mesenchymal stem cells, and bone marrow-derived mononuclear cells showed that CDCs were superior in terms of paracrine factor secretion, angiogenesis, cardiomyogenic differentiation, ischemic tissue preservation, antiremodeling effects, and functional benefit [18]. A Phase I clinical trial has also been performed using CDCs [19].

Sca-1+ CSCs

Sca-1+ CSCs were identified as another predominant cardiac stem cell population in the adult mouse heart that express stem cell antigen-1 (Sca-1) but not c-kit or blood lineage markers [20]. When treated with oxytocin, Sca-1+ CSCs expressed genes of caridac transcription factors including Nkx-2.5, GATA4 and contractile proteins such as sarcomeric α-actin, cardiac troponin I, and MHC, at the same time, a small fraction (~1%) of Sca-1+ cells exhibited spontaneous beating activity [21]. Furthermore, transplantation of Sca-1+ cells into the acutely infarcted mouse heart resulted in functional promotion by secretion of various cytokines and proteins related to caridac proliferation and regenaration, which show their reparative roles in MI [22,23].

SP CSCs

Side population (SP) cells are characterized by their expression of the ATP binding cassette transporter ABCG2 and their ability to exclude Hoechst 33342 dye [24,25]. Hierlihy et al. was the first to isolate SP cells and found that these cells comprised ~1% of all cells in the mouse heart [26]. SP CSCs are subdivided into two distinct populations according to their cell phenotype, namely, CD31-/Sca-1+ SP CSCs and CD31+/Sca-1+ SP CSCs. However, only CD31-/Sca-1+ SP CSCs show high cardiomyogenic potential [27]. Still, SP CSCs demonstrate their ability to differentiate into mature cardiomyocytes after 2–3 weeks of co-culture with adult rat ventricular cardiomyocytes [28]. Two years later, SP CSCs from neonatal rat hearts were successfully induced into functional cardiomyocytes through oxytocin or trichostatin A without co-culture with other cell types [29]. Recently, human SP Cells were isolated from biopsies of left atrium [30,31]. The properties of these cells will need to be further characterized in the future.

Islet-1+ CSCs

Islet-1+ CSCs could be isolated from neonatal mouse hearts, and these cells could express the cardiac transcription factors Nkx2.5 and GATA4, but not Sca-1, CD31, or c-kit [32-34]. Islet-1+ cells are crucial for the formation of the right ventricle, atria, and outflow tract [35]. However, Islet-1+ cells can be found only in neonatal and fetal tissues, yet reduce to low or nonexistent levels in adult hearts, which limits their clinical potential [36].

Mechanisms of CSCs in Cardiac Regeneration

Until now, the mechanisms involved in cardiac repair caused by CSCs transplantation have been summarized as Cardiomyo Angiogenesis and paracrine mechanisms.

Cardiomyo angiogenesis

Lots of animal researches and clinical trials have demonstrated that transplanted CSCs could differentiate into new myocytes and vessels. Current researches inform that IP3R-mediated Ca2+ oscillations control CSCs growth and their regenerative potential. In the unfavorable environment of the necrotic tissue post-myocardial infarction, the highly expressed ATP increases the frequency of Ca2+ oscillations among neighboring CSCs, which initiates and enhances the engraftment, proliferation, and regeneration of a myocyte progeny [13,37]. Additionally, the latest research revealed that the heart contains two distinct subpopulations of CSCs: myogenic CSCs (mCSCs), characterized by expression of c-kit, which are mainly responsible for regenerating cardiomyocytes [38], and vasculogenic CSCs (vCSCs), which express c-kit as well as KDR, are more committed to the turnover of coronary vessels [39]. Both of these two subpopulations possess the fundamental properties of stem cells: self-renewing, clonogenic, and multipotent [40]. All this evidence described above provides convincing proof that the improvement of cardiac function by CSCs therapy is mediated partially by cardiomyo angiogenesis mechanism if not all.

Paracrine and exosomes

Although transplantation of CSCs has showed its beneficial effects in mediating cardiac protection, it is believed that the positive outcomes of stem cell transplantation are regulated mainly through production and secretion of growth factors and cytokines by the engrafted stem cells [41]. The paracrine factors secreted by the large number of injected stem cells could contribute to rearrange the post-ischemic microenvironment and promote angiogenesis, inhibit apoptosis, and stimulate myocyte proliferation [42-44]. CSCs possess growth factor receptor systems such as IGF-1/IGF-1R, HGF/c-Met, and SDF-1/CXCR4, when integrated with growth factors, the downstream signalling pathways will be activated to induce cell

migration, proliferation and differentiation [45,46]. Several growth factors and cytokines have been identified, and these include VEGF, HGF, IGF-1, SDF-1, etc. They play their role in cardiac functional improvement through corresponding receptors and signalling pathways. For example, delivery of IGF-1 exerts a prosurvival effect on CSCs through induction of the IGF-1 receptor and PI3K/Akt/GSK-3beta signalling [47]. While VEGF shows it's prosurvival and anti-apoptosis potential via activating SDF-1/CXCR4 axis and downstream STAT3 and ERK1/2 pathway [48-50]. Overall, paracrine effectors are crucial regulators involved in CSCs homing, expansion and differentiation.

Exosomes are membrane vesicles with a diameter of 40–100 nm, which are emerging as an attractive vector of paracrine signals delivered by CSCs. Exosomes are stored intracellularly in endosomal compartments and are secreted when these multivesicular structures fuse with the cell plasma membrane [51-54]. Exosomes carry a specific set of proteins derived from the plasma membrane, endocytic pathway, and the cytosol, which play important roles in cell penetration, invasion and fusion events, and regulate exosome docking and membrane fusion [55,56]. Exosomes also contain annexins, metabolic enzymes, ribosomal proteins, signal transduction molecules, adhesion molecules, ATPases, cytoskeletal and ubiquitin molecules, growth factors, cytokines and miRNA molecules [57], among which, miRNAs, an important regulators in CSC-mediated cardiac repair after MI are best studied. A research have recently reported that miRNA families plays important roles in the transition of cellular proliferation in CSCs in vivo,and may be an crucial modulator in the process of bone morphogenetic protein (BMP)-2-regulated myocardial differentiation due to their repression of cardiac progenitor genes IsI1 and Tbx1 [58-60]. In addition, miRNA could repress CMC progenitor cell death via targeting receptor interacting protein 1 [61]. Altogether, exosomes act as vectors for the intercellular exchange of biological signals and information, which mediate cell activation, phenotypic changes, and reprogrmming of cell function. Exosomes may be a key mechanism by which cardiac progenitors communicate with each other and deliver paracrine signals to neighboring cells [62-65].

Experimental and Preclinical Research on CSCs

In the last years tremendous effort has been undertaken to evaluate CSCs for their safety, feasibility, and efficacy on cardiac repair and regeneration, including small animal experimental studies and preclinical large animal trials.

Experimental research

Small animal models of MI have been widely used to study the effects of transplanted CSCs and they did document the structural and functional benefits.

Transplanting CSCs into a rat model with a 90-min coronary occlusion following by 4 hours of reperfusion, Dawn et al. found that CSCs were able to induce regeneration, and decrease myocardial infarct size by 29% [66]. Another study by Wang et al. revealed that delivery of Sca-1+/CD31- cells into the acutely infarcted mouse heart attenuated functional decline and adverse structural remodeling as evidenced by an increased left ventricular ejection fraction, a decreased end-diastolic and systolic dimension, a significant increase of myocardial neovascularization, and modest cardiomyocyte regeneration [67]. Smith et al. transplanted human CDCs into the border zone of myocardial infarcts in immunodeficient mice. 20 days later, the percentage of viable myocardium within the infarct zone was greater in the CDC-treated group(24.9 ± 1.1%) than in the control group(17.7 ± 1.8%, *P*0.01); likewise, left ventricular ejection fraction was significantly higher in the CDC-treated group (42.8 ± 3.3% vs 25.0 ± 2.0% for control group) [68]. Abundant studies finished in recent years further confirmed that administrated CSCs in the setting of MI produced beneficial structural and functional effects in small animal models [69-72].

Preclinical research

Similar results have already been obtained in large animal models. The pig, which is more similar in tissue biology, size, and physiology to the human than the rodent models commonly used has proven a very productive and frequently used preclinical large animal model for regenerative therapy. Johnston et al. administered CDCs to both healthy and infarcted pigs at 4 weeks after MI through intracoronary infusion. 8 weeks later, CDCs treatment formed new cardiac tissue, reduced relative infarct size, attenuated adverse remodeling, and improved hemodynamics [73]. A randomized, blinded, and placebo-controlled study showed that Intramyocardial injection of autologous CDCs effectively halted the deterioration in LVEF and efficiently improved echocardiographic and hemodynamic indexes after large scale of anteroseptal myocardial infarction [74]. In a study by Bolli R et al., autologous CSCs (n=11) or vehicle (n=10) were infused into the infarct-related artery of pigs 3 months after MI. One month later, CSCs-treated pigs exhibited significantly greater LVEF (51.7 ± 2.0% versus 42.9 ± 2.3%, P<0.01), systolic thickening fraction in the infarcted LV wall, and maximum LV dP/dt, as well as lower LV end-diastolic pressure. The expression of cardiac markers as troponin I, troponin T, myosin heavy chain, connexin-43, and α-sarcomeric actin was a strong reflection of myocardial regeneration. Some engrafted CSCs also formed vascular structures and expressed α-smooth muscle actin [75].

In summary, all these animal studies have shown that transplantation of autologous CSCs improves regional and global left ventricular function and promotes cardiac and vascular regeneration, thus laid a solid foundation for clinical trials.

Clinical trials

Cell-based therapies to regenerate the damaged myocardium using CSCs have been performed in the recently completed SCIPIO and CADUCEUS clinical trials.

In the open label, randomised phase 1 SCIPIO trial, sixteen patients with post infarction left ventricular dysfunction (ejection fraction ≤40%) who had undergone coronary artery bypass grafting, received 500,000–1 million of autologous c-kit+ CSCs intracoronary, nearly 4 months after surgery. In the control group no treatment was given. The primary endpoint was short-term safety of CSCs and the secondary endpoint was efficacy. LVEF increased progressively from a mean of 30.3% before CSC infusion to 38.5% 4 months after transplantation, whereas the LVEF did not change in the control patients, during the corresponding time interval. Moreover, in the eight patients who completed the 1 year of follow-up, LVEF increased by 12 EF points vs. baseline. Cardiac MRI of seven of the treated patients showed that infarct size decreased by 24% at 4 months and 30% at 1 year [76].

In the prospective, randomized CADUCEUS trial, patients with left ventricular ejection fraction of 25–45% were consecutively enrolled in

the treatment and control groups. Autologous CDCs up to 25 million were infused into the infarct-related artery of the 17 patients assigned as treatment group, 1.5-3 months after myocardial infarction. 8 patients received standard care and acted as the control group. Compared with controls at 6 months, MRI analysis of patients treated with CDCs showed significant reductions in scar size and mass, increases viable heart mass, regional contractility, and regional systolic wall thickening. However, changes in end-diastolic volume, end-systolic volume, and LVEF did not differ between groups at 6 months [19].

Additionally, two promising clinical trials utilize CSCs for treatment of ischemic heart diseases are still in progress, the results have yet to be reported.

In the ALCADIA trial (NCT00981006), 6 patients of ischemic heart failure after CABG surgery received implantation of autologous CDCs together with a biodegradable gelatin-hydrogel infused with basic fibroblast growth factor (bFGF). Preliminary data at 6 months reported an improvement in LVEF (from 26.7% to 35.8% by 3-D echo and 22.6% to 34.7% by MRI), a decrease in infarct size by MRI (from 23% to 19.7%), as well as a decrease in wall motion score (from 17.2 to 6.6) [77].

The phase I/II "Allogeneic heart stem cells to achieve myocardial regeneration" (ALLSTAR) trial (NCT01458405), led by Eduardo Marban, is ongoing to test the safety of allogeneic CDCs. It is the first time to investigate the allogeneic use of CSCs in humans, and the results are awaited [78].

New Strategies to Promote CSCs Survival, and Proliferation

Growing evidence has suggested that CSCs exert great effects on cardiac repair post-MI. But only a small percentage of donor cells could successfully be engrafted into the damaged myocardium due to the harsh microenvironments after infarction. The unfavourable microenvironment of the necrotic myocardium together with diffuse inflammatory infiltrates interferes with homing, survival, and growth of the administered cells, which are critical variables of successful myocardial repair. Therefore, it is imperative to look for some new strategies to enhance the survival rate and long-term engraftment of CSCs after transplantation.

Delivery of biologic factors

Great efforts have been made to create more conducive myocardial environment for CSCs proliferation. Different cytokines or growth factors are used to boost cell survival, persistence, and proliferation. In the damaged dog and pig heart, CSCs transplantation as well as in situ activation by co-administered IGF-1and HGF has been shown to be a practical and effective strategy to prolong cell survival, induce cardiovascular regeneration, and improve left ventricular function. Such myocardial reconstitution caused by combination delivery of IGF-1 and HGF can promote a significant restoration of dead tissue, resulting in a marked recovery of contractile performance of the infarcted heart [79,80].

Gene transfection

Based on the observations that HGF gene transferred into human bone marrow- and adipose tissue-derived stem cells highlights great regenerative effects [81-84], a novel powerful therapeutic strategy, gene therapy, has been applied to enhance the ability of CSCs to promote myocardial regeneration. Overexpression of SDF-1 in the infarcted mice heart by rAAV1-SDF-1a-eGFP infection resulted in more CSCs retention to the infarcted myocardium, a higher percentage of proliferation, and reduced infarcted area via CXCR4/PI3K pathway [85]. Targeted delivery of human VEGF gene via complexes of magnetic nanoparticle-adenoviral vectors exhibited higher capillary and arteriole density and lower collagen deposition and significantly improved left ventricular function [86].

Precondition with low oxygen and growth factors

CSCs preconditioned by exposure to low oxygen ex vivo are more resistant to ischemic microenvironment. A study in 2009 provided evidence that CSCs subjected to ischemic preconditioning markedly augmented c-kit+ cells recruitment to the ischemic myocardium and enhanced protection against ischemic cardiac injury after myocardial infarction. Four weeks after treatment, infarct size and heart function were significantly better in mice administered hypoxia-preconditioned CSCs than in mice treated with cells cultured under normoxic conditions. Furthermore, these effects were largely abolished by the addition of a CXCR4 inhibitor, indicating that the benefits of hypoxic preconditioning are mediated by the SDF-1/CXCR4 axis [87]. Subsequent experiments have also reported similar results that implantation of low O2 pre-cultured CSCs into infarcted hearts of mice led to greater cell engraftment and better functional recovery compared with that in normoxic stem cells [88-90]. A newly published article indicates that hypoxic preconditioningeffect [91].

An alternative method to promote survival is to precondition CSCs with growth factors prior to delivery into the recipient heart. Preconditioning Sca-1+ CSCs with IGF-1 before transplantatation enhanced cell survival via PI3K/Akt-dependent caspase-3 downregulation and reprogramed cardiomyogenic differentiation [92]. CSCs activated by IGF-1and HGF, ex vivo, formed conductive and intermediate-sized coronary arteries together with resistance arterioles and capillaries [38].

Engineered cell delivery

With the rapid progress in the study of biological materials, tissue engineering has gradually become an alternative strategy on cardiac cell therapy. The injectable biomaterials such as hydrogels, gelatines, nanofibers, and self-assembling peptides have been used as vehicles for cell delivery. These biomaterials can provide a scaffold that mimics natural extracellular matrix under physiological conditions, reducing cell washout from the injection site and preventing apoptosis triggering attributable to anoikis. They also possess a reservoir for controlled release of growth factors. In addition, embedded in bio-engineered tissues and supported by extracellular matrix, transplanted cells would have a better chance to survive and engraft in the cardiac microenvironment in comparison to direct exposure to injured tissue via injection [93]. In the rat MI model, nanofibers were used to deliver IGF-1 along with CPCs. Compared with infarcts exposed to CPCs or NF-IGF-1 alone, combination therapy resulted in a greater increase in the ratio of left ventricular mass to chamber volume and a better preservation of +dP/dt, -dP/dt, and ejection fraction. The number of newly formed myocytes with combination therapy was 32% and 230% higher than with CPCs and NF-IGF-1, respectively. Similarly, the length density of newly formed coronary arterioles with both CPCs and NF-IGF-1 was 3% and 83% greater than with CPCs and NF-IGF-1 alone, respectively [94]. Similar progress has been made that

biomaterials carrying cytokines such as bFGF, IGF-1, and VEGF modulate the post-ischemic microenvironment to enhance CSCs engraftment and differentiation. This novel strategy demonstrates significant functional improvements after myocardial infarction and may potentially represent a therapeutic approach to be studied in a clinical trial [95-100].

Summary and Conclusions

The findings that the adult heart harbors a regenerative multipotent cell population conclusively dispel the notion of the heart as a terminally differentiated organ without self-renewal potential, representing a paradigm shift in cardiovascular biology. Although initial encouraging results have been achieved from preclinical and clinical studies that administration of CSCs can induce cardiogenesis and neovasculogenesis, additionally, improve recovery of the damaged heart function, there still remain many challenging problems to be solved. Currently, the conditions of isolation, augmentation, and purification of CSCs differ among different laboratories. Time consuming, and the reliability of autologous CSC culture and expansion make the clinical application of CSC transplantation much difficult, especially in their application in the acute post-MI phase. So it is necessary to establish a standard protocol for isolation and culture of CSCs in vitro, which is simple, effective, and reproducible. Available routes of CSC delivery include intravenous, intracoronary, epicardial, endocardial, and coronary sinus injection, each of which has its own advantages and disadvantages, respectively. Despite major advances made in delivering cells to the ischemic heart, low engraftment and survival rate still remains as one of the major hurdles of current cell delivery methods. Subsequent years have seen the prosperity of new ways to deal with the problems of cell survival, persistence, and proliferation, including cell preconditioning or genetic modification prior to CSC delivery or codelivery of CSCs directly into the myocardium with growth factors or degradable biomaterials such as nanofibers and hydrogels. Furthermore, the mechanisms underlying the differentiation of CSCs have not been well understood yet, making it difficult to impose precise regulation on their directed differentiation. Other controversies remaining in cell dose, optimal time for injection and whether the application of gene therapy will lead to cancerization wait to be handled. Though faced with many challenges, CSC therapy, acting as an exciting and dynamic area of research, has shown its great potential to improve recovery of myocardial infarction. With further study of regulation systems and signal transduction mechanisms and development of more large-scale, randomized and double-blind controlled trials, CSC transplantation will bring significant and long-term impact on socioeconomics and patient well-being.

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