

# Local activation of cardiac stem cells for post-myocardial infarction cardiac repair

Zhuzhi Wen, Zun Mai, Haifeng Zhang, Yangxin Chen, Dengfeng Geng, Shuxian Zhou, Jingfeng Wang \*

Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou, China

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## Abstract

The prognosis of patients with myocardial infarction (MI) and resultant chronic heart failure remains extremely poor despite continuous advancements in optimal medical therapy and interventional procedures. Animal experiments and clinical trials using adult stem cell therapy following MI have shown a global improvement of myocardial function. The emergence of stem cell transplantation approaches has recently represented promising alternatives to stimulate myocardial regeneration. Regarding their tissue-specific properties, cardiac stem cells (CSCs) residing within the heart have advantages over other stem cell types to be the best cell source for cell transplantation. However, time-consuming and costly procedures to expand cells prior to cell transplantation and the reliability of cell culture and expansion may both be major obstacles in the clinical application of CSC-based transplantation therapy after MI. The recognition that the adult heart possesses endogenous CSCs that can regenerate cardiomyocytes and vascular cells has raised the unique therapeutic strategy to reconstitute dead myocardium *via* activating these cells post-MI. Several strategies, such as growth factors, microRNAs and drugs, may be implemented to potentiate endogenous CSCs to repair infarcted heart without cell transplantation. Most molecular and cellular mechanism involved in the process of CSC-based endogenous regeneration after MI is far from understanding. This article reviews current knowledge opening up the possibilities of cardiac repair through CSCs activation *in situ* in the setting of MI.

**Keywords:** cardiac stem cells • myocardial infarction • endogenous regeneration

## Introduction

Myocardial infarction (MI) with resultant chronic heart failure (CHF) is a leading cause of mortality and morbidity in developed countries. Despite recent improvements in disease prevention and combinative therapy for MI and CHF, the 1 year mortality rate for patients with acute MI with subsequent CHF is still depressed [1]. Adult stem cell therapy has recently emerged as a promising outlook for patients

after MI. Since Makino *et al.* [2] induced cardiomyocytes (CMCs) from bone marrow stromal cells by 5-azacytidine treatment *in vitro* in 1999, several types of stem cells, including adult stem cells derived from the heart itself, have been used in an explosive manner. However, controversies exist concerning the ability of bone marrow-derived adult stem cells and peripheral tissues adult stem cells, to

\*Correspondence to: Jingfeng WANG, M.D.,  
Department of cardiology,  
Sun Yat-sen Memorial Hospital of Sun Yat-sen University,  
107 Yangjiang West Road,

Guangzhou 510120, China.  
Tel.: +(8620)8133 2475  
Fax: +(8620)8133 2475  
E-mail: syswjf@hotmail.com

acquire cardiac cell lineages and reconstitute the myocardium lost after infarction. Clinical application of embryonic stem cells (ESCs) is limited by their pluripotent nature, teratomas potential and ethical concerns. In addition, despite that cardiac stem/progenitor cells (CSCs) can be generated from induced pluripotent stem cells (iPSCs), the clinical application of iPSCs for cell therapy of MI with subsequent CHF will not become feasible until the issues of specific teratogenic precursors and teratoma formation of these cells have been mastered [3, 4]. Thus, cardiac-specific stem cells that can reconstitute lost myocardium may be the most important and suitable cells for cardiac repair after MI.

The dogma that the adult heart is a postmitotic organ and cannot renew by itself has been challenged by recent studies. It has been reported that human CMCs can renew, despite with a gradual decrease with age, and fewer than 50% of CMCs can be exchanged during a normal life span [5]. The notion of the adult heart as terminally differentiated organ without self-renewal potential has also been challenged by recent studies providing the existence of resident CSCs, including side population (SP) cells, c-kit-positive (c-kit<sup>POS</sup>) cells, Sca-1-positive (Sca-1<sup>POS</sup>) cells, cardiospheres cells and Isl1-positive (Isl1<sup>POS</sup>) cells, according to their properties and surface markers [6–15]. Recent studies have revealed that adult CSCs derived from human and animal hearts are self-renewing, clonogenic and multipotent, giving rise to CMCs, vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) *in vitro* and after transplantation into infarcted heart *in vivo* [7, 10, 16–18]. Cardiac-specific CSCs offer promise of enhanced cardiogenesis compared to other cell source, which makes them a logical cell source and the most important cell type for cell transplantation therapy in the setting of MI with subsequent CHF [16, 17, 19, 20].

However, it is time-consuming and costly to expand isolated CSCs prior to cell transplantation and the reliability of cell expansion may also be the major obstacle in the clinical application of CSC-based transplantation therapy after MI. It is noteworthy that the adult heart has an inherent ability to replace its parenchymal cells continuously by resident CSCs [21], which opens new opportunities for endogenous cardiac repair following MI [10]. Several studies have provided convincing proof that cardiac repair by endogenous CSC activation is reasonable and may be recommended as a promising therapeutic strategy post-MI, without the need for cell expansion *in vitro* and consequent transplantation [22]. We have observed that the activation of an endogenous reserve of CSCs niches is one of the most important mechanisms of mesenchymal stem cell (MSC)-mediated cardiac repair after MI [1]. Accumulating evidence has also demonstrated that the resident CSC pool may be activated by adjacent cells *via* cell-to-cell interactions, local growth factor administration, microRNA (miRNA, miR) modulators and pharmaceutical preparations and others, to reconstitute dead myocardial tissue and recover cardiac function [1, 18, 20, 23–26].

However, the cellular and molecular mechanisms of resident CSC-mediated cardiac repair following MI are yet to be understood. This review focuses on current knowledge of the experimental studies and/or clinical trials about CSCs *in vitro* and *in vivo*, and potentiates the prospects of resident CSC activation as a promising strategy for cardiac repair post-MI.

## Identification of CSCs

For a long time, the heart has been considered a terminally differentiated organ without any regenerative potential. This notion has been challenged by the existence of a subpopulation of CSCs which can commit to the CMC, VSMC and EC lineages [10, 13–15]. Several cell types with cardiomyogenic potential, such as SP cells, c-kit<sup>POS</sup> cells, Sca-1<sup>POS</sup> cells, cardiospheres cells and Isl1<sup>POS</sup> cells, residing in the heart, are identified to be CSCs [6–15]. Quantitative data in the animal and human heart have demonstrated that there is one CSC per ~30,000–40,000 myocardial cells [19]. These cells mainly accumulate in the atria and apex and are less numerous at the base and midportion of the left ventricle [19, 27], which provides great possibility to simply isolate CSCs from human atrial appendages for clinical trials in repairing MI [28].

The c-kit<sup>POS</sup> CSC pool was the first stem cell identified in the rat heart and, up to date, this CSC pool is still the most extensively characterized [19]. Beltrami *et al.* [10] have early reported that resident adult Lin-negative (Lin<sup>NEG</sup>) c-kit<sup>POS</sup> CSCs are self-renewing, clonogenic and multipotent, giving rise to CMCs, VSMCs and ECs. The subpopulation of CSCs with cardiomyogenic potential has also been derived from small human samples of myocardium, despite differentiation into the major three lineages at a lesser extent [17] and the number of these cells were significantly prompted by acute cardiomyopathic injury following MI in the region of infarction relative to remote myocardium [29]. The Sca-1<sup>POS</sup> CSC pool has been indicated as another predominant stem cell population in the heart [30, 31], and it has been discovered to significantly increase after MI [32]. Using Hoechst 33342 dye exclusion, Hierlihy *et al.* [33] were the first to isolate a responsive SP cells residing in the adult myocardium, and this cell pool was discovered to influence adaptation of the post-natal heart. Cardiac SP cells may acutely deplete, both within the infarct and non-infarct areas, but are subsequently reconstituted to baseline levels within 7 days after MI [8]. Messina *et al.* [34] have also isolated and expanded a pool of CSCs termed cardiospheres from subcultures of post-natal atrial or ventricular human biopsy specimens and from murine hearts, and cardiosphere-derived cells (CDCs) were discovered to show biophysical and cytochemical evidence of cardiogenic differentiation [35]. It is notable that cardiospheres may contain a mix of heart-derived cell subpopulations including CSCs expressing c-kit and Islet-1, and supporting cells [36], and c-kit<sup>POS</sup> cells are able to form cardiospheres [37]. Therefore, cardiospheres may be suggested unsuitable as a source of CSCs with cardiomyogenic potential and need to be further characterized with respect to phenotype and differentiation potential before initiating human trials [38]. The Isl1<sup>POS</sup> CSCs have also been identified in post-natal rat, mouse and human myocardium [39]. Highly efficient conversion to a mature cardiac phenotype with stable expression of myocytic markers in the absence of cell fusion, intact Ca<sup>2+</sup>-cycling and the generation of action potentials, indicate that these cells are authentic, endogenous CSCs.

Except that CSCs have been identified within the myocardium, growing evidence has revealed that epicardium may be the other novel source of CSCs. Epicardial progenitors have been revealed to contribute to the CMC lineage in the developing heart [40]. The C-kit<sup>POS</sup> cells

in both human and animal epicardium have also been identified as myocardial and vascular precursor cells [41]. This c-kit<sup>POS</sup> pool localized in the subepicardium was demonstrated much more than in the myocardium, and MI could induce a significant increase in the absolute number and proliferation of these cells [41, 42].

Despite that several categories of cells termed as CSCs have been described, c-kit<sup>POS</sup> CSCs displayed greater growth potential than Sca-1<sup>POS</sup> or MDR1<sup>POS</sup> cells *in vitro* [19] and may be the only class of resident cells with the biological and functional properties of tissue-specific adult stem cells [43]. It is notable that c-kit<sup>POS</sup> CSCs may be the only stem-progenitor cells proven to fulfil the requirement to define a bona fide stem cell, and these cells express MDR1, which is a P-glycoprotein of the same family of membrane transporters, as Abcg2 and Sca-1 [44]. This would suggest that c-kit<sup>POS</sup> CSC represent a more primitive cell population with the potential to generate SP and Sca-1<sup>POS</sup> cells. However, other authors proposed the possibility that Sca-1<sup>POS</sup> rather than c-kit<sup>POS</sup> should be considered as the true adult CSCs [31]. Therefore, it is of importance to have a better understanding of the origin, biology and physiology of these different CSC types, which may be a cardinal step both for understanding their potential role in cardiac pathophysiology and for inspiring suitable technologies in clinical applications.

## Transplantation of CSCs

The MI leads to a significant loss of cells and formation of scar tissue. The remaining CMCs and vascular cells are unable to reconstitute the necrotic tissue, and cardiac function deteriorates during the ensuing course. Since Orlic and his colleagues reported that locally delivered bone marrow cells could generate *de novo* myocardium *in vivo* in infarcted mice in 2001 [45], several types of stem cells have been used as cell transplantation sources for MI therapy in animal experiments and/or clinical trials. ESCs and bone marrow-derived cells have been extensively studied and characterized, and dramatic advances have been made in the clinical application of bone marrow-derived cells in MI with subsequent heart failure [19]. However, the ability of bone marrow-derived cells to acquire cardiac cell lineages and reconstitute the dead myocardium after MI still remains controversial. Unfortunately, malignant tumour formation after transplantation of short-term cultured bone marrow MSCs was observed up to 30% in experimental MI mice [46], and clinical application of ESCs or iPSCs is limited by their high risk of teratoma formation and ethical concerns. CSCs may exert greater effects on cardiac repair post-MI transplantation than those of bone marrow mononuclear cells, skeletal myoblasts and adipose tissue-derived MSCs [47]. Therefore, CSCs may represent a logical source to exploit in cardiac regeneration therapy because, unlike other adult stem cells, they are likely to be intrinsically programmed to generate cardiac tissue *in vitro* and to increase cardiac tissue viability *in vivo* [48].

Till now, despite that debate still remains, several cell types derived from either human or animal hearts with cardiomyogenic potential termed as CSCs has been identified to regenerate lost myocardium and to improve cardiac performance after transplantation into MI hearts. Cultured resident adult Lin<sup>NEG</sup> c-kit<sup>POS</sup> CSCs or their

clonal progeny, when injected into ischaemic hearts, reconstitute well-differentiated myocardium, formed by blood-carrying new vessels and CMCs with the characteristics of young cells, encompassing approximately 70% of the ventricle [10]. When engrafted into injured heart, CSCs inclined to migrate and home to infarcted myocardium than to normal myocardium, and exerted their capability to differentiate into CMCs, ECs and VSMCs in injured heart with and without fusion to host cells [30, 35, 49]. Compared with the treatment of placebo, transplantation of human CSCs or their progeny in animal MI model results in the formation of new cardiac tissue, reduces relative infarct size, attenuates adverse remodelling and improves haemodynamics [50, 51]. Moreover, transplantation of human CSCs promoted greater myocardial regeneration and functional improvement in infarcted myocardium than transplantation of cardiac fibroblasts [35, 36], bone marrow mononuclear cells, skeletal myoblasts and adipose tissue-derived MSCs [47]. Despite no long-term engraftment and benefit to cardiac function in a MI mouse model [52], beneficial effects derived from the transplantation of CSCs have also been discovered by other authors [53].

Growing evidence has suggested that paracrine effects rather than cell differentiation of engrafted stem cells may mainly be responsible for their beneficial efficiency for post-MI treatment, because donor stem cells have poor viability and will rapidly and massively die due to harsh microenvironments in the infarcted myocardium. Together with their spontaneous commitment to cardiac and angiogenic differentiation, transplanted CDCs recruited endogenous regeneration and improved tissue resistance to ischaemic stress by secreting several paracrine factors [54]. CDC-conditioned media exerted antiapoptotic effects on neonatal rat ventricular myocytes, and proangiogenic effects on human umbilical vein ECs [54]. CDC-conditioned media has also been found to improve the contractile behaviours of isolated adult CMCs in a concentration-dependent manner and to normalize angiotensin (Ang) II-induced contractile dysfunction [55]. Other studies have also authenticated that CSCs improve post-infarct myocardial function mainly through paracrine factors to induce corresponding receptors and signalling pathways [56, 57]. In addition, CSCs may also release exosomes containing many growth factors, cytokines and miRNAs, to display their paracrine effects [58].

CSC-based transplantation therapy may be emerging as a novel approach for myocardial repair over conventional cardiovascular therapies. CSCs with predominantly myogenic and vasculogenic properties can be harvested from relatively small myocardial samples [59], and if appropriate techniques are used, direct culture and expansion of CSCs from myocardial tissue is simple, straightforward and reproducible [60]. Intracoronary infusion of autologous c-kit<sup>POS</sup> lineage<sup>NEG</sup> CSCs and autologous CDCs have recently been reported to effectively improve left ventricular systolic function and reduce infarct size, accompanied by no CSC-related adverse effects, in patients with CHF after MI in two randomized phase 1 clinical trials [61, 62].

However, time consuming, costly price 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. Improved methods for the expansion of human CSCs may improve cell production efficiency to avoid the high frequency of chromosomal abnormalities [63]. Nevertheless, techniques

to characterize culture-expanded stem cells, and then to deliver them and promote their engraftment into damaged hearts are still unsafe and inefficient. Many researches have reported that c-kit<sup>POS</sup> CSCs exhibit mesenchymal markers and produce osteoblasts and adipocytes [27, 64, 65]. Consistently, the spontaneous differentiation of CSCs into cells other than cardiac lineages, such as adipocyte and skeletal myocyte, has also been observed [66]. The formation of malignant tumour from transplanted stem cells alarms us that more attention should be paid to the unwanted differentiation together with other adverse potentials of stem cell transplantation after MI [46].

Endogenous cardiac regeneration from resident CSCs may avoid unwanted differentiation and malignant proliferation, coupled with fewer side effects. Stimulation of endogenous cardiogenic progenitor activity but not transdifferentiation to CMCs by exogenously delivered c-kit<sup>POS</sup> cells or by cell fusion is a critical mechanism of cardiac cell transplantation therapy [67]. The injected single cell would have no competitive growth advantage with respect to the remaining endogenous CSCs [19]. In light of these considerations, it is reasonable to develop strategies to improve the success of endogenous cardiac regeneration by activating resident CSCs, which may permit therapeutic myocardial repair without the need for cell expansion *in vitro* and consequent transplantation [68].

## Strategies to activate CSCs *in situ*

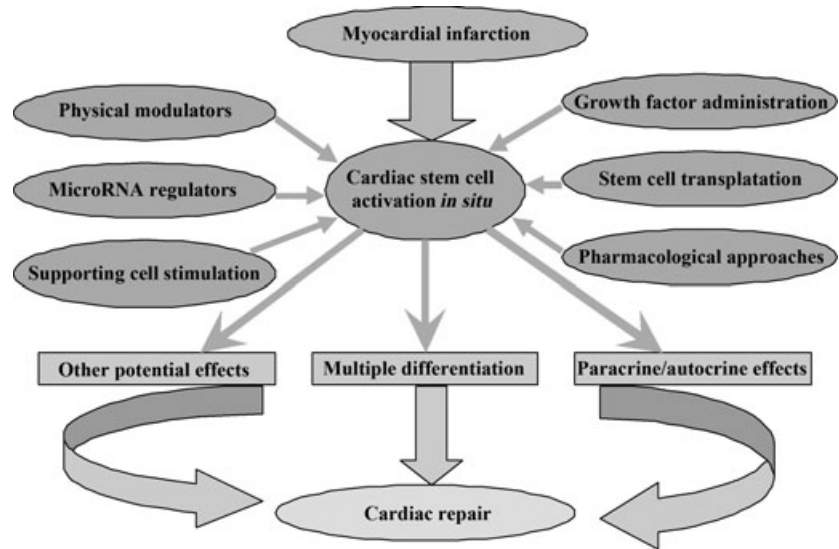
Despite that the transplantation of exogenous cardiac-regenerating cells has been predominant in the cardiac stem cell therapy post-MI in the last decade; myocardial regeneration *via* activation of endogenous CSCs has recently attracted increasing interest. Adult mammalian myocardium, including that in humans, contains a small pool of CSCs that can replenish the CMC population and the coronary microcirculation [14], and this cell pool is from the heart itself rather than from other organs that have a direct spontaneous role in myocardial homeostasis [19]. The heart homeostasis of CMC regeneration and death regulated by CSCs' ability to acquire the distinct cell lineages of the myocardium occurs physiologically, which is sufficient to maintain cell homeostasis and adequate pump performance, and these cellular processes are enhanced post-MI. The abundance of CSCs was found to increase after MI, and increase many more in the failure hearts than that in the healthy hearts, indicating that the human hearts in pathological conditions potentiate their myocardial regeneration and maintain their proliferative capability through activating the progenitors and precursors of cardiac cell lineages [29, 32, 68, 69]. However, the abundance of this CSC pool may gradually decrease in MI follow-up patients [13]. More p16(INK4a)-p53-positive senescent CSCs may account for the loss of functionally competent CSCs in chronic MI and underlie the progressive functional deterioration and the onset of terminal failure. Therefore, the possibility of activating CSCs *in situ* and maintaining the number of functionally competent CSCs may be a major challenge to develop clinically effective therapies of myocardial regeneration in the future research. Accumulating evidence has implied that several strategies discussed after (Fig. 1) may be implemented as novel modulators for resident CSC activation after MI.

## Locally activating CSCs by supporting cells *via* cell-to-cell communication

The myocardium possesses interstitial structures with the architectural organization of stem cell niches that harbour long-term label-retaining cells, providing functional evidence of resident CSCs in the myocardium [70]. Despite that contracting new cardiac myocytes exhibited an immature phenotype and frequent electric coupling with the cocultured neonatal rat ventricular myocytes, the formation of new human cardiac myocytes from human CSCs was at a relatively high frequency [68], which confirms that CSCs possess the potential to differentiate into functional cardiac phenotypes by cardiac microenvironment. The ability of resident CSCs to acquire a cardiomyogenic phenotype is subject to temporal limitations, and CMCs may be much more important than cardiac fibroblasts as modulators for CSCs in this process [71]. The modulation of the inflammatory response may enhance the regenerative response through increasing the stem cell pool and promoting its differentiation towards a myogenic lineage [31]. Cardiac myofibroblasts (MFs) involving in the inflammatory response may play a key role in infarct repair and scar formation following MI. Accumulated evidence has shown that atria tissues are the main source of CSCs [19, 27], and MFs have been demonstrated to co-localize with CSCs in remodelled human atrial tissues of valvular heart disease [72]. Cardiac MFs produce and secrete a large number of cytokines themselves, which help to maintain the inflammatory response to heart injury and may also activate resident CSCs [73]. Injection of extracellular matrix emulsion into the infarcted myocardium increases neovascularization and preserves cardiac function, potentially mediated by enhanced recruitment of c-kit<sup>POS</sup> cells along with MFs [74]. Furthermore, fibroblasts take part in the process of cardiac repair after MI partly by differentiating into MFs [73]. The recently described telocytes (TCs) may be another important supporting cell pool for post-MI activation of CSCs within the heart. TCs have been found in the myocardium, epicardium, endocardium and CSC niches, and form an interstitial system to assemble all cardiac cells in an integrative network [75]. CMC progenitors and TCs sustain a continuous cardiac renewal process in the adult mammalian heart, and the complex nanoscopic junctions between TCs and resident stem cells may be essential for the decision of stem cells to proliferate, differentiate and mature into new CMCs or other cardiac cell types [75, 76]. TCs, co-residing with CSCs in adult heart, may be a novel, possible target for therapeutic strategies aimed at potentiating cardiac repair and regeneration after MI, at least partly through nursing and guiding myocardial precursors to form the correct three-dimensional tissue pattern [77].

Stem cells do not exist in the absence of supporting cells within the niche, thus the direct physical interactions between stem cells and their non-stem cell neighbours in the niche are critical in keeping stem cells in this specialized compartment and in maintaining stem cell characteristics. CSCs and supporting cells represented by CMCs and fibroblasts in the niches may interact structurally and functionally through gap junction channel protein-mediated passage of small molecules and signals involved in cell-to-cell communication, and these gap junctions interfere with the activation, commitment and migration

**Fig. 1** Resident cardiac stem cells activation after MI can lead to endogenous cardiac repair through multiple differentiation, paracrine/autocrine effects and other potential effects. Several proposed modulators, such as growth factors, physical activations, pharmacological approaches, mircoRNAs, stem cell transplantation and supporting non-stem cell stimulation, may enhance and maintain these beneficial effects (see text for details).



of the niches of stem cells [19, 70]. In cardiac precursors, Notch, an evolutionarily conserved cell-to-cell communication modulator, prevents cardiogenic differentiation, favours proliferation and may facilitate the expansion of a transient amplifying cell compartment [78]. CSCs in the niches express Notch1 receptor which can interact with its receptor ligand Jagged1 expressed in the supporting cells [79]. The ephrin A1-ephA2 system between CMCs and human CSCs can promote CSC migration after MI [80]. Cell-to-cell calcium transients synchronous and the relative concentration of proteins, such as proteins atrial natriuretic factor, connective tissue growth factor and interleukin (IL)-1 receptor-like 1, may be crucial for cellular intertalk between CSCs and supporting cells [35, 81]. Growth factors may be important regulators involved in the process of CSC-recapitulated niche-like microenvironment being rich in stemness and cell-matrix interactions that rationalize stem cell's enhanced functional potency for myocardial repair [82]. Most notably, the knockdown of CSCs led to deprivation of myocardial trophic factors, accompanied by the compromised cardiomyogenesis and angiogenesis [83]. The interactions of ligands with its receptors between CSCs and supporting cells may possibly provide a novel strategy for the management of the infarcted heart *via* growth factor-stimulated CSCs *in situ* [80]. In addition to growth factor, the high expressed miRNAs in microvesicles or exosome may be new mechanisms of cell-to-cell communication between CSCs and supporting cells [26, 84]. For example, TCs release shed vesicles and/or exosomes, mediated transfer of genetic information, thus sending macromolecular signals involved in this cellular mode of communication [75, 85].

### Locally activating CSCs by transplanted stem cells

In most cases, the incidence of myocardial and vascular regeneration, either by transdifferentiation or cell fusion, appears too low minor to

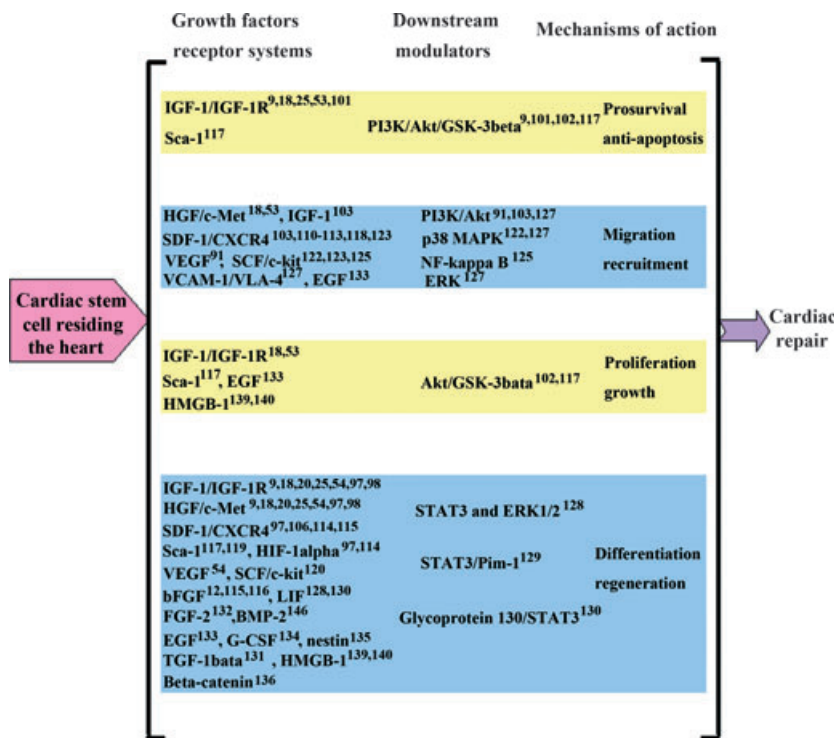
explain the significant recovery of cardiac function. The activation of resident CSCs by paracrine factors released by engrafted stem cell may at least partly account for the beneficial effects after cell transplantation. The stimulation of the proliferation and differentiation of endogenous CSCs was part of the regenerative repertoire of bone marrow MSC therapy for MI [1]. Bone marrow cells can home into the heart and give rise to cells that share properties of resident c-kit<sup>POS</sup> CSCs in the damaged heart following MI [86], and the subpopulation of these cells that differentiate towards CMCs may be CSCs [87]. Loffredo *et al.* [67] have also recently discovered that it is bone marrow-derived c-kit<sup>POS</sup> cells rather than MSCs that stimulates endogenous CMC progenitors and promotes cardiac repair during cell therapy following MI. Consistently, administration of exogenous CSCs was associated with increased proliferation and expression of cardiac proteins by endogenous CSCs, accompanied by increased recruitment of endogenous c-kit<sup>POS</sup> cells in the infarcted hearts [88, 89]. The paracrine factors secreted by the large number of injected stem cells could contribute to rearrange the post-ischaemic microenvironment and render it more suitable for resident CSCs homing, expansion and differentiation. For example, MSC-derived conditioned medium enriched in growth factors had protective effects on CSCs and enhanced their migration and differentiation [90]. The trophic factors from stem cell-conditioned medium are responsible for cardiac regeneration, independent of stem cell differentiation or stemness, which may provide a viable option for efficiency from resident CSCs cardiogenesis in future research.

### Local activation by growth factors and receptor systems

The heart has an endogenous reserve of CSCs possessing growth factor receptor systems, when activated with growth factors or stem cell-conditioned medium enriched with growth factors, these cells are

able to reconstitute dead myocardial tissue and recover cardiac function [16, 20, 44, 91–93]. Soluble factors released in the pericardial fluids following myocardial necrosis may play a role in the process of the reactivation of an embryonic program in epicardial c-kit<sup>POS</sup> cells [94], further supporting that activating resident CSCs by growth factors is of great feasibility. Till now, many growth factors and signalling modulators have been demonstrated to activate resident CSCs to repair infarcted heart (Fig. 2). Linke *et al.* [18] have discovered that a resident CSC pool with their early committed progeny possesses a hepatocyte growth factor (HGF)-c-Met and an insulin-like growth factor 1 (IGF-1)-IGF-1 receptor system that can be activated to induce their migration, proliferation and survival. When injected, HGF and IGF-1 can regulate resident CSCs to promote a significant restoration of dead tissue by the newly formed myocardium contained arterioles, capillaries and functionally competent myocytes in a dose-dependent manner [18, 20, 25]. Local activation of CSCs by IGF-1/HGF may also be able to reduce arrhythmogenesis *via* inducing the mobilization of CSCs and to counteract the renin-angiotensin system (RAS) in CSCs following MI [95, 96]. Locally delivered CSCs activated by HGF/IGF-1

before engraftment generate large *de novo* coronary arteries for restoration of blood supply to the ischaemic myocardium [97], and preconditioning with IGF-1 also reprograms Sca-1<sup>POS</sup> stem cells for prosurvival signalling and cardiomyogenic differentiation in the infarcted heart [9]. HGF and IGF-1 play different roles in the processes of CSC chemoattractant, proliferation and survival [53]. IGF-1 receptor can also identify a pool of human CSCs with superior therapeutic potential for their stronger ability to improve cardiomyogenesis and vasculogenesis than unselected CSCs [98]. Accordingly, stem cell transplantation with local myocardial IGF-1 delivery further enhance the recovery of myocardial structure and function than stem cells alone after MI [92, 99]. CSCs could even exert a paracrine survival effect on CMCs through induction of the IGF-1 receptor and signalling pathway [57], thus enhancing CSCs to release autocrine factors vascular endothelial growth factor (VEGF), IGF-1 and HGF, which may further potentiate their regeneration ability after MI, because the auto/paracrine loop is responsible for maintenance of the activated state of the CSCs for some time after the disappearance of the primary stimulus [54, 66].



**Fig. 2** Proposed modulators of growth factors and receptor systems coupled by downstream signalings that may be used as important therapeutic targets to activate resident cardiac stem cells (CSCs) to repair infarct heart. These modulators can activate CSCs *in situ via* different mechanisms of action, such as prosurvival, migration, proliferation and differentiation, resulting in cardiac repair following MI (see text for details). IGF-1, insulin-like growth factor 1; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor; PI3K, phosphatidylinositol-3-kinase; GSK-3, glycogen synthase kinase-3; SDF, stromal cell-derived factor; CXCR-4, CXC chemokine receptor 4; HIF-1alpha, hypoxia-inducible factor 1alpha; bFGF, basic fibroblast growth factor; SCF, stem cell factor; MAPK, mitogen-activated protein kinase; NF, nuclear factor; VCAM/VLA-4, vascular cell adhesion molecule/very late antigen-4; ERK, extracellular signal-regulated kinase; LIF, leukaemia inhibitory factor; STAT-3, signal transducer and activator of transcription 3; FGF-2, fibroblast growth factor 2; EGF, epidermal growth factor; G-CSF, granulocyte colony-stimulating factor; TGF-1beta, transforming growth factor-1beta; HMGB-1, high-mobility group box 1.

The IGF-1 treatment improved myocardial function and the improvement was associated with preservation of myocardial structure [100]. IGF-1-mediated nuclear phospho-Akt and telomerase delaying cellular ageing and death may partly account for this preservation, resulting from the preservation of functional CSCs undergoing cardiac commitment [101]. Preconditioning Sca-1<sup>POS</sup> cells with IGF-1 under oxygen glucose deprivation further enhanced cell survival through phosphatidylinositol-3-kinase (PI3K)/Akt-dependent caspase-3 down-regulation [9]. VEGF signalling may induce CSCs migration *via* the activation of PI3K/Akt in a concentration-dependent manner, and its role of homing CSCs is inhibited by either the VEGF receptor blocker or the PI3K/Akt inhibitor [91]. Thereby, Akt signalling may be a crucial modulator for human CSC maintenance in the heart [102]. Fischer *et al.* [89] have recently demonstrated that the overexpression of nuclear Akt in CSCs can enhance recruitment of endogenous CSCs, but the inability of CSCs overexpressing Akt to undergo lineage commitment hinders their capacity to provide functional or structural benefits to infarcted hearts. Inhibition of Akt pathway impaired the human CSC proliferation and induced apoptosis, whereas inhibition of glycogen synthase kinase-3 (GSK-3) enhanced their growth and survival, indicating that Akt/GSK-3beta may be crucial modulators for human CSC maintenance in human heart [102].

Elevated myocardial IGF-1 and phospho-Akt could also promote the expression of stromal cell-derived factor (SDF)-1alpha, resulting in massive mobilization and homing of c-kit<sup>POS</sup> and MDR1<sup>POS</sup> cells into the infarcted heart [103]. SDF-1alpha/CXC chemokine receptor (CXCR) 4 axis is a well-characterized chemokine for the mobilization and differentiation of CSCs in the injured heart [104–106]. Cardiac haeme oxygenase-1 gene transfer post-MI resulted in a notable increase in the number of c-kit<sup>POS</sup> stem cells recruited to the infarcted area after ligation, which may at least partly be promoted by coinduction of VEGF and SDF-1 in infarcted hearts [107]. Independent of the proangiogenesis effect, VEGF may also mediate cardiac repair *via* SDF-1-dependent and SDF-1-independent cascades [83, 108, 109]. Localized SDF-1alpha gene release or local delivery of SDF-1 significantly induces c-kit<sup>POS</sup> stem cell migration and homing [110–112], and the capability of SDF-1 homing CSCs can be enhanced by its overexpression [113]. Besides, clone-specific levels of SDF-1 expression both predict and promote development of a vasculogenic phenotype of CSCs *via* an autocrine mechanism, and hypoxia-inducible factor 1alpha could promote the synthesis and secretion of this growth factor, resulting in the acquisition of vascular lineages from c-kit<sup>POS</sup> CSCs [97, 114]. The activation of the SDF-1alpha/CXCR4 axis may also be considered as a downstream modulator of exogenously administered basic fibroblast growth factor (bFGF) to promote CSC-mediated myocardial regeneration, thereby improved the cardiac function after miniswine acute MI [115, 116].

Akt signalling activation may also be an important regulator involved in *ex vivo* expansion and apoptosis of CSCs *via* Sca-1 knock-down [117]. The Sca-1 might be a directly or indirectly essential component to promote CSC proliferation, differentiation and survival, and is also required to upregulate the secreted paracrine effectors that augment neoangiogenesis. Sca-1 plays a crucial role in the maintenance of cardiac integrity and in the recruitment, exhaustion of the precursor pool [118]. The expressed levels of Sca-1 in CSCs are

associated with the diverse differentiation potential of these cells, and may at least partly account for their unwanted differentiation into osteogenic and chondrogenic lineages [119]. Similarly, besides surface marker role, c-kit, the transmembrane tyrosine kinase receptor for stem cell factor (SCF), exerts novel functions to promote CSC differentiation and regulate CMC terminal differentiation [120]. The c-kit dysfunction impairs myocardial healing after infarction [121]. SCF/c-kit signalling may mediate the migration of CSCs *via* activation of p38 mitogen-activated protein kinase (MAPK), and both the antibody against SCF receptor and p38 MAPK selective inhibitor SB203580 can block the induction of CSC migration by SCF in the MI rat model [122]. The rapid induction of SCF during myocardial ischaemia strengthened the notion that SCF was involved in the activation of resident primitive c-kit<sup>POS</sup> cells and, thereby, in the increased formation of myocytes in the heart after MI [19]. Intramyocardial administration of SCF sustainably directs more lin<sup>NEG</sup>/c-kit<sup>POS</sup> stem cells to the heart following MI [123], and this cardiomyogenic lineage cells origin from the myocardium rather than bone marrow [124]. Inducing CSCs home to the injured myocardium by SCF may at least partly depend on the activation of nuclear factor (NF)-kappaB [125], because SCF levels can be decreased by the hyperhomocysteinaemia *via* inhibition of tumour necrosis factor-alpha-induced activity of NF-kappaB, resulting in the reduced migration of CSCs [126].

The soluble vascular cell adhesion molecule/very late antigen-4 signalling pathway may also induce migration of Sca-1<sup>POS</sup> cells and prevent CMC death from oxidative stress through activation of Akt, extracellular signal-regulated kinase (ERK), and p38 MAPK [127]. It is proposed that leukaemia inhibitory factor (LIF), a member of IL-6-related cytokines, regulates the commitment of CSCs into the EC lineage, contributing to neovascularization in the process of tissue remodelling and regeneration by activating both signal transducer and activator of transcription 3 (STAT3) and ERK1/2 [128]. The STAT3/Pim-1 signalling pathway has also been shown to play a crucial role in LIF-stimulated endothelial differentiation of cardiac resident Sca-1<sup>POS</sup> cells both *in vitro* and *in vivo* [129]. LIF and IL-11 transcripts were upregulated in post-infarct myocardium, accompanied by the induction of cardiac Sca-1<sup>POS</sup>/VE-cadherin<sup>POS</sup> cells, and the glycoprotein 130/STAT 3 may be a critical regulator in this process [130]. Other mediated factors, such as proteins of beta-catenin and nestin, and growth factors of fibroblast growth factor 2 (FGF-2), epidermal growth factor, granulocyte colony-stimulating factor, transforming growth factor-1beta (TGF-1beta) and others, may also provide potentially therapeutic options to be studied in clinical trials in human MI, due to their abilities to enhance CSC engraftment and differentiation [12, 105, 131–136].

Growth factors may be delivered locally to stimulate resident CSCs and promote myocardial regeneration, and this treatment could be repeated over time to reduce progressively tissue scarring and expand the working myocardium [53]. However, the differentiation of CSCs to CMCs does not occur when cells are challenged with soluble growth factors alone [137]. Without the recreation of a microenvironment critically featured by a fine-tuned combination of specific biological and physical factors, soluble growth factors alone cannot drive the differentiation of CSCs to CMCs, indicating that cardiac microenvironment coupled by growth factors in combination are determinants

of CSC differentiation. It is of importance to consider that only the synergistic cooperation of biochemical, topographic, chemical and physical factors could induce stem cells to adopt the desired phenotype [137]. Understanding the environment of the CSC niche may further obtain greater benefit from the full differentiation of resident CSCs, and miRNAs may be another important regulator in resident CSC-mediated cardiac repair after MI, due to their ability to regulate the expression levels of proteins that govern the process of resident cardiac regeneration.

## MicroRNAs as regulators

Emerging evidence potentiates miRNAs to be important regulators in resident CSC-mediated cardiac repair after MI, due to their ability to regulate the expression levels of proteins that govern the process of resident CSC activation. MiRNAs, a large family of post-transcriptional regulators of gene expression, are approximately 22 nucleotides in length, and their expression is tightly controlled in a tissue-specific and developmental stage-specific manner and some of them are highly and specifically expressed in cardiovascular tissues [26]. Gain- and loss-of-function studies have revealed that signature expression patterns of miRNAs play an important role in various physiological and pathological processes following MI, despite that their roles for diagnostic, prognostic and therapeutic applications in MI still need to be systematically evaluated. It has been shown that about half of miRNAs expressed in human heart are significantly dysregulated in acute MI patients in comparison with healthy controls and some miRNAs show the highest sensitivity and specificity for the discrimination of cases from controls [26].

Current evidence has revealed that in mouse models of MI, systemic administration of an antagomir designed to inhibit miR-92a targeting several proangiogenic proteins, including the integrin subunit alpha 5, led to enhanced blood vessel growth and functional recovery of damaged myocardium [138]. Exogenous high-mobility group box 1 protein induces myocardial regeneration after MI *via* enhanced c-kit<sup>POS</sup> CSC proliferation and differentiation [139, 140], and its beneficial effect on myocardial regeneration may partly attribute to increase the expression of miR-206 [141]. Hosoda *et al.* [23] have recently reported that the commitment of human CSCs to the myocyte lineage and the generation of mature working CMCs are influenced by miR-499. The authors reported that miR-499 traversed gap junction channels and translocated to structurally couple human CSCs favouring their differentiation into functionally competent cells. The repression of target genes Sox6 and Rod1 by miR-499 in human was demonstrated to account for the enhanced cardiomyogenesis *in vitro* and after MI *in vivo*. Furthermore, human CSCs overexpressing miR-499 showed greater aggregate volume of the regenerated myocyte mass and myocyte cell volume in animals when injected, accompanied by an additional positive effect on cardiac performance. Similarly, miR-499 together with miR-1 was demonstrated highly upregulated in differentiated cells in cultured human CMC progenitor cells by miRNA expression profiling by Sluijter *et al.* [142]. Also, transient transfection of these two miRNAs in human CMC progenitor cells markedly reduced proliferation rate and enhanced differentiation into CMCs,

likely *via* the repression of histone deacetylase 4 or Sox6. Consistently, small interference RNA-mediated knockdown of Sox6 strongly induced myogenic differentiation. The overexpressions of these two miRNAs also resulted in the upregulation of important cardiac myosin heavy-chain genes in embryoid bodies, and miR-499 overexpression simultaneously caused the upregulation of cardiac transcription factor Mef2c [143]. Takaya *et al.* [144] have also revealed that miR-1 and miR-133, exhibit directly transcriptional regulation by serum response factor (SRF) and Mef2 in the heart. MiR-1-1 and miR-1-2, specifically expressed in CSCs, are direct transcriptional targets of muscle differentiation regulators including SRF, MyoD and Mef2, accompanied by targeting Hand2, a transcription factor that promotes ventricular CMC expansion [145].

The miR-17-92 cluster plays important roles in the transition of cellular proliferation in c-kit<sup>POS</sup> 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 *Isl1* and *Tbx1* [146, 147]. MiR-29a could regulate cardiomyogenic differentiation by targeting secreted frizzled related protein 2 which is a member in the Wnt signalling and can govern cardiomyogenic differentiation by inhibiting a positive transcriptional autofeedback loop of *Wnt3a* [148, 149]. Also, Wnt signalling was reported to limit cardiac SP cell renewal, block endogenous cardiac regeneration and impair cardiac performance by the injection of recombinant *Wnt3a* protein into MI mice [150]. Other miRNAs, such as miR-21, miR-24 and miR-221 together could improve the engraftment of transplanted CSCs [151], and miR-155 could repress CMC progenitor cell death *via* targeting receptor interacting protein 1 [152]. MiRNAs may also help to avoid and prevent potential deleterious effects of unwanted differentiation and tumourigenesis, for example, miR-1 blocked the growth of rhabdomyosarcoma xenografts [26] and miR-669a and miR-669q prevented cardiac progenitors differentiating into skeletal muscle differentiation [153].

Therapeutic genes modification in CSCs and CMC progeny may improve the ability of these cells to repair infarcted heart [154]. CSC function could be altered and differentiation directed by miRNA modulation, thereby enhancing cardiomyogenic differentiation with subsequent cardiac recovery. However, more direct research is required to precisely ascertain the role of miRNAs in the course of endogenous cardiac regeneration from resident CSCs in the host myocardium.

## Pharmaceutical preparations and other modulators

The reservoir of CSCs possessing receptor systems can also be activated through the administration of systemic drugs, without the need for cell expansion *in vitro* and consequent transplantation [22]. Novel pharmacological approaches may be investigated to better the cardiac post-ischaemic matrix to create a proper microenvironment, for the infarcted myocardium is an inadequate milieu for the growth, differentiation and maturation of resident CSCs. For example, pravastatin may improve function of hibernating myocardium by mobilizing c-Kit<sup>POS</sup> bone marrow progenitor cells and promote myocytes to reenter the growth phase of the cardiac cell cycle [155]. CSCs express the



receptor of growth hormone releasing hormone (GHRH), and GHRH-agonist therapy substantially improved cardiac performance and reduced infarct size, partly *via* a regenerative process [156]. Pharmacological inhibition of Akt alleviated blockade of lineage commitment in CSCs, and cancer chemotherapy induces cardiotoxicity by targeting CSCs, further confirming pharmacological approaches as modulators of the reservoir of CSCs [89, 157].

Cardiac RAS is activated following MI and locally produced Ang II plays a pivotal role in the pathogenesis of myocardial repair and remodelling after MI. The documentation that a local RAS is present in human CSCs and that the formation of Ang II, together with the expression of Ang II type 1 receptor (AT1R), increasing with age in human CSCs provides evidence in favour of the role of this octapeptide in human CSC senescence and death [98]. It is proposed that the increased Ang II which induces contractile dysfunction post-MI may be normalized by the activation of the resident CSCs to release growth factors or cytokines [55]. The presence of AT1R identified senescent human CSCs with impaired growth reserve and increased susceptibility to apoptosis [98]. In addition, decreasing the attenuation of the IGF-1/IGF-1 receptor and HGF/c-Met systems may counteract the RAS in CSCs, resulting in decreased cellular senescence, growth arrest and apoptosis [96]. The post-MI expression of AT2Rs was mainly induced in cardiac c-kit<sup>POS</sup> precursor cells, and the cell population of cardiac c-kit<sup>POS</sup>AT2<sup>POS</sup> accumulated in peri-infarct zone has characteristics of self-renew and cardiogenic differentiation [158]. AT2R stimulation inhibited apoptosis of CMCs *in vitro* and *in vivo*, and led to an increased c-kit<sup>POS</sup>AT2<sup>POS</sup> cell population in the infarcted myocardium in rats with acute MI. The modulation of components of the cardiac RAS by cardiac-selective overexpression of AT2R protects heart function from ischaemic injury and attenuates the upregulation of AT1R induced by MI [159]. Therefore, it can be postulated that the utility of AT1R blockage and AT2R agonist may improve the impaired growth reserve and decrease susceptibility to apoptosis of CSCs. However, it is metoprolol rather than losartan that can increase the number of c-kit<sup>POS</sup> CSCs following MI treatment [24].

Isoproterenol injury activates CSCs to repair injury myocardium through differentiating into new CMCs during cardiac repair [160]. CSCs expressed a decreased and inverted complement of beta1/beta2-adrenoreceptors and absence of ryanodine receptor 2, resulting in better resistance to the hyperadrenergic state in CHF [37, 161]. These findings suggest that endogenous CSCs possess intrinsic abilities to repair infarcted heart partly through their resistant response to the hyperadrenergic state and differentiation, and beta-adrenoreceptor blockers may further enhance these abilities. Female gender was associated with a higher number of c-kit<sup>POS</sup> CSCs in human heart tissue than male gender [27], which suggests that sex-specific hormone may at least account for this difference. Consistently, cardiac oestrogen receptor was upregulated in post-infarct cardiac c-kit<sup>POS</sup> cells accumulating in peri-infarct myocardium in MI rats, and when activated it supports survival of CMCs through post-infarct cardiac c-kit<sup>POS</sup> cells [162]. GHRH-agonist and oxytocin therapy may substantially improve cardiac performance and reduce infarct size through the interactions with their receptors expressing in CSCs [49, 65, 156]. Hyaluronan treatment could stimulate the differentiation of epicardial cells, a promising source of CSCs [163], and hyaluronan mixed esters

of butyric and retinoic acids (HBR) have also been reported to increase the secretion of VEGF and HGF which can activate endogenous CSCs [164], resulting in endogenous regeneration after MI. In addition to its effects on paracrine factor secretion, HBR therapy may also lead to a high throughput of cardiogenesis by targeting Smad proteins and activating mitogen-activated protein kinase kinase kinase 1 signalling cascades [163, 165]. Erythropoietin could potentially reduce the risk of heart failure *via* restoring endothelial differentiation of CSCs [166], and drugs to normalize hyperhomocysteinaemia may also be used as therapeutic choice for cardiac repair *via* CSC homing after MI [126]. Besides drugs, physical modulators, such as low-frequency electromagnetic fields tuned at Ca<sup>2+</sup> ion cyclotron energy resonance and spontaneous calcium oscillations, may drive CSC differentiation, engraftment and expansion [167, 168].

## Conclusion

This review provides systemic insight into the identification of a CSC pool, the beneficial effects of the transplantation of CSCs, most important, the possibility of activating resident CSCs for cardiac repair following MI. Stem cell-based therapy is emerging as a novel approach for myocardial repair over conventional cardiovascular therapies, but it may only be considered as an adjunctive therapy at present. CSCs, through both cell transplantation and *in situ* activation, have the capacity to reconstruct dead myocardium and restore anatomical integrity and ventricular function. Albeit little evidence has recommended the pros or cons between the transplantation of CSCs and endogenous CSC activation *in situ*, the latter tended to be more reasonable and safer because of its intrinsic advantage to avoid unwanted differentiation and malignant proliferation coupled with other potential side effects.

Cellular therapy of the infarcted heart with a variety of different CSC types has shown encouraging effects on cardiac function. Although great endeavour has been made to distinct stem cells from progenitor cells within the heart, the most common misunderstanding originates from the uncertain difference between these two different cell pool has still been confusing most researchers at this time [169]. There is an exact difference between these two cell types, but it is still difficult to distinguish them from each other. Till now, different CSC types have been characterized and classified by their different cell surface markers rather than on biological properties displayed in their original environment *in vivo* [31]. The same stem cell population can display different functional phenotypes without consensus cell surface markers, due to the lack of strictly standardized procedures for isolating and expanding stem cells. None of the surface markers that have been utilized to characterize the cells are specific for CSCs, and the expressed surface markers may differ between species and could also change in different laboratories [170]. The use of these markers, each supposedly identifying a specific stem cell, created confusion in the scientific community regarding which cell is the real CSC and what is its origin [169]. It is highly speculated that the different putative adult CSCs reported so far, do not represent different cell types but, instead, different developmental and/or physiological stages of a unique resident adult CSC [169, 170]. The origin of these cells may

be intrinsic cells present in the myocardium from embryonic and foetal life or cells from an extracardiac pool of circulating progenitors, possibly coming from the bone marrow, which have colonized the myocardium in post-natal life, where they acquire tissue-specific properties [31, 169]. These two hypotheses need not necessarily be mutually exclusive, as growing evidence has suggested that extracardiac stem cells can exert a beneficial effect on CSCs residing within the heart. However, the origin of all these cells is not fully completed, and few studies have focused on the comparison of the beneficial effects of these cells termed as CSCs with different surface antigens for MI with resultant heart failure treatment. Therefore, consentaneous and standardized protocols should be made to isolate and expand antigen-specific CSCs prior to their clinical applications.

The recognition that a stem cell compartment exists in the adult human heart answers only partly the question of whether these cells retain the capacity to divide and differentiate throughout life or whether the multiple variables that affect human beings, including age, diseases and the interrelated effects of genes, environment and probabilistic changes, interfere with the growth of human CSCs, limiting their therapeutic efficacy [171]. The differentiation and expansion of CSCs may result from multiple signalling parameters operating in a tightly regulated spatiotemporal pattern. Cell-to-cell communication between the engrafted stem cells or supporting cells within the heart and resident CSCs has been proved to play important roles in the activation of resident CSCs. And paracrine/autocrine-mediated growth factors and miRNA release are crucial regulators involved in the process of cell-to-cell communication. The reservoir of CSCs possess receptor systems that can be activated through the administration of growth factors and systemic drugs, and miRNAs that regulate the

expression of growth factors and other proteins, thus, are proposed regulators for the activation of resident CSCs after MI.

However, the acclaimed paradigm shift of parenchymal cells replaced by resident CSCs or by other cells that are recruited into the infarct heart may be limited [21]. Growth factors or miRNAs, or drugs alone cannot acquire full beneficial effects from stimulating resident CSCs. The host environment including genomic and proteomic substrates is important to regulate molecular signal pathways involved in the course of activation of resident CSCs to integrate with adult CMCs fully differentiated from CSCs. A comprehensive understanding of positive and negative modulators can then result in the identification of the most appropriate factors to optimally harness the enormous potential of resident CSCs to achieve efficient myocardial regeneration. Many of the above issues have not been extensively tested yet in *in vivo* MI models, let alone in clinical practice. Optimal answers to all of these issues may pave the way for potential utility of endogenous CSCs as non-invasive stem cell therapeutic options for clinical MI treatment a reality in coming future.

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## Conflict of interest

There are no conflicts of interest.

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