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REVIEW

Challenges for heart disease stem cell therapy

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Abstract: Cardiovascular diseases (CVDs) are the leading cause of death worldwide. The use of stem cells to improve recovery of the injured heart after myocardial infarction (MI) is an important emerging therapeutic strategy. However, recent reviews of clinical trials of stem cell therapy for MI and ischemic heart disease recovery report that less than half of the trials found only small improvements in cardiac function. In clinical trials, bone marrow, peripheral blood, or umbilical cord blood cells were used as the source of stem cells delivered by intracoronary infusion. Some trials administered only a stem cell mobilizing agent that recruits endogenous sources of stem cells. Important challenges to improve the effectiveness of stem cell therapy for CVD include: (1) improved identification, recruitment, and expansion of autologous stem cells; (2) identification of mobilizing and homing agents that increase recruitment; and (3) development of strategies to improve stem cell survival and engraftment of both endogenous and exogenous sources of stem cells. This review is an overview of stem cell therapy for CVD and discusses the challenges these three areas present for maximum optimization of the efficacy of stem cell therapy for heart disease, and new strategies in progress.

Keywords: mobilization, expansion, homing, survival, engraftment

Introduction

The recovery of function after a myocardial infarction (MI) is dependent on increasing blood flow and regeneration of tissue. Stem cells (SCs) can provide cellular precursors for cardiomyocyte differentiation, endothelial and supporting cells, as well as signals for activation of cells and prevention of apoptosis. The results of clinical trials have been encouraging, however either no change or only small increments in recovery were found. Recent reviews of completed clinical trials (2002–2010) for SC therapy report improvements of 10% or less in about half of the studies.[1–4] In the review by George,[1] 13 studies of SC therapy for acute MI were described. In the eight randomized controlled studies, bone-marrow (BM) cells were administered by intracoronary injection and left ventricular ejection fraction (LVEF) measured 3–6 months following the MI. In five of the randomized controlled trials, there was only an average increase of 6% (3%–12%) in cardiac function. Mozid et al[2] reported two additional studies of BM SC therapy for acute MI,[5,6] and only one study showed improvement (5%) of LVEF function. Mozid et al[2] also described eight clinical trials of SC therapy for chronic ischemic heart failure. There was improvement in LVEF in three of the four studies in patients treated with BM SCs and improvement in two

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of the four studies in patients transplanted with autologous skeletal myoblasts. Wen et al[4] performed a meta-analysis of eight randomized controlled trials and concluded that BM cell therapy provided only moderate (6%–10%) but definite improvements in LVEF. SC therapy has the potential to provide gains not only for MI, but also for chronic ischemia and heart failure. Currently, there are 33 ongoing clinical trials described on the ClinicalTrials.gov Website[7] (see Table 1). While autologous BM cells are still the major source of SCs in the ongoing studies, new SC sources are rigorously being investigated. SC therapy for cardiovascular disease (CVD) is an intensive area of research, and collective improvements in the source and number of SCs, and better mobilizing and homing agents, are needed to increase the effectiveness of this emerging therapy.

Challenges for SC therapy

Improved identification and expansion of autologous SCs and their role in cardiac recovery

In the 1960s, Till et al,[8] while studying the components responsible for regenerating blood cells, defined two required properties of SCs: (1) self-renewal – the ability to go through numerous cycles of cell division while maintaining the undifferentiated state; and (2) potency – the capacity to differentiate into specialized cell types. SCs are identified by their capacity to form colonies in culture and by cell surface markers that are cell specific. The majority of clinical trials of SC therapy for heart disease have used BM cells, particularly the mononuclear cells (MNCs) (Figure 1). In the ongoing trials listed

Table 1 Ongoing clinical trials of stem-cell therapy for heart diseases

Condition	Stem cells	Phase	Acronym	ClinicalTrials.gov NCTID
Congestive heart failure	Skeletal myoblasts	II/III	MARVEL	NCT00526253
Old MI	Skeletal myoblasts	II	PERCUTANEO	NCT00908622
Angina, coronary disease	Bone marrow	II		NCT01214499
Ischemic heart disease	Bone marrow	II		NCT00690209
CAD, AMI	Bone marrow	I/II	REPAIR-ACS	NCT00711542
MI, ischemia	Bone marrow	I/II		NCT01267331
AMI	Bone marrow	II/III	REGEN-AMI	NCT00765453
CAD	Bone marrow	II/III		NCT00130377
Chronic ischemic heart failure	Bone marrow	II/III	REGEN-IHD	NCT00747708
MI	Bone marrow/AC 133	III		NCT01167751
Congestive heart failure	Bone marrow	I/II		NCT01061580
Non-ischemic dilated cardiomyopathy	Bone marrow	I/II	POSEIDON-DCM	NCT01392625
Dilated cardiomyopathy	Bone marrow	II	NOGA-DCM	NCT01350310
Cardiomyopathy	Bone marrow	II	REGENERATE-DCM	NCT01302171
Ischemic heart failure	Bone marrow/PBC	III	ESCAPE	NCT00841958
Left ventricular dysfunction	Bone marrow	II	TIME	NCT00684021
Left ventricular dysfunction	MSC, bone marrow	I/II	TAC-HFT	NCT00768066
Ischemia, left ventricular dysfunction	MSC	I/II	MESAMI	NCT01076920
MI	Mesenchymal precursors	I/II		NCT00555828
AMI, heart failure	MSC	III	ESTIMATION	NCT01394432
Chronic ischemic heart disease	MSC	II	MyStromalCell	NCT01449032
Congestive heart failure	MSC	I/II		NCT00644410
Dilated cardiomyopathy	CD34+	II		NCT00629018
AMI	CD133+		SELECT-AMI	NCT00529932
MI	CD133+	II/III		NCT01187654
MI, CAD	CD133+	I/II	PERFECT	NCT00950274
CAD	CD133+	III		NCT01049867
MI, heart failure	CD133+	II	IMPACT-CABG	NCT01033617
AMI	Adipose tissue-derived	II/III	ADVANCE	NCT01216995
Heart failure	Cardiac progenitor	I	TICAP	NCT01273857
Congestive heart failure	Cardiac	I	ALCADIA	NCT00981006
MI	Cardiosphere	I	CADUCEUS	NCT00893360
CAD, congestive heart failure	Cardiac	I	SCIPIO	NCT00474461

Source: ClinicalTrials.gov Website.[7]
Abbreviations: AMI, acute myocardial infarction; CAD, coronary artery disease; MI, myocardial infarction; MSC, mesenchymal stem cell; PBC, peripheral-blood cell.

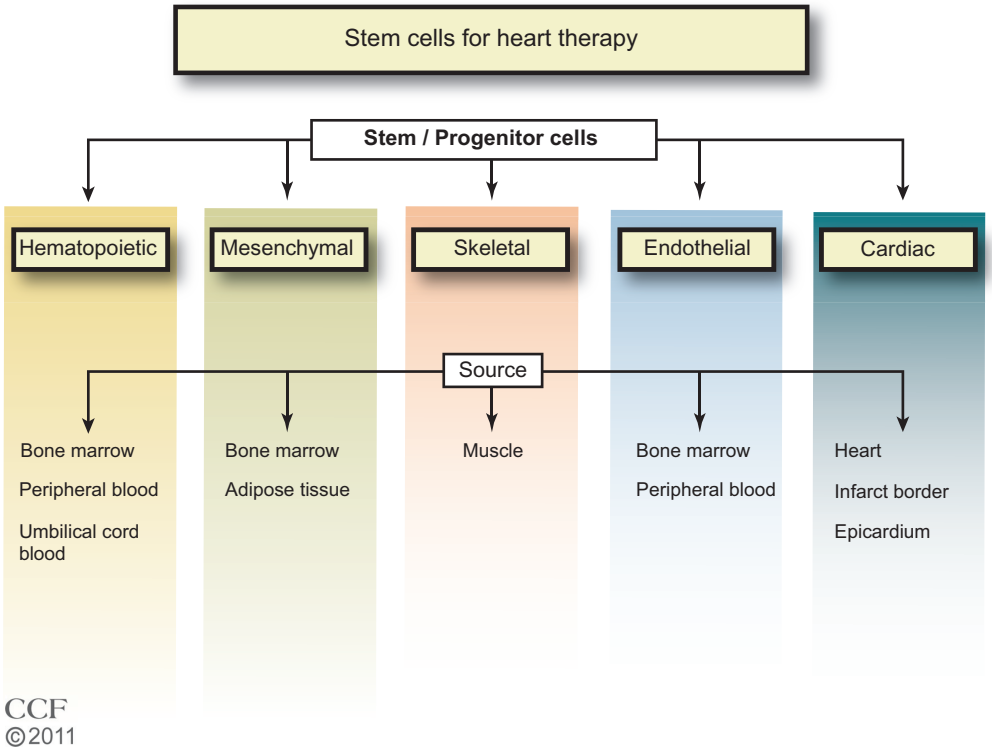

Figure 1 Types of stem cells in use for heart disease therapy.[1–7]

in Table 1, other types of SCs are being tested, including specific BM, CD34+ or CD133+, and mesenchymal cells. One study tests adipose tissue-derived SCs, and three trials are testing cardiac progenitor/stem cells.

Skeletal myoblasts

Skeletal myoblasts isolated from muscle biopsies were the first cells used for the SC therapy for cardiac recovery.[9] In a comparison of rats with chronic MI, treated with human skeletal myoblasts or BM-derived CD133+ progenitors, improvements in cardiac function were similar with the two cell types.[10,11] In trials of skeletal myoblast treatment[3] in patients with chronic ischemic heart failure, there were improvements in LVEF in two of four studies (SEISMIC, TOPCARD-CHD).[3] While the initial evaluation in clinical studies of skeletal myoblast treatment showed there was improved function, the effect was not sustained, and the cells were not electrically integrated into the heart.[12] Enthusiasm for this approach has waned. However, second-generation products are now being developed.[9,13] Six trials of skeletal myoblast therapy have been discontinued, but currently there are two active trials with skeletal myoblasts (Table 1) for patients with an old MI (PERCUTANEO) or congestive heart failure (MARVEL).

Hematopoietic progenitor/stem cells (HPSCs)

In clinical trials for MI or ischemic heart disease, BM, peripheral blood (PB), or umbilical cord blood (UCB) have been used as the source of SCs.[1,3] Autologous BM and PB have an advantage over UCB cells since UCB cells may be at risk for immunological rejection. However, the UCB have a high proliferation potential.[12] Autologous BM cells from aging individuals may have reduced transplant efficiency, and UCB cells would be advantageous.[14,15] A limitation of the PB is the low yield of SCs. BM is the major source of adult SCs and the best characterized. The BM cells have long been used in therapeutic BM replacement for blood diseases.[16–18] BM SCs provide the myeloid and lymphoid lineages that give rise to blood cells.[19] The cell surface markers that identify hematopoietic SCs (HSCs) for humans include: CD34+, CD59+, Thy1/CD90+, CD38lo/–, c-kit/CD117+, and lin–. There are differences in mouse HSC markers; namely, CD34lo/–, Sca-1+, Thy1.1+/lo, and CD38+, but with c-kit+ and lin– as common markers. The lineage negative designation includes the absence of 13–14 cell surface markers found on mature cells. BM has been the major source of SCs for reported and ongoing clinical trials. Currently, studies are underway that isolate subsets of the

BM cells such as CD34+, and CD133+ for use in therapy. Whether these subsets of SCs will have an advantage in heart disease recovery remains to be seen.

Endothelial SCs

Stages of lineage development of endothelial SCs and their sites of origin are less well defined than those for the hematopoietic lineage.[20] The endothelial progenitor cells (EPCs) found in the PB are thought to originate in the BM from a subset of SCs or from the myeloid precursors. There is considerable controversy with regard to the identification of the EPCs.[21] Some investigators have identified the EPCs as CD34+ cells and/or CD133+ cells,[22] while others view these cells as HPSCs.[23,24] Recently,[25,26] a consensus definition of EPC markers was suggested for cross-study comparisons and with the cell surface markers CD31+, CD34 bright, and CD45, AC133, CD14, CD14a, CD235a, Live/Dead Violet negative. Of importance for identification of the EPC is the ability to become endothelial cells (ECs) in culture. While CD34+ and/or CD133+ cells in culture may become ECs, the CD34+ and/or CD133+ cells could be a mixture of subpopulations. However, the cells identified as CD34+ and/or CD133+ may be more effective in providing paracrine factors and stimulating neovascularization than the commonly used BM MNCs. Tongers et al[27] recently described the results of a clinical trial for patients with refractory angina treated with intramyocardial autologous CD34+ cells, finding significant improvements in angina frequency and exercise tolerance. There is one clinical trial currently underway for treatment with CD34+ in patients with dilated cardiomyopathy, and five clinical trials underway for the treatment of MI, CAD, and heart failure with CD133+ cells. One study, NCT01187654, will compare the treatment of CD133+ cells and BM MNC in MI patients. This comparison could be informative as to whether the CD133+ cells have an advantage over the more frequently used BM MNC. Bissels et al[28] found that microRNAs were expressed differentially in CD133+, CD34+, and CD133- cells involved in differentiation, prevention of apoptosis, and cytoskeletal remodeling.

Mesenchymal SCs (MSCs)

The MSCs are found in the BM and other tissues. MSCs are positive for CD44, CD73, CD90 (Thy1), and CD105, and negative for the hematopoietic markers, CD45, lineage markers, EC (CD31), and macrophage (CD11b/MAC-1).[29] The MSCs have advantages over HSCs.[27,30] Compared with HSCs, MSCs are more abundant, readily proliferate in culture, and are easily differentiated into different cell types, such as adipocytes, fibroblasts, osteocytes, and myoblasts. Further, studies suggest that MSCs may be more potent for cardiac repair than HPSCs.[31] Although the MSCs can be differentiated into cardiomyocytes, immortalization was important and could increase the potential of tumor formation.[15] In addition to BM, adipose tissue can also be used as an abundant source of MSCs.[32,33] The MSCs from UCB, adipose tissue, and BM expressed the same cell surface markers; however, there are some differences in the percentage of certain markers and colony heterogeneity. Gaebel et al[34] compared treatment of MI in mice with MSCs from UCB, adipose tissue, and BM. Cells from BM, adipose tissue, and UCB CD105+ showed improvements in heart functions, decreased infarct size, and capillary density. UCB CD105 treated mice had reduced collagen deposition compared with BM and adipose tissue cells, and BM and UCB CD105 cells additionally had reduced apoptosis when compared with mice treated with adipose tissue cells. This study suggests that the function of the MSCs may be dependent on the source. Clinical trials with MSCs[35–37] are promising, and currently there are 19 clinical trials underway.[7,38] In a recent randomized, double blind, placebo-controlled study[37] with MSC therapy after acute MI; there was improvement in the global assessment of cardiac function at 6 months in 45% of the patients.

Cardiac progenitor cells (CPCs)

Although it had been believed for a long time that cardiac myocytes were terminally differentiated, dividing myocytes found in the heart implied that there are resident or noncardiac cardiomyocyte progenitor cells.[39] There have been intensive efforts to identify the cardiomyocyte stem and progenitor cells in the last 10 years.[39] Purified cardiomyocytes isolated from rodent hearts dedifferentiate and divide, expressing SC markers such as c-kit, Sca-1, Isl1, and Abcg2.[40–45] CPCs have been isolated from human myocardial biopsies.[46,47] These same cells can organize into spheres and re-differentiate into myocytes and ECs.[48] Yamada et al[49,50] suggested that $CD133^+$ cells from brown adipose tissue were highly effective in differentiation into cardiomyocytes compared with HPSCs, and that mouse BAT CD133+ cells efficiently induced BM SCs into cardiomyocytes (CD45- CD31- CD105+) differentiation. There are four ongoing clinical studies to test autologous CPCs (Table 1); one study (ALCADIA) will use cardiac-derived SCs to treat ischemic cardiomyopathy, and two studies will take advantage of the cardiosphere-derived stem/progenitor cells (derived from cell outgrowth of autologous cardiac biopsy) for patients with a recent MI (CADUCEUS) or heart failure (TICAP). In the SCIPIO trial, patients with

ischemic cardiomyopathy are treated with c-kit+lin– CPCs derived from the right atrial appendage, and initial results from 16 patients report that LVEF increased and infarct size decreased.[51]

Adipose tissue-derived SCs (ASCs)

Cells isolated from adipose tissue can be separated by centrifugation into adipocytes and stromal vascular cells. The stromal vascular fraction may contain preadipocytes, pericytes and EPCs, adult multipotent MSCs, circulating blood cells, fibroblasts, ECs, smooth-muscle cells, and immune cells. This stromal vascular fraction may differentiate into a number of cell lineages, including the adipocytes, cartilage, bone skeletal muscle, neuronal cells, ECs, cardiomyocytes, and smooth-muscle cells.[52,53] ASCs are defined as CD44 and CD105 positive, and Cd11b, CD34, and CD45 negative cells. Although there is disagreement regarding the capacity of ASCs to differentiate into ECs, freshly isolated human ASCs also consist of EPCs (CD11b, CD34, and CD45 positive cells) and when cultured they have a cobblestone appearance and take up acetylated low-density lipoprotein. Bai et al[54] found that human freshly isolated adipocytes or cultured adipose tissue-derived cells underwent cardiomyogenesis through a fusion-independent pathway. Takahashi et al[55] reported that in rat femoral artery injury, ASCs did not differentiate into ECs, but were able to inhibit neointimal formation by the secretion of paracrine factors. There is one ongoing clinical trial (NCT01216995) testing adipose tissue-derived cells in patients after an acute MI.

Induced pluripotent stem (iPS) cells

Another potential source of SCs is iPS cells.[56] This source relies on in vitro de-differentiation of adult cells to embryonic-like SCs and then reprogramming using specific culture conditions to induce cardiac lineage cells including cardiomyocytes, smooth-muscle cells, and ECs. Adult cells most commonly used for iPS cells are fibroblasts and may be derived from a variety of tissues such as dermal, liver, stomach, pancreas, and neural and hematopoietic cells. Endogenous non-BM SC and iPS cells have been characterized in animal models and some have been identified in adult humans. Defining these cells and their requirements for proliferation and mobilization will provide additional options for enhanced efficacy of SC therapy.

Embryonic SCs (ESCs)

The ESCs are the ideal SCs, due to the fact that cultures of embryonic cells when stimulated can develop into >200 adult cell types.[38,57,58] Current efforts focus on establishing the conditions for directed differentiation of cells by altering the chemical composition of the culture medium, altering the culture surface, or inserting genes.[58] A major challenge is the potential of uncontrolled differentiation when injected directly into an animal, and the potential for tumor formation. The promise of ESCs is to genetically modify lethal debilitating chronic disease. There are currently four clinical trials in progress of human ESCs for spinal cord injury and macular degeneration, but unfortunately none for cardiac disease.[38]

Expansion of SCs

A critical step for improved SC therapy is the expansion of accessible SCs (Figure 2). The homing of cells to injured tissues is very inefficient, and increasing the number of cells that are available for treatment would be beneficial. Autologous BM cells, adipose tissue, myocardial, and UCB are cultured ex vivo to increase the number of cells. Culturing the tissue also allows selection of specific cells. The ESCs and iPS cells require additional steps prior to expansion of a preparation. The iPS cells require de-differentiation as an initial step and then both iPS cells and ESCs are induced to differentiate prior to expansion. SCs in culture form colonies, and proliferation without differentiation requires a specific sequence and timing of the availability of growth factors and cytokines.[59–66] In addition, these cells must maintain their pluripotency. Cells need to be free of feeder-cells, serum proteins, and microbial agents. Large-scale expansion with maintenance of pluripotency and transplant safety is required.[58,67] Currently, effective cell culture proliferation is limited,[61] and further studies are needed to understand the requirements for expansion. New approaches are being investigated including the use of nanofibers with growth factors, mesenchymal stromal cells in cultures of HSCs, and genetic manipulation of UCB HSCs.[68–72] To improve SC therapy, improved methods of SC ex vivo expansion are required.

Identify mobilizing agents with improved effectiveness

SC niches

Intensive studies are underway to identify new sources of stem and progenitor cells for therapy. In addition to BM, SC niches have been identified (Figure 3) in heart. The SC niches are defined as a microenvironment with one or more SC that regulates self-renewal and progeny in vivo.[73,74] Self-renewal occurs in all tissues and in addition to BM, niches of SCs have been identified in heart, arteries, veins, gonads, intestine, epidermal tissue, and neural tissue.[73,75–77] The

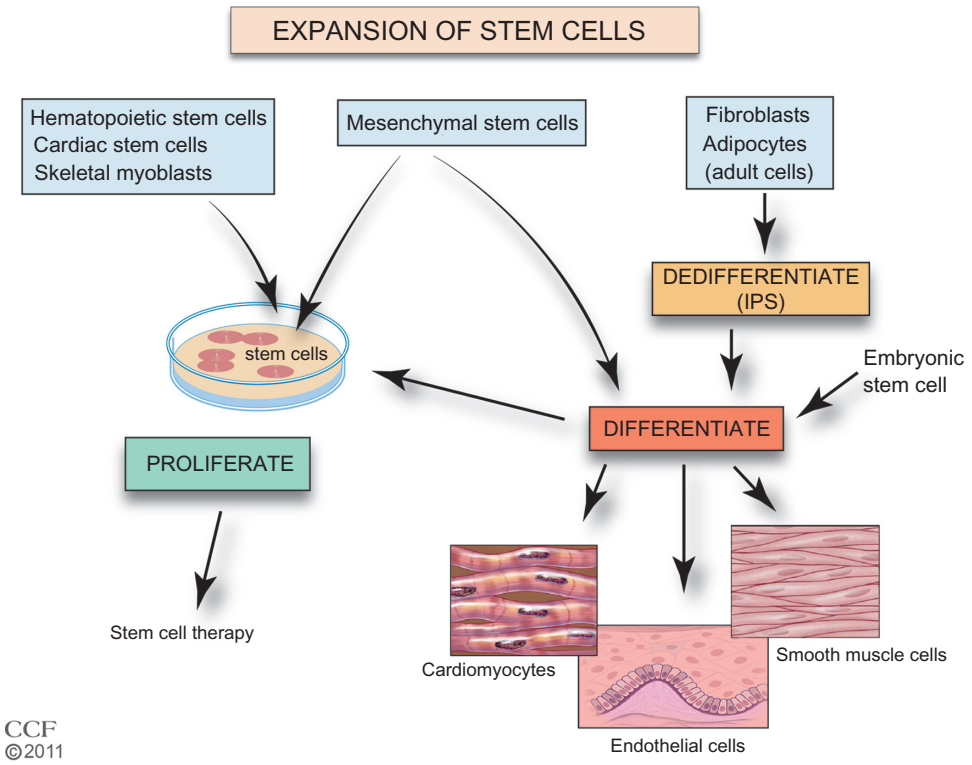

Figure 2 Expansion of stem cells.
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Notes: Currently, increased numbers of autologous hematopoietic, mesenchymal, cardiac, endothelial, and skeletal stem cells can be generated by expansion in culture with proliferation specific conditions. Adult cells such as fibroblasts or adipocytes may be dedifferentiated in culture to stem cells (iPS cells). MSCs, iPS cells, and ESCs can be induced to differentiate and proliferate in cell culture. Use of differentiated MSCs, iPS cells, and ESCs is in preclinical development.
Abbreviations: ESC, embryonic stem cell; iPS, induced pluripotent stem; MSC, mesenchymal stem cell.

non-BM SCs were initially defined by immunofluorescence in tissue, but given the number of markers needed, this became untenable, and isolation and identification of SCs by flow cytometry using multiple markers simultaneously has made it possible to isolate and investigate the function of these cells. Recently, lineage mapping has been utilized to locate niches in animal models by genetically labeling SC markers and identifying their location in adult tissue.[78,79] An example of lineage mapping is the recent study of Tamura et al[78] of neural crest-derived SCs found in the heart that migrate and differentiate into cardiomyocytes after MI. The lineage mapping has been utilized for locating SC niches in a variety of developing organisms.[79] The number of quiescent SCs is small, and better detection methods are necessary. Further, identifying the regulation and recruitment of these endogenous SCs in adults is critical.

Mobilization of BM SCs

In the BM, SCs reside in an endosteal niche along with stromal cells, mesenchymal cells, and ECs. The SCs are retained in the BM with high concentrations of stromal-derived factor (SDF)-1, the major chemoattractant for SCs. The SDF-1 SC receptor, CXCR4, is found in low concentrations. Stimulation with cytokines or growth factors may interrupt ligand/receptor balance. With a decrease in SDF-1 and an increase in CXCR4 expression, a signaling gradient with the PB allows the egress of the SCs from the BM (Figure 4). Granulocyte colony-stimulating factor (G-CSF) is widely used clinically for SC mobilization and sometimes in conjunction with other factors[57,80] including granulocyte-macrophage colony-stimulating factor, stem cell factor, fms-like tyrosine kinase (Flt)-3 ligand, and interleukin-1, -3, -6, -7, -8, -11, and -12 (Figure 3). AMD3100, an inhibitor that blocks SDF-1 binding to CXCR4; CTCE-0021, a CXCR4 agonist; recombinant human growth hormone, a pleiotrophic cytokine; parathyroid hormone; pegfilgrastim, pegylated G-CSF with a prolonged half-life, and thrombopoietin, a cytokine that regulates megakaryocytopoiesis, are also being investigated.[80]

In addition to cytokines and growth factors, proteases such as neutrophil elastase, cathespin G, plasmin, and matrix metalloproteinase (MMP)-9 have been implicated in BM SC mobilization.[81–86] After G-CSF treatment, these proteases increase in BM as well as in plasma; however, studies[83] in mice deficient in neutrophil elastase or cathespin G suggest

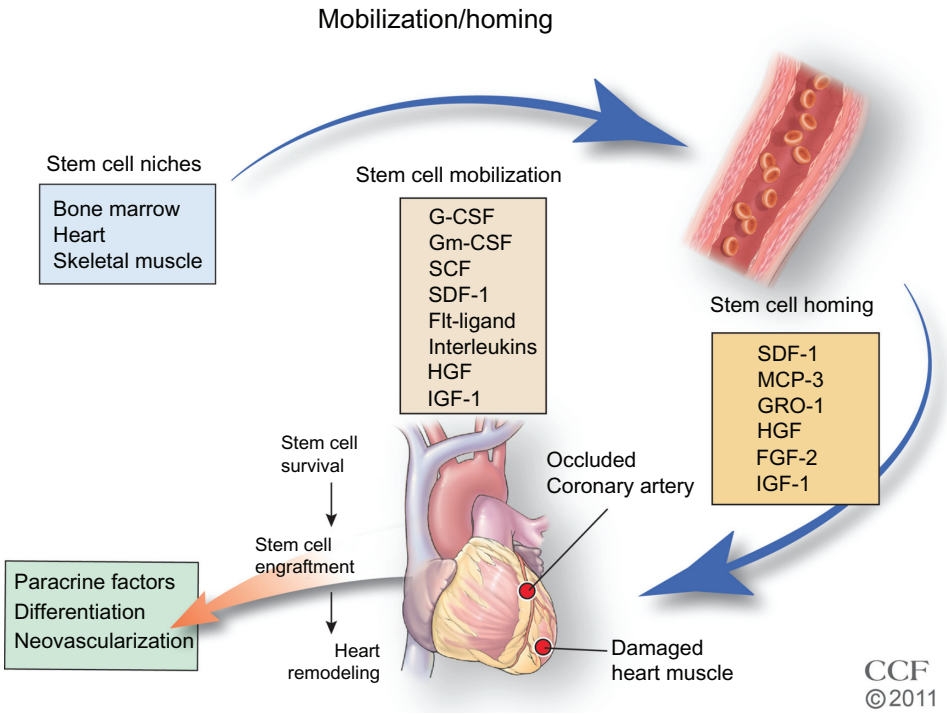

Figure 3 Stem cell mobilization and homing. Growth factors and cytokines stimulate the mobilization of the stem cells from their niche to injured tissue.

Notes: Flt-ligand is a growth factor; interleukins refer to interleukin-1, -3, -6, -7, -8, -11, and -12 cytokines; homing factors MCP-3, GRO-1, HGF, FGF-2, and IGF-1 are produced in the heart and promote endogenous and exogenous stem cells homing to the injured tissue; survival and implantation of stem cells in the tissue may result in differentiation, secretion of paracrine factors, and/or stimulation of angiogenesis to restore blood flow and remodel tissue.
Abbreviations: G-CSF, granulocyte colony-stimulating factor; Gm-CSF, granulocyte-macrophage colony-stimulating factor; SCF, stem cell factor/c-kit ligand; SDF-1, stromal cell-derived factor 1; MCP-3, monocyte chemotactic protein-3; GRO-1, growth regulated oncogene 1; HGF, hepatic growth factor; FGF-2, fibroblast growth factor; IGF-1, insulin-like growth factor.

these two proteases are not required for HPSC mobilization. The results of studies[81,83,87,88] in MMP-9 deficient mice are not consistent. While some studies[83,88] report MMP-9 is not required, other studies[81,86,87] suggest MMP-9 plays an important role. These differences may be due to the differences in genetic background of the mice and to differences in the dose of the mobilizing agent. In a recent study,[86] the authors of this present paper report that plasmin/MMP-9 is a major proteolytic pathway required for SC mobilization from BM (Figure 5). Plasmin activation of MMP-9 regulates the SDF-1/CXCR4 signaling. In addition, plasmin also promotes direct degradation of the ECM during SC mobilization.[85] G-CSF induced HSC MMP-9 degrades BM SDF-1.[83,89,90] The increase in the number of SC mobilized with G-CSF treatment may not be sufficient for the cardiac remodeling after MI, and some patients are resistant to G-CSF.[91–93] AMD3100, an inhibitor of CXCR4, is a promising HSC mobilizer under clinical investigation. Studies report mild and reversible side effects[94–96] and that it works synergistically with G-CSF to increase CD34+ cells and total white blood count.[94,96–98] However, Dai et al recently reported that chronic AMD100 exacerbates cardiac dysfunction after MI in mice.[99]

Mobilization of CPCs

A number of cardiomyocyte progenitor pools have been identified[76] that have common and unique markers, including: side population (SP) CPCs; c-kit+ CPCs; Sca-1+ CPCs; cardiospheres and cardiosphere-derived cells; stage specific-embryonic antigen-1+ (SSEA-1+) CPCs; LIM-homeodomain transcription factor+ (Islet-1+) CPCs; and epicardium-derived cells. The CPCs demonstrate greater proliferation potential in the infarct border compared with the necrotic core. These cells have the potential to differentiate into cardiomyocytes, smooth-muscle cells, and ECs, but the stimulatory factors for differentiation vary. The SP CPCs[100,101] can be stimulated by SDF-1 and are both c-kit and Sca-1 positive, but are also positive for the ATP-binding cassette transporter (ABCG2). The cardiac SP cells are a mixture of subpopulations, and proof that these cells are SC is not definitive. The c-kit marker was used to identify and isolate HSCs, but their ability to differentiate into cardiomyocytes is controversial.[102,103] Cells positive for c-kit isolated from human and rodent tissue express specific cardiac transcription factors, GATA4, GATA5, MEF2C, and Kkx2, and when cultured express mature cardiomyocyte markers, cardiac actinin, cardiac myosin, desmin, and connexin 43.[45,104]

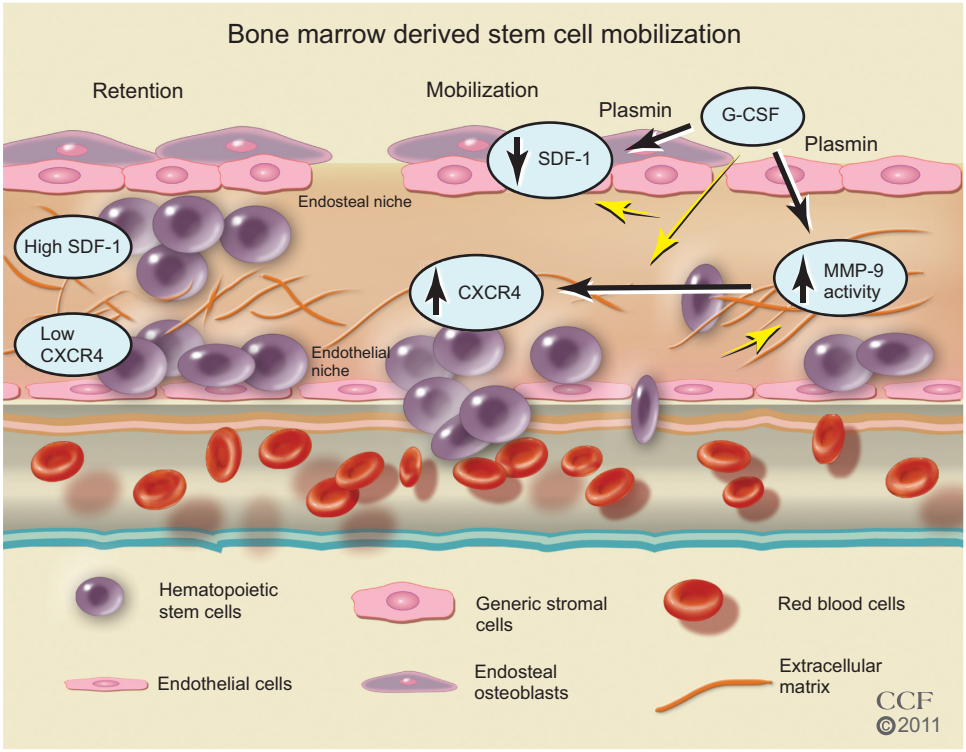

Figure 4 Bone marrow-derived stem cell mobilization. Bone marrow stem cells may be mobilized by reducing the ligand SDF-1 and increasing the stem cell receptor CXCR4 to create a chemotatic gradient with the peripheral blood. G-CSF treatment increases MMP-9 to regulate changes in SDF-1/CXCR4 pathway, which is dependent on plasmin activation of MMP-9.

Abbreviations: CXCR4, C-X-C receptor 4; G-CSF, granulocyte colony-stimulating factor; MMP-9, matrix metalloproteinase-9; SDF-1, stromal-derived factor-1.

The CPCs may be stimulated by insulin-like growth factor-1 (IGF-1); hepatic growth factor (HGF) high-mobility group box protein-1 (HMGB1), a chromatin-binding protein secreted by necrotic cells, and SDF-1.[105] The CPCs possess growth factor receptors and when activated increase proliferation, migration, and differentiation. Tamoxifen-treated double-transgenic mice[48] expressed dedifferentiated cardiomyocytes that expressed CPC markers and ~2/3 expressed c-kit. Studies in zebrafish and mammalian development suggest the potential of the epicardium-derived cells, the epithelial cells in the outermost layer of the heart, to develop into cardiomyocytes in vivo.[106] Smart et al[107] reported that in mouse heart, thymosin β4 can release the quiescent EPDCs. Development of small molecules to release the cells is underway.[106] Isl1+ CPCs are prominent during development, and in the postnatal rat, mouse, and human myocardium, Isl1+, c-kit-, Sca-l-, and CD31- cells have been defined as cardioblasts. Both iPS cells and ESCs give rise to this lineage in vivo. The Isl-1+ cells are rare in the myocardium and the possibility of endogenously recruiting or in vitro expansion appears to be limited. The SSEA-1+ CPCs[108] give rise to myocardial and endocardial cells during development in the neonatal and adult rat heart, but can progress to more committed c-kit+, Sca-1, and abcg2+ cells. When transplanted into rat heart, improved regeneration of infarcted myocardium results. Sca-1+ CD31+ cells are found in the heart as small interstitial cells that lack the HSC lineage markers of c-kit, Flt-1, Flk-1, CD45, and CD34.[41] Using transgenic mice, cardiac Sca-1+ cells were found to play a role in the regulation the signaling required for efficient myocardial regeneration.[42,109] Studies with ESCs and their requirements for cardiomyocyte differentiation may shed light on the factors that induce differentiation and proliferation of the endogenous CPCs.[110] A better understanding of SC mobilization from cardiogenic niches may lead to more effective agents for not only recruiting cells for ex vivo expansion, but to mobilize endogenous sources.

Strategies for improving SC homing, survival, and engraftment in the injured heart

SC delivery

Available routes of SC delivery include intravenous, intracoronary, epicardial, endocardial, and coronary sinus injection.[2,111] While the intravenous injection of SCs is the least invasive

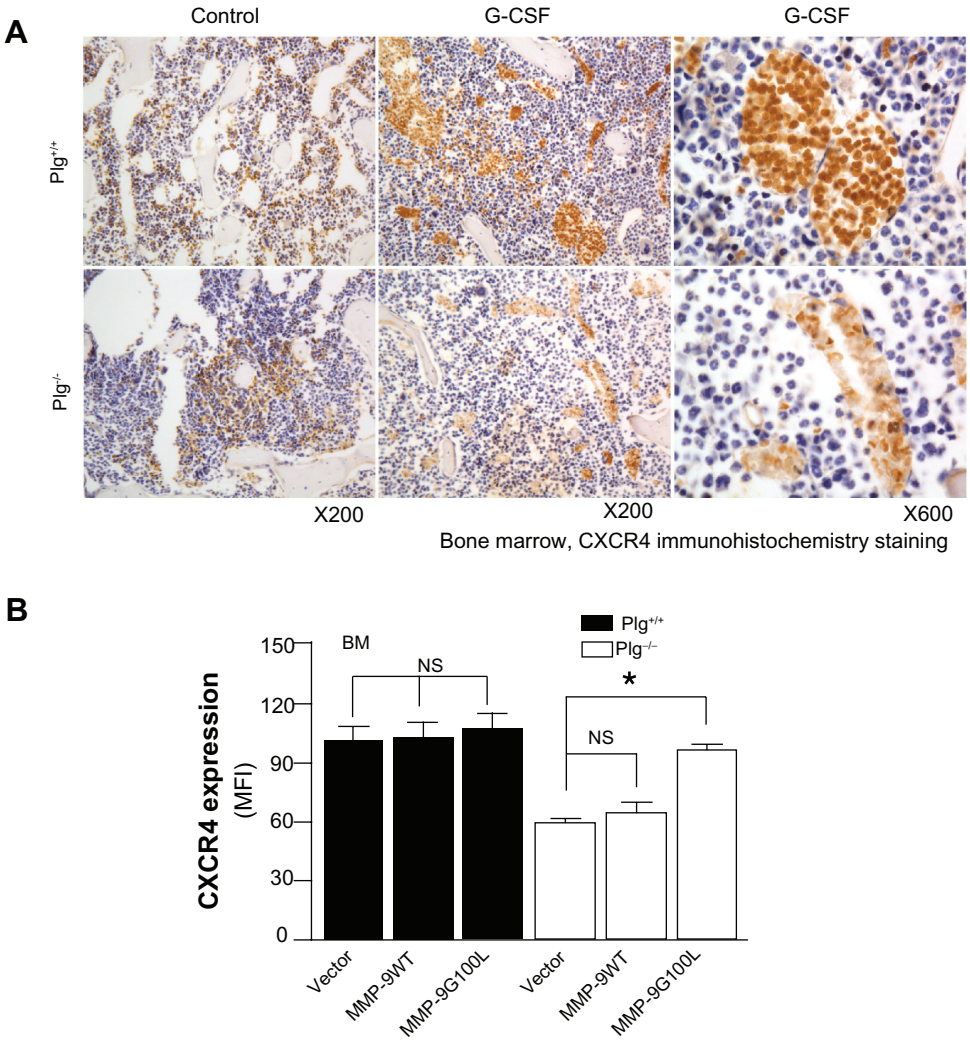

Figure 5 Plasminogen regulates CXCR4 after G-CSF stimulation. (**A**) CXCR4 immunostaining of bone marrow from Plg+/+ and Plg–/– mice treated with saline (control) or G-CSF. CXCR4 expressing cells (brown color) increased two fold after G-CSF treatment in Plg+/+ mice, but CXCR4 did not change in Plg–/– mice. (**B**) Lentivirus expression of act MMP-9 in Plg–/– restored CXCR4 expression. Plasminogen activation of MMP-9 is required for CXCR4 expression after G-CSF treatment.
Note: Reproduced with permission from Gong Y, Fan Y, Hoover-Plow J. Plasminogen regulates stromal cell-derived factor-1/CXCR4-mediated hematopoietic stem cell mobilization by activation of matrix metalloproteinase-9. *Arterioscler Thromb Vasc Biol.* 2011;31(9):2035–2043.
Abbreviations: CXCR4, C-X-C receptor 4; G-CSF, granulocyte colony-stimulating factor; MFI, mean fluorescence intensity; MMP-9, matrix metalloproteinase-9; Plg, plasminogen.

method of delivery, retention of cells in the lungs is problematic. After an MI, intracoronary injection through a catheter is the preferred method of delivery. The epicardial and transendocardial are more invasive, but the most reliable. The transendocardial administration uses a percutaneous catheter-based approach. The coronary sinus delivery provides access to the infarcted and ischemic tissue, but may not be available to all patients. In the clinical trials, SCs were delivered by either bolus or multiple intracoronary injections, but only a small percentage reached the heart.[1,112] At least 90% of injected cells die by apoptosis. Alternative methods of delivery are being investigated, such as use of biodegradable scaffold-based engineered tissue.[113,114] An advantage is the variable size, but problematic issues are thickness of the patch and toxicity of the degraded material. Only limited improvement in cardiac function has been noted. A recent study[115] tested sheets of cardiomyocytes progenitor cell and reported an increase in cardiogenesis and improved function. The development of safe and more effective materials for use in SC delivery is necessary.

Homing

Homing is the migration of SCs from endogenous and exogenous sources through the blood or tissue to a destination where they

differentiate and replace or repair injured tissue. After an MI, expression of several factors has been observed, including transient increases in cardiac cytokines, SDF-1, MCP-3, GRO-1, that are chemo-attractants for SCs.[116–123] After acute MI, the expression of these factors leads to SC homing to the infarcted tissue. However, many of the homing factors are expressed for only a short period of time after MI. SDF-1, the most studied homing factor, is expressed by the injured cardiac tissue for less than 1 week[123] and MCP-3 for less than 10 days after MI.[124] In preclinical studies, genetic engineering of these factors into delivered SCs is effective in increasing SC homing.[123,125] For example, the delivery of SDF-1 to the myocardium, either through cell-based gene therapy,[123,126] gene transfer,[127] or protein-enhanced[128] homing of SCs, results in revascularization and improvement in cardiac function. Furthermore, overexpressing SDF-1 receptor CXCR4 in SCs leads to greater homing of SCs and improved left ventricular function when the cells were delivered within 24 hours of MI.[129–131] Studies in animals show that engineering cells to induce the expression of SC homing factors or their receptors in myocardial tissue can promote SC homing from BM to the injured myocardium; however, these have not to date been tested in humans.[132]

Survival/engraftment

Survival and engraftment of SCs is perhaps the most important challenge for SC therapy, and the factors necessary for effective survival and engraftment are not necessarily the same as those required for homing. After an MI, there is an enormous loss of cardiomyocytes and supporting cells that need to be replaced. The environmental signals that may guide SCs to the cardiomyocyte lineage or to the secretion of paracrine factors may be absent in the infarcted tissue, and SCs may provide these signals. Many studies have focused on strategies to optimize SC migration through injured myocardial tissue. Proteases, adhesion molecules, and integrins are important in regulating SC migration through injured myocardial tissue and modulation of the connective tissue microenvironment to improve SC engraftment.[133–136]

Several proteases have been identified to have significant effects on SC mobilization or SC migration and engraftment in cardiac tissue. SDF-1 and other factors induce the secretion of matrix metalloproteinase MMP-2 and MMP-9.[137–139] Of significant interest, proteolytic enzymes, including neutrophil elastase, cathepsin G, and MMP-2/9, also negatively regulate cell migration by cleaving the N-terminal region of SDF-1 or cleaving CXCR4.[90,139–142] Those proteolytic enzymes are involved in spatial temporal changes in the locomotion machinery of SCs, thus mediating SC recruitment and engraftment.

Integrins are also key factors for adhesion, rolling and transmigration of SCs across the endothelium. The HSCs express several adhesion molecules including multiple integrins. In particular, a dominant role for the α4β1 integrin very-late antigen [VLA]-4 interaction with vascular cell adhesion molecule (VCAM)-1 has been suggested by studies in which exposure to blocking antibodies to VLA-4 or VCAM-1 significantly reduced the engraftment of transplanted HSCs.[143–145] CD18 expression by the EPCs is necessary for its interaction with EC surface ICAM-1, and a CD18 neutralizing antibody significantly inhibits SC engraftment after acute MI.[146] These studies suggest the potential targets for the genetic enhancement of SC recruitment and engraftment.

Several other strategies have been proposed: identifying natural mediators; pre-translational directed differentiation of SCs to cardiomyocytes; activation of growth factors (FGF-2, IGF-1a)[132] and antiapoptotic factors (p-Akt, SDF-1, BCl-1, and PDGF); and genetically engineered SCs.[125,132] The challenge to improve survival in SC therapy is to identify effective ways to increase the number of cells that reach and survive in the injured heart area.

Assessment of SC therapy

The goals of SC therapy are to: replace lost cardiomyocytes; increase ECs to improve blood flow; provide paracrine cytokines and growth factors; and improve measurable cardiac function, including an increase in LVEF; decrease left ventricular end-diastolic diameter; increase myocardial perfusion; and importantly increase exercise capacity. In clinical trials, methods to measure cardiac function include echocardiography, single photon emission computed tomography, and magnetic resonance imaging (MRI).[1,3,37,147–149] These methods are well established, but more sensitive methods are necessary to evaluate SC homing and engraftment. Techniques to evaluate the timing and specific role of narrow populations of cells, such as MRI[150–152] and SC labeling with genetic[153,154] and immunofluorescence detectable tags[155] are being investigated in animal models. The lineage/fate mapping[110,156–158] has proved to be an informative tool, and further studies in animal models and ex vivo SC labeling of cells for therapy will continue to be valuable.

Conclusion

SC therapy is an exciting and dynamic area of research with the potential to improve recovery of CVD, the leading cause of

death. While animal models clearly show benefits of SC therapy to improve cardiac function after MI and ischemic heart failure, clinical trials have been disappointing. However, the results of clinical trials are promising. Better methods are needed to improve the isolation and identification of SCs, increase ex vivo expansion of SCs, and increase delivery effectiveness. A clearer understanding of mobilization and homing of SCs is needed to identify new and more effective agents. Delineating the function of specific SCs in remodeling injured tissue and how resident cardiac SCs may be enhanced is needed to improve SC engraftment and survival.

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Disclosure

The authors report no conflicts of interest in this work.

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