

A Review of the Basis of Autologous Stem Cell Therapy for Coronary Artery Disease

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Abstract

Until recently, the myocardium has been viewed as a terminally differentiated organ without potential for regeneration. Although dramatic advances have been made in the treatment of coronary artery disease resulting in greatly improved morbidity and mortality in these patients, further progress in treatment is limited by the inability to repair concomitantly damaged cardiac tissue. This limitation has led to increasing use of stem cell (SC) therapies with the assumption that replacement or repair of damaged vascular and cardiac tissue could lead to improvement in myocardial function.

Although multiple experimental animal models and clinical trials of cell-based cardiac therapy have delivered promising results, the mechanisms of their effect are unclear. SC, depending on their lineage, possess the ability to differentiate into cells of various tissues. Although the differentiation of SC into functional cardiomyocytes has been difficult to demonstrate and fraught with controversy, differentiation into functioning endothelium with improved blood flow has been better illustrated and accepted. Studies in animal models have demonstrated improvement in myocardial function after targeted repair of myocardium via implantation of progenitor cells by various delivery methods, whether derived from peripheral blood, bone marrow (BM), umbilical cord blood, or embryonic sources.

Herein is a review of the use of autologous SC therapy for coronary artery disease.

Introduction

While death rates of cardiovascular disease have been in decline for the past 30 years, it remains one of the highest causes of mortality in the United States, accounting for 1 in every 2.9 deaths annually. Each day, 2400 Americans will die of cardiovascular disease, which amounts to 1 death every 37 seconds [1]. Each year, 785,000 Americans will suffer a new myocardial infarction (MI) and 470,000 will experience a recurrent attack, while an additional 195,000 are estimated to undergo silent infarctions, which mostly go unreported [2]. Approximately 16% of individuals experiencing a first MI die acutely and of the 84% of patients that do survive their MI, an estimated 15 years of life is lost following the MI [3].

MI is a permanent and irreversible injury in which there is a rapid loss of myocytes at the cellular level [4]. As little as 15 minutes of coronary artery occlusion can irreversibly depress left ventricular function [5]. The goal of current medical therapy for MI is prompt reperfusion and establishment of patency to the infarct-related artery, which has been proven to limit myocardial damage [6]. This is accomplished using medical, interventional, or surgical strategies, which acutely salvage some ischemic myocardium and spare further loss of cardiac function. Even if administered beyond the time-frame required for myocyte rescue, reperfusion strategies have still proven to decrease left ventricular remodeling and dilatation [7], which are major contributors to reduced patient survival and poor prognosis [8].

While all of these current therapies can improve revascularization and acutely rescue ischemic myocardium at risk leading to a reduction in cardiovascular mortality, they are still limited by their inability to regenerate already dead myocardium. Recent advances in stem cell (SC) technology may be key to treatment of patients following MI and further improving their prognosis by myocardial regeneration.

Regenerative potential of the heart

The heart was previously thought to be a static tissue incapable of differentiation and repair. The scar following MI was seen as being incapable of cell division and differentiation to repair the damage with associated permanent loss of myocytes and heart function. To

the contrary, recent evidence confirms that resident myocardial SC are present, can divide and proliferate, are capable of repopulating the damaged heart, and lead to cardiomyogenesis after transplantation [9].

In the last decade, many groups have shown that several sources of adult SC are capable of inducing cardiac repair. Evidence of resident cardiac SC began to formulate in studies of human patients who had died within 4-12 days after MI. Beltrami et al. stained postmortem hearts with Ki-67 (a marker of cellular proliferation), examined them for histological evidence of cellular proliferation and division (mitotic spindles, contractile rings, karyokinesis or cytokinesis) and demonstrated that 4% of nuclei in the infarct border zone and 1% of distant nuclei from viable sites in the myocardium were Ki-67+ implying an underlying regenerative process [10]. They also determined that 60,500 myocytes in the healthy control hearts were actively proliferating compared to 1,976,000 dividing myocytes in the post-infarction heart [11], which was a dramatic change from the previous notion that the heart was incapable of any regeneration.

Some of the most convincing early evidence of the existence of cardiac progenitor cells outside the heart involved studies of patients who had undergone gender-mismatched heart transplants, in which hearts from female donor were implanted into male recipients, and at the time of death (ranging from 4 to 552 days after transplant), all donor hearts displayed Y-chromosome-positivity, suggesting that SC from some location outside the heart (i.e. peripheral blood or bone

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marrow) must have migrated to the myocardium to induce repair [12-14]. One study reported Y-chromosome positivity in as high as 7-10% of myocardial nuclei [12], while another study reported much lower Y-chromosome-positivity of about 0.04% of myocardial nuclei [13]. A third study reported no Y-chromosome positive nuclei out of over 6000 myocytes examined, but they did report extensive Y-chromosome positivity in endothelial cells within the myocardium, suggesting the contribution of extra-cardiac progenitor cells to neovascularization in the ischemic tissue [14]. All three of these recent studies used co-localization of the Y-chromosome with markers of mature cardiomyocytes (including c-kit, MDR1, Sca-1, or sarcomeric myosin) to contradict an older study, which reported that Y-chromosome+ cells were the result of infiltrating lymphoid cells and not new endothelial cells or new myocytes [15].

Mechanism of action of stem cells

Mechanisms of chemokine-mediated stem cell migration have been proposed to explain the homing of extra-cardiac cells into the ischemic heart. Stem cell factor (SCF) and granulocyte-colony stimulating factor (G-CSF) can both induce BM cell migration *in vivo* using a mouse MI model. Cytokine mobilized cells were also able to induce significant tissue regeneration and improved cardiac function by 4 weeks post-MI, although this improved function could also be attributed to endogenous cardiac progenitor cells [16]. The CXCR4 receptor and its ligand, stem cell-derived factor-1 (SDF-1) also play an important role in the homing of extra-cardiac progenitors. SDF-1 expression is markedly upregulated in the hypoxic myocardium, and its expression is induced by hypoxia in other organs including the liver and kidney [17]. Hematopoietic BM progenitor cells with the CD34 surface marker for primitive SC also express the CXCR4 receptor, which may mediate their chemotaxis toward high SDF-1 concentrations in ischemic tissues [18]. Flow cytometry analyses have demonstrated that these same CD34+ mononuclear cells are upregulated following MI and peak at day 7 when compared to normal patient controls. While this study did not look at SDF-1 specifically, the authors did note that vascular endothelial growth factor (VEGF), a potent stimulator of angiogenesis, was upregulated following MI in these patients. The VEGF gene has a known hypoxia-responsive element in its promoter and therefore, VEGF-mediated signaling in response to MI may also attract bone marrow SC [19] (Figure 1).

In concurrence with these studies on SC chemotaxis, several reviewers have outlined a three-step process of SC engraftment: 1) chemokine-mediated mobilization, 2) homing to the site of injury, and 3) transdifferentiation into functionally mature cells of the endogenous tissues [20-22]. SC homing to the site of injury is supported by the studies showing increased expression of chemokines such as G-CSF, SDF-1, or VEGF in the ischemic myocardium. Transdifferentiation and successful tissue engraftment may depend on the specific type of SC as well as on the local environmental niche of the endogenous tissue.

Evidence of stem cell-mediated repair in preclinical models

Various studies have been focused at generating successful models for cell transplantation into the ischemic and failing heart. To date, two potential sources of adult SC have been heavily examined for their ability to mediate cardiac repair: peripheral blood (PB), and BM. Many preclinical studies have been undertaken to examine the efficacy of these cells for cardiac repair, which have demonstrated their ability to induce neovascularization, decrease infarct size, and improve overall cardiac function.

Bone marrow-derived progenitor cells: There is much evidence

that endothelial progenitor cells (EPC) derived from the adult BM support postnatal neovascularization and contribute to maintenance and support of the ischemic myocardium. G-CSF-mobilized human CD34+ BM cells transplanted into rats following LAD ligation were shown to improve cardiac function and induce angiogenesis, with formation of new capillaries positive for human markers [23]. The authors argued that these newly formed vessels were crucial to enhanced cardiac function by providing trophic support or secreting paracrine factors to prevent further apoptosis of myocytes. Another study using labeled BM-derived EPC from transgenic Lac-Z mice showed formation of new Lac-Z+ vessels after transplantation into wild-type MI mice [24].

In addition to neovascularization, BM-derived cell transplantation following MI can improve cardiac function in animal models. Rat BM progenitor cells cultured with 5-azacytidine (a DNA-demethylating agent) can form myotubules positive for troponin I and myosin heavy chain *in vitro*, engraft into the infarcted left ventricle following cryoinjury to improve cardiac function, as measured during *ex vivo* perfusion, and further induce formation of new vessels within the ischemic zone [25]. Another study showed that G-CSF-mobilized human CD34+ BM progenitor cells transplanted into rats following LAD ligation improved cardiac function measured by echocardiography, and displayed new capillaries positive for human markers [23].

Another widely-studied population of pluripotent BM-derived SC can be identified by their expression of the c-kit (CD117) surface receptor, which appears on primitive cells of the hematopoietic lineage, and their lack of expression of all other blood lineage markers (CD2, CD3, CD7, CD16, CD33, CD38, CD45, CD56, and glycophorin A), which is termed "lineage negative" or "lin-" [26]. When c-kit+/lin- cells are isolated from male transgenic mice overexpressing green fluorescent protein (GFP) and implanted into the border zone of female wild-type mice following LAD ligation, myocytes positive for both GFP and the Y-chromosome are visible in the infarct zone within 9 days of transplantation. This study concluded that these new myocytes were the result of transdifferentiation, because GFP and antibodies to the Y-chromosome were shown to co-localize with stains for several known

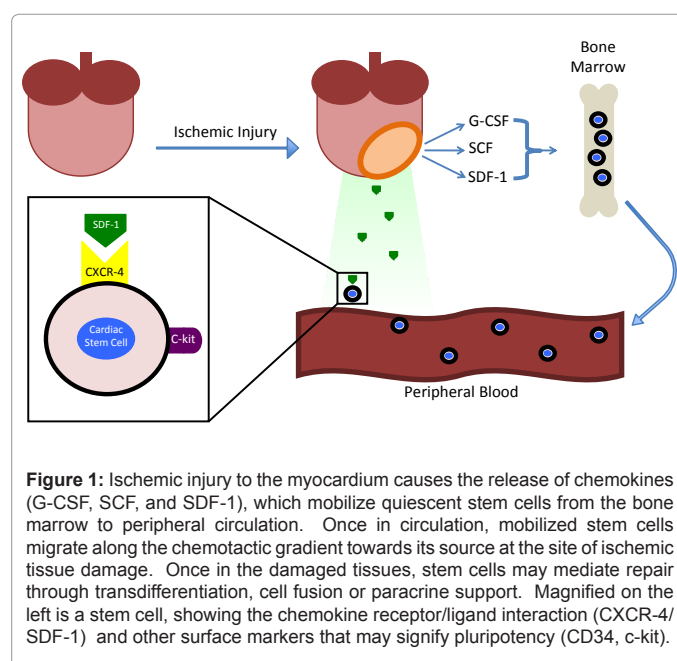


Figure 1: Ischemic injury to the myocardium causes the release of chemokines (G-CSF, SCF, and SDF-1), which mobilize quiescent stem cells from the bone marrow to peripheral circulation. Once in circulation, mobilized stem cells migrate along the chemotactic gradient towards its source at the site of ischemic tissue damage. Once in the damaged tissues, stem cells may mediate repair through transdifferentiation, cell fusion or paracrine support. Magnified on the left is a stem cell, showing the chemokine receptor/ligand interaction (CXCR-4/ SDF-1) and other surface markers that may signify pluripotency (CD34, c-kit).

markers of mature cardiomyocytes including cardiac myosin, myocyte enhancer factor-2 (MEF-2), the cardiospecific transcription factor GATA-4, and the gap junctional protein connexin 43 (Cx43). Mice receiving cell transplants also demonstrated improvement in cardiac function, with lower left ventricular end-diastolic pressure, lower left ventricular developed pressure, and increased index of left ventricular contractility (dp/dt) [27].

Mesenchymal stem cells (MSC) are another subset of highly pluripotent progenitor cells that may be cultured from BM. Human MSC labeled with β -galactosidase were transplanted into the left ventricle of immunodeficient mice, and observed to integrate into the host myocardium becoming indistinguishable from surrounding endogenous myocytes, and even expressing contractile proteins such as desmin, α -myosin heavy chain, α -actinin, and phospholamban [28], which led this group to conclude that BM-derived MSC are capable of transdifferentiation into myocytes.

Peripheral blood-derived progenitor cells: Like BM-derived SC, there is much evidence that PB-derived EPC can support postnatal neovascularization in the ischemic myocardium. Labeled human and mouse PB-derived CD34+ EPC were both shown to induce new blood vessel formation when injected into the ischemic hindlimb of mice following unilateral femoral artery ligation. The same observations were reported using rabbit PB-derived CD34+ EPC injected into the rabbit ischemic hindlimb [29]. EPC can be mobilized into peripheral circulation following ischemic tissue injury or after administration of chemokines such as G-CSF. This group administered G-CSF in ischemic hindlimb models of rabbits or mice to mobilize EPC into peripheral circulation and subsequently demonstrated enhanced neovascularization at a distant location in the cornea of either species following a micropocket surgical procedure [30].

The process of new vessel formation in adults, known as angiogenesis, is thought to be mediated by VEGF, which can induce differentiation of PB EPC *in vitro*, causing them to express markers of mature endothelial cells. VEGF can mobilize EPC *in vivo* into peripheral circulation within 1 day of administration, with cell numbers peaking at 4 days and remaining elevated in circulation through 7 days [31]. Another group examined a specific subset of CD133+/KDR+ PB EPC, where CD133 is thought to denote a more primitive subset of the CD34+ cell population, while KDR is the VEGF receptor 2. When these cells were injected subcutaneously with the A549 lung cancer cell line, angiogenesis and tumor growth were markedly enhanced compared to animals injected with A549 cells alone [32].

In addition to their support of neovascularization in ischemic tissues, PB-derived progenitor cell transplants can improve cardiac function in MI models. When labeled human EPC were injected into athymic nude rats following LAD ligation, cardiac function improved on echocardiography and labeled cells appeared in newly formed capillaries within the ischemic myocardium [33]. In a mouse MI model, transplanted human PB-derived CD34+ EPC injected into the infarct zone showed evidence of transdifferentiation into cardiomyocytes, smooth muscle cells, and endothelial cells on immunohistochemical analysis 2 months post-MI. Transdifferentiation was demonstrated by the co-expression of human leukocyte antigen (HLA) with markers of mature myocytes, smooth muscle, and endothelial cells [34].

The proposed theory, that SC actually transdifferentiate into functional cardiomyocytes, is highly controversial. Criticisms of this proposed mechanism range from questions about the histological analyses performed by some studies, to a proposed alternative model

for SC engraftment involving fusion of transplanted cells with existing endogenous myocytes. Other groups advocate a third model, in which cardiac repair is mediated by paracrine support of transplanted SC through their secretion of soluble growth factors or stimulatory effects on angiogenesis.

Taylor et al., challenged the data presented by Quaini *et al* regarding gender mismatched transplants [12], questioning the true specificity of their "cardio-specific" markers and claiming drastically overestimated numbers of new myocytes in their samples, since they relied on non-specific markers including Mef2D (also expressed by T cells), GATA-4 (also expressed in the spleen and blood cells) and even β -myosin heavy chain (also detected in other blood lineage cells). They also noted that many of the hearts examined were from patients who died between 4 and 28 days following transplantation, which coincides with that of acute allograft rejection, leading her to surmise that many of these Y-chromosome-positive cells may actually belong to nuclei of invading lymphocytes [35].

Other groups have attributed the appearance of new myocytes following SC transplantation to fusion of labeled SC with existing myocytes. To address this point, Nygren and colleagues transplanted cells from Lac-Z transgenic mice into GFP transgenic mice, and they demonstrated the formation of new myocytes with dual Lac-Z/GFP positivity [36]. They suggested that their study provided indisputable evidence for cell fusion, but there were criticisms regarding the population of cells they used for transplantation with either whole BM, or BM selected for c-kit+/lin-/CD34-cells. Since CD34 is a widely accepted marker of more primitive stem cells with greater proliferative potential, it is possible that they removed many pluripotent cells from their transplantation pool. Additionally, since they did no selection on the whole BM samples, they likely had relatively few pluripotent SC in this sample as well.

Perhaps a more reasonable explanation for the improvement in cardiac function is the idea that transplanted progenitor cells provide trophic support to endogenous myocytes either through enhancement of neovascularization in the ischemic myocardium, or through secretion of paracrine factors that prevent apoptosis and support ischemic myocytes [37]. One group transplanted BM SC from Lac-Z- or GFP-transgenic mice into wild-type MI mice following LAD ligation and failed to show any evidence of engraftment either through transdifferentiation or fusion. They did, however, observe similar improvements in cardiac function to the study by Orlic D et al. [16], and attribute this finding to the paracrine effects of these SC [38]. Another group used MSC transformed with the prosurvival gene Akt1 and demonstrated their ability to trophically support hypoxic myocytes *in vitro* and *in vivo* in a rat MI model. They suggested that paracrine factors with known hypoxia-responsive elements, such as VEGF, FGF-2, HGF, IGF-1, or TB3, were most likely responsible for the observed improvements in cardiac function following MI [39].

Clinical trials using stem cells

Despite the variance in accepted mechanisms of action, recent data from clinical trials involving SC transplants into the ischemic myocardium provides strong evidence that the cell-based therapy approach to treating MI produces real and sustainable improvements, despite the lack of conclusive evidence for one particular cellular mechanism. Several clinical trials utilizing BM-derived progenitor cells to repair damaged myocardium have been completed with variable results. Three studies to date have produced inconclusive results, with negligible effects of BM SC on cardiac function, while five studies

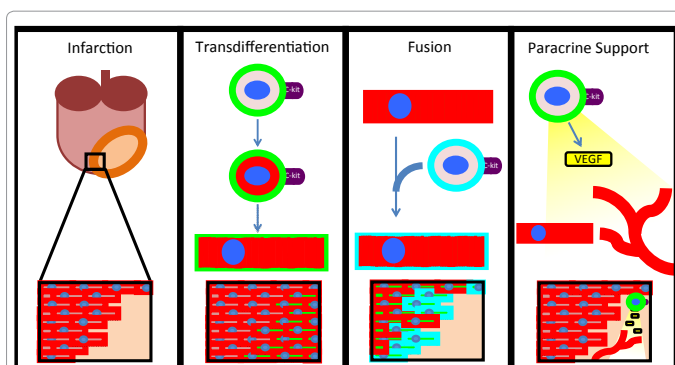


Figure 2: Infarction represents a loss of myocytes at the cellular level where each functional myocyte represents a discrete pumping unit, and loss of myocytes on a large scale results in depression of global cardiac function, ultimately leading to heart failure and poor long-term prognosis. Three mechanisms of stem cell-mediated repair have been proposed. 1) Transdifferentiation: GFP-labeled c-kit⁺/lin⁻ stem cells (circled in green) transplanted into wild-type animals after MI have demonstrated formation of new myocytes positive for GFP expression. These cells initially synthesize unorganized α -actin (red cytoplasm), and upon differentiation, lose surface expression of c-kit, and organized sarcomeres (red lines) are visible histologically. 2) Fusion: LAC-Z⁺ stem cells (circled in cyan) transplanted into GFP transgenic animals have demonstrated myocytes co-expressing LAC-Z and GFP. 3) Paracrine Support: Secretion of VEGF (yellow) and other cytokines from transplanted stem cells have been shown to trigger formation of new blood vessels which results in improvement of post-MI cardiac function through paracrine mechanisms.

have shown consistent beneficial effects of such cell transplantation in ischemic myocardium.

Fuchs et al. conducted a multicenter trial [40] in which 10 patients underwent autologous BM SC transplantation through percutaneous transendocardial injection with an electromechanical mapping and injection catheter (Biosense Webster, Johnson & Johnson). No changes in cardiac function were shown, but patients did experience improvements in angina symptoms compared to untreated control subjects. Additionally, patients who received transplants displayed better myocardial perfusion, but this effect was only apparent on stress testing and not at rest. However, this study was an initial pilot study to test the feasibility of intra myocardial injection of SC, and no control group was included [40].

The Autologous Stem-Cell Transplantation in Acute MI (ASTAMI) Trial [41] was a much larger trial of 100 patients who were treated with intracoronary infusion of BM-derived progenitor cells or control (no bone marrow aspiration or sham catheterization) at a median of 6 days following percutaneous coronary intervention (PCI) for MI. Left ventricular ejection fraction (LVEF) increased only modestly (+0.6%) over control groups when measured by single-photon emission computed tomography (SPECT), but LVEF was actually shown to decrease (-3.0%) when measured by gadolinium-enhanced magnetic resonance imaging (MRI). No difference in end-diastolic volume or infarct size was observed using either measurement tool, and the group could not conclude any significant effects of the transplants on LVEF [41].

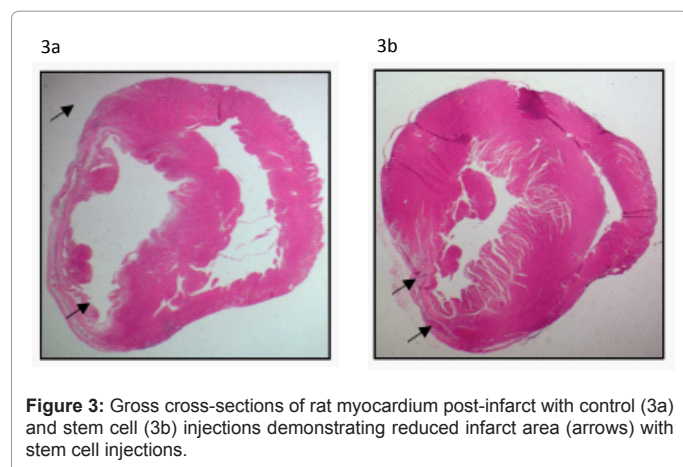
A third clinical trial [42] involved 67 patients who received either autologous BM SC transplantation via intracoronary infusion or placebo (bone marrow aspiration and sham injection with no cells) 1 day following PCI after ST-segment elevation MI. This group reported only modest improvements in LVEF in the BM transplant group (+1.036%) compared with the untreated controls. The patients receiving cell therapy did show significant reduction in infarct size and improved

regional systolic function over the control group. The researchers attributed these modest changes to the paracrine effects of transplanted BM cells rather than to direct transdifferentiation or cell fusion, which they would have expected to produce more dramatic changes in LVEF. However, due to lack of any cell labeling or histology, these arguments could not be validated [42] (Figure 2).

Five recent completed clinical trials generated much more substantial results than these first three. Fernandez-Aviles et al. [43] conducted autologous intracoronary BM-derived progenitor cell grafts on 20 patients at 13.5 ± 5 days following PCI for acute MI, with an additional 13 control patients receiving no treatment. At 6-month follow-up, the transplanted group showed decreased end-systolic volume on MRI with improved regional and global LV function and increased thickness of the infarcted wall. Samples of the cells from each patient cultured on cryo-injured mouse heart slices were shown to engraft and transdifferentiate *in vitro* into mature myocytes that expressed the connexin-43 gap junctional protein [43]. In the BOOST trial [44], 60 patients received either optimal medical treatment (with no sham injection) or intracoronary infusion of bone marrow progenitor cells in addition to optimal medical treatment 4-8 days following PCI for MI. In this trial, the BM cell transplant recipients experienced a 6% improvement in LVEF above the control group when analyzed by MRI [44].

The Transplantation of Progenitor Cells and Regeneration Enhancement in Acute MI (TOPCARE-AMI) trial [45] conducted a similar study of 20 patients comparing the administration of BM- to PB-derived progenitor cells given 4 days post-MI with optimal medical therapy (ACE inhibitors and β -blockers). Both cell types showed significant improvements in global LVEF, reduction in end-systolic volume and improved myocardial viability measured by fluorodeoxyglucose-positron emission tomography (FDG-PET) compared to controls, and no major differences were observed between the two cell types [45]. This group conducted another clinical trial, the TOPCARE for Chronic Ischemic Heart Disease (TOPCARE-CHD) trial [46], comparing BM- and PB-derived progenitor cells, but this time examined re-transplantation 3 months after MI to see if either population of cells could repair chronic scar tissue. Three months after the initial transplant, the patients received a second infusion, this time with cells of the other type (i.e. the patients initially receiving bone-marrow derived cells received a second infusion of peripheral blood-derived cells and vice versa). In both follow-up exams, one 3 months after the initial infusion (3 months post-MI) and the other 3 months after the cross-over transplantation (6 months post-MI), patients receiving BM-derived cells showed the most significant improvement in LVEF and New York Heart Association (NYHA) classification over both the PB-treated and control groups. They therefore concluded that BM-derived progenitor cells may be better able to improve cardiac function in the chronic infarct than PB-derived cells [46].

The largest clinical trial to date was the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute MI (REPAIR-AMI) trial [47], a double-blind, randomized, placebo-controlled multicenter trial, that enrolled a total of 200 patients suffering from acute MI, and were given either BM-derived progenitor cells or a placebo coronary infusion at 3-6 days following PCI for MI. At 4-month follow-up, the BM cell transplant group had a 5.5% improvement in global LVEF compared to a 3% improvement in the placebo group; and patients with the lowest baseline LVEF going into the transplant procedure appeared to receive the greatest benefit, as measured by angiography [47]. At 1 year follow-up, the primary endpoint of death, recurrence of MI, re-



hospitalization for heart failure, or necessity for revascularization were all significantly reduced in the patients receiving BM transplants [48] (Figure 3).

Disadvantages of current cell therapy models

The ability of autologous adult-derived SC, either from PB or BM, has shown the potential to enhance neovascularization and improve cardiac function following MI in many preclinical animal models and several human clinical trials, with many studies showing promising results [49]. The use of autologous tissues provides several advantages over allografts from different donors [50], in that any immune rejection can be avoided. However, there are several disadvantages that must be discussed, from the risks associated with harvesting and implantation procedures to the ability of these cells to function once implanted in the failing heart.

The procedures required for autologous SC transplantation could cause additional harm to a patient who is already coping with active disease. BM aspiration may add to the patient's stress and mortality, as does the surgical transplantation itself. There is risk of embolization caused by introduction of the cells into coronary circulation and risk of developing arrhythmias following implantation [51]. Some reviewers have pointed out the potential risk of malignant transformation of these stem cells, but this event has yet to be reported in any study with BM-derived cells [52]. The exogenous handling of BM samples may also negatively impact the patient such that the required culturing time for the progenitor cells may not necessarily coincide with the timetable needed for optimal success of cell therapy (the cells may not be ready in time for intervention). The use of cell culturing or exogenous expansion of adult-derived SC [53] also adds sterility issues to the procedure, and the sample could be exposed to any number of pathogens during this process.

The quantity and quality of SC isolated from adult patients with active disease has been called into question. There is an age-associated decline in the ability of coronary microvasculature to dilate in response to endogenous factors such as acetylcholine [54], nitric oxide or calcium ionophores, which is thought to be caused by chronic exposure of the arterial endothelium to high pressures and pulsatility [55]. These types of age-related decline in endothelial function may be linked to the limited functionality of the EPC in BM or PB that are tasked with maintaining this vascular system.

More recent evidence has shown that EPC from older or unhealthy patients may be present in more limited numbers and have poorer

functionality [56]. The number of circulating EPC and CD34+/KDR+ cells were determined to be reduced by 40% and 48%, respectively, in patients with active coronary artery disease. Additionally an increased atherosclerotic risk score (a composite of age, sex, hypertension, diabetes, smoking, family history of coronary artery disease, or high low density lipoprotein cholesterol levels) was associated with decreased counts of both cell types [57]. Another study found a similar correlation between reduced EPC count and elevated Framingham risk score for heart disease, which also takes into account factors like elevated serum cholesterol, hypertension and diabetes. This study also showed that EPC isolated from patients with cardiovascular disease risk factors, even without the presence of active disease, demonstrated increased markers of senescence measured by β -galactosidase activity [58]. Indeed cell aging and senescence of adult-derived SC may be the cause behind these observations. Along these lines, another group questioned whether transfection of adult-derived cells with the catalytic subunit of human telomerase, called telomerase reverse transcriptase (hTERT), could rescue the cells from their senescent phenotype and make them more effective at enhancing neovascularization in a hindlimb ischemia model *in vivo*. They determined that adult-derived EPC with constitutive activation of the hTERT gene were able to produce a 4-fold enhancement of neovascularization in the ischemic mouse hindlimb compared to wild-type EPC controls [59].

Another factor contributing to decreased function of adult-derived SC could be age-related decline in cytokine expression. While CD34+/CXCR4+ EPC have been shown to be elevated in peripheral circulation in patients with active coronary artery disease, one study showed that expression of the chemotactic ligand SDF-1 is actually decreased when this cell count is elevated, which could impair homing of these cells to the ischemic myocardium [60]. Likewise, signal transduction of CXCR4 may be affected by age. While the surface expression of the CXCR4 receptor itself was found to be similar in EPC cultured from patients with coronary artery disease compared to healthy donors, downstream signaling appeared to be diminished. The CXCR4 receptor signal is transduced by the classic Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway; however, in diseased patients phosphorylation of the downstream JAK-2 domain is significantly reduced on immune blotting, and cells from these patients show decreased chemotaxis in response to SDF-1 [61].

Recently, various micro RNA (miR) have been shown to play an integral role in the preconditioning of SC and promoting their survival post-engraftment in the infarcted heart through various mechanisms [62]: miR-21 has been shown to be a key determinant in an anti-apoptotic signaling pathway [63], while miR-33 has been shown to mediate down regulation of the tumor suppressor p53, resulting in decreased apoptosis and increased survival [64]. Further research is required to clarify the role of miR in stem cell survival post-transplantation.

Summary

Over the past decade, the old dogma of a static and unchanging myocardium has been challenged, and advancements in our understanding of SC biology have led to new models for cell transplant-mediated repair. While promising research has progressed the field of autologous adult-derived SC transplantation for myocardial repair, there remain many disadvantages to these procedures that have yet to be resolved. Additional sources of SC, such as embryonic SC or umbilical cord blood, also show promise in the field of cardiac regeneration, and are being considered as well. Despite work that remains to be accomplished, there is already a multitude of promising evidence that

use of SC, either from adult, umbilical cord, or embryonic sources, could provide optimal therapies to repair the damaged and failing heart.

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