Cell Transplantation for Ischemic Heart Disease

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Abstract

Recent studies support the notion that cardiomyocyte regeneration may occur during physiological and pathological states in the adult heart. These data highlight the possibilities that myocardial regeneration may occur via cardiomyocyte proliferation and/or differentiation of putative cardiac stem cells. To date, various cell types have been used for cardiac repair, including skeletal myoblasts, bone marrow-derived cells, mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), umbilical cord blood (UCB) stem cells, cardiac stem cells, and embryonic stem cells (ESCs). This chapter will review each of these different stem cell populations in regards to the potential treatment of heart disease. We will examine the in vitro and in vivo animal studies, and then briefly discuss the cell therapy clinical trials that are currently underway for the treatment of ischemic heart disease.

Keywords

Embryonic stem cells • Adult stem cells • Skeletal myoblasts • Bone marrow-derived stem cells • Mesenchymal stem cells • Endothelial progenitor cells • Umbilical cord blood stem cells • Cardiac stem cells

Abbreviations

CPCs	Cardiac progenitor cells
EPCs	Endothelial progenitor cells
ESCs	Embryonic stem cells
HGF	Hepatocyte growth factor
hiPSCs	Human induced pluripotent stem cells
IGF-1	Insulin-like growth factor
LV	Left ventricular
MI	Myocardial infarction
MSCs	Mesenchymal stem cells
Sca-1	Stem cell antigen-1
SDF-1	Stromal cell-derived factor-1
SP	Side population
UCB	Umbilical cord blood
VEGF	Vascular endothelial growth factor

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40.1 Introduction

Although coronary interventions and associated medical therapies have improved postinfarction cardiac function in patients with coronary artery disease, approximately half of the patients will still progress to end-stage or advanced heart failure [1]. To date, cardiac transplantation remains the only definitive therapy for replacing the lost muscle, but it is a widespread approach limited by the inadequate supply of donor hearts (approximately 2000 donor hearts are available each year in the USA). An alternative potential therapy for limiting postinfarction left ventricular (LV) remodeling, and thus the development of congestive heart failure, is the directed replacement of infarcted myocardium with the new myocardium being generated from transplanted stem cells.

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Recent studies have provided evidence to support the notion that cardiomyocyte regeneration may occur during physiological and pathological states in the adult heart. These data highlight the possibility that myocardial regeneration may occur via cardiomyocyte proliferation and/or differentiation of putative cardiac stem cells [2]. To date, various cell types have been used for cardiac repair including skeletal myoblasts, bone marrow-derived cells, mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), umbilical cord blood (UCB) stem cells, cardiac stem cells, and embryonic stem cells (ESCs). This chapter will review each of these different stem cell populations with regard to their potential treatment of heart disease. We will begin by examining the in vitro and in vivo animal studies, and then briefly discuss the cell therapy clinical trials that are currently underway for treating ischemic heart disease. We will conclude by summarizing selected techniques that have been used to enhance the beneficial effects of stem cell transplantation.

40.2 Cells for Myocardial Repair in Ischemic Heart Disease

40.2.1 Embryonic Stem Cells

ESCs can differentiate into all three developmental germ layers (including the mesoderm, which is the source of the cardiac lineage) and can proliferate with self-renewal in an unlimited fashion. Thus, ESCs have the potential of producing a limitless number of cells and cell types for regenerative therapy. However, ESCs must be differentiated into specific cell lineages before transplantation as the ESCs themselves are tumorigenic [3], and the cells derived from ESCs must be administered with immunosuppressive therapy [4] because ESCs can only be obtained from an allogenic source. Yet, the use of human ESCs (hESCs) is limited due to ethical concerns regarding the need to destroy human embryos in order to produce hESCs.

40.2.1.1 Mouse ESCs

The cardiogenic potential of mouse ESCs was first demonstrated in 1985 when these cells were cultured in suspension and formed 3D cystic bodies, termed *embryoid bodies*, which differentiated into cell types of the visceral yolk sac, blood islands, and myocardium [5]. Currently, such cells are separated from their feeder layer and then resuspended in leukemia inhibitory factor-free culture medium at a low density [6]. Mouse ESCs are then cultured in small drops which are formed on the lid of tissue culture dishes. When kept in this hanging droplet setting for 2 days, the cells aggregate and form differentiating embryoid bodies [6]. Embryoid bodies are then transferred into ultralow attachment dishes where they further differentiate. Spontaneous contracting cells (cardiomyocytes) can be observed between 7 and 8 days of differentiation [6]. This process of cardiac differentiation can be further enhanced by the use of selective growth factors and inhibitors of signaling pathways.

Importantly, mouse ESCs have been shown to engraft and regenerate myocardium after an experimentally induced myocardial infarction (MI) [7–9]. These cells can form cardiomyocytes that electrically couple with the host myocardium, endothelial cells, and blood vessels [7–9]. More recently, multipotent cardiac progenitor cells (CPCs) derived from mouse ESCs have been characterized from three independent laboratories [10–12]; Brachyury+/Flk+ and Isl1+ CPC cell lines were shown to differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells, while Nkx2-5/c-kit CPCs could differentiate into cardiomyocytes and smooth muscle cells.

40.2.1.2 Human ESCs

Human ESCs were first isolated from the human blastocyst in 1998 [3], and later it was shown that they could differentiate into cardiomyocytes [13]. Human ESCs also form embryoid bodies when cultured in suspension form; these are positive for cardiomyocyte markers such as myosin heavy chain, α-actinin, desmin, and troponin I [13]. Electrophysiological studies showed that most of the human ESC-derived cardiomyocytes resemble human fetal ventricular myocytes that can propagate action potentials [14]. Human ESCs can also differentiate into endothelial and smooth muscle cell lineages.

Initial in vivo studies have demonstrated that human ESC-derived cardiomyocytes can form new myocardium in the uninjured heart of athymic rats [15] or immunosuppressed pigs [14]. It was shown that the size of the graft could be increased fourfold by prior heat shock treatment of the cells [15]. When human ESCs are implanted in animal models that have a slow heart rate (such as in pigs or guinea pigs), they can form pacemakers when the native pacemaker (node) is dysfunctional, implying electrical integration with surrounding cardiomyocytes [14, 16]. However, when these cells are transplanted in the setting of MI, only 18 % form myocardial grafts and these grafts also contain substantial noncardiac elements [17]. To enhance the yield and purity of cardiomyocytes from human ESCs, Laflamme et al. developed a new technique to direct the differentiation of human ESCs into cardiomyocytes using sequential treatment of high-density undifferentiated monolayer cultures with activin A and bone marrow morphogenic protein 4 [17]. This protocol has yielded greater than 30 % cardiomyocytes, as compared to less than 1 % with the embryoid body-based system which used serum to induce differentiation [17]. Furthermore, Percoll gradient centrifugation, which allows specific enrichment of human ESC-derived cardiomyocytes, resulted in cultures containing 82.6 ± 6.6 % cardiomyocytes

[17]. Moreover, this laboratory used a prosurvival engraftment cocktail to improve graft survival in infarcted hearts. This cocktail included Matrigel to prevent anikis, a cell-permeant peptide from Bcl-XL to block mitochondrial death pathways, cyclosporine A to attenuate cyclophilin D-dependent mitochondrial pathways, a compound that opens ATP-dependent K+ channels (pinacidil) to mimic ischemic preconditioning, insulin-like growth factor (IGF-1) to activate Akt pathways, and the caspase inhibitor ZVAD-fmk [17]. Importantly, transplantation of human ESC-derived cardiomyocytes, in combination with this prosurvival cocktail into infarcted hearts, resulted in myocardial grafts with improved ventricular function [17]. The other intriguing aspect of this study was that almost all noncardiac human ESC-derived cells died by the 4-week period [17].

40.2.2 Human Induced Pluripotent Stem Cells

The immunogenicity and ethical concerns associated with hESCs have led to the development of human induced pluripotent stem cells (hiPSCs), which possess an ESC-like capacity for differentiation and self-replication but can be generated from an individual patient's own somatic cells. The somatic cells are reprogrammed with pluripotency factors such as Oct3/4, Sox2, Klf4, and c-Myc; however, hiPSCs (like hESCs) can be tumorigenic and must be differentiated into specific cell types before administration. Effective protocols for differentiating hiPSCs into smooth-muscle cells (hiPSC-SMCs) have been available for several years [18, 19], while methods for obtaining sufficiently large, pure, and stable populations of hiPSC-derived endothelial cells (hiPSC-ECs) [20] and cardiomyocytes (hiPSC-CMs) [21, 22] have recently been established. Studies in pigs with experimentally induced ischemia-reperfusion injury indicate that all three hiPSC-derived cell lineages are retained at the site of administration for at least 4 weeks after injection, and that the combined treatment can lead to improvements in contractile performance, myocardial wall stress, and cellular metabolism [22]; furthermore, treatment with hiPSC-CMs alone was not associated with arrhythmogenic complications such as those reported when hESC-derived cardiomyocytes (hESC-CMs) were administered to monkeys [23], perhaps because the number of cells administered was much smaller (i.e., 10 million hiPSC-CMs versus 1 billion hESC-CMs).

Although hiPSCs can in principle be used to generate cells of any lineage, the efficiency of the differentiation protocol and function of the hiPSC-derived cells after transplantation may be influenced by epigenetic factors that the hiPSCs retain from their tissues of origin [24, 25]. Thus, hiPSC-derived cells may be more effective for regenerative myocardial therapy if the hiPSCs were reprogrammed from cardiac lineage cells rather than from other organ-specific lineages. Notably, Zhang et al. [26] have successfully generated hiPSCs from cardiac fibroblasts which were obtained from the hearts of patients who were undergoing open-chest surgery. When these cardiac lineage hiPSCs (hciPSCs) were used to generate sheets of cardiomyocytes, the efficiency of the differentiation protocol exceeded 92 %, compared to 60–85 % when dermal- or cord blood lineage hiPSCs have been used [21]. Approximately 30 % of the hciPSC-derived cardiomyocytes were retained for at least 28 days after administration to the infarcted hearts of immunodeficient mice.

40.2.3 Adult Stem Cells

40.2.3.1 Skeletal Myoblasts

Skeletal myoblasts can be derived from myogenic stem cells also known as satellite cells. These myogenic stem cells are quiescent and located in a niche; they are sublaminar and sandwiched between the basal lamina and the plasmalemma of the skeletal muscle fibers. In response to injury, the myogenic stem cells become activated, they proliferate and differentiate, and typically completely restore the skeletal muscle architecture [27]. Previous studies have demonstrated that skeletal myoblasts form viable, long-term skeletal myotube grafts following transplantation into adult hearts [28]. In one study, it was shown that transplantation of autologous skeletal myoblasts in cryoinfarcted rabbit myocardium leads to myoblast engraftment by 3 weeks with subsequent improvement in systolic performance [27]. Importantly, as these cells are specified and committed to the skeletal muscle lineage, they do not differentiate into cardiomyocytes [29], and thus they are also not electromechanically coupled to each other or to the surrounding cardiomyocytes of the host [30, 31].

40.2.3.2 Bone Marrow-Derived Stem Cells

The bone marrow contains many adult stem cells which have been used to treat hematological disorders for decades. It has recently been shown that bone marrow-derived stems can traverse cell lineage boundaries and, upon appropriate stimulation, transdifferentiate into hepatocytes, endothelial cells, skeletal muscle, and/or neurons [32–34]. Yet, the ability of bone marrow-derived cells to differentiate into cardiomyocytes remains controversial. For example, Bittner et al. were the first researchers to suggest that cardiac muscle cells may be derived from bone marrow cells [35]. Goodel et al. demonstrated that transplantation of murine bone marrow side population (SP) cells (c-kit+, Sca-1+, CD34−/low) resulted in donor-derived cells with cardiomyocyte morphologies, as well as smooth muscle and endothelial cells which were

found in the heart following left anterior descending coronary artery ligation [36]. Orlic et al. [37] demonstrated that transplantation of GFP-labeled $Lin^{-}c\text{-}kit^{+}$ cells (presumably containing both hematopoietic stem cells and MSCs) into the ventricular wall after left anterior descending coronary artery ligation resulted in improved function of the ventricle, and they also detected a large number of GFP^{+} cells that coexpressed myocardial proteins in the myocardium. In contrast to these findings, other laboratories using genetic mouse models to label cell populations (and their derivatives) have shown that lineage negative, c-kit-positive cells were not able to differentiate into cardiomyocytes [38, 39]. Alternatively, Anversa and colleagues have shown, using similar genetic techniques, that c-kit+ bone marrow cells can engraft in the injured myocardium and differentiate into cells of the cardiogenic lineage, forming functionally competent cardiomyocytes and vascular structures [40].

40.2.3.3 Mesenchymal Stem Cells

Phenotype and Differentiation Potential

In the late 1980s and through the 1990s, Caplan's laboratory identified a subset of cells within the bone marrow which gave rise to osteoblasts and adipocytes. These cells were termed MSCs [41]. MSCs are present in many different organs of the body including muscle, skin, adipose tissue, and bone marrow. They can be isolated from the bone marrow by a simple process involving Ficoll centrifugation and adhering cell culture in a defined serum-containing medium. In the early studies, MSCs were shown to be expanded for 4–20 population doublings only [42], with preservation of the karyotype, telomerase activity, and telomere length [43, 44]. Phenotypically, these cells were negative for CD31, CD34, and CD45, unlike hematopoietic progenitors from bone marrow, and were positive for CD29, CD44, CD71, CD90, CD105, CD106, CD120a, CD124, SH2, SH3, and SH4 [45, 46]. In the bone marrow, only 0.001–0.01 % of the initial unfractionated bone marrow mononuclear cell population consists of MSCs [33, 36]. However, in a number of rodent studies, the adherent fibroblastic cells obtained from the unfractionated mononuclear class of the bone marrow are termed MSCs [47, 48].

MSCs were reported to have the potential to differentiate into any tissue of mesenchymal origin [41]. MSCs derived from rodent marrow aspiration have been shown to differentiate into cardiomyocyte-like cells in the presence of 5-azacytidine [49, 50]. The cellular morphology changes from spindle-shaped to ball-shaped, and finally a rod-shaped form; thereafter, these cells fuse together to form a syncitium which resembles a myotube [51]. In addition, these cells exhibit markers of fetal cardiomyocytes [50]; specific transcription factors of the myocyte and cardiac lineage including GATA4, Nkx2.5, and HAND 1/2 can be detected [49]. Yet compared to native cardiomyocytes, there are noteworthy differences in those which are derived from MSCs. First, the β-isoform of cardiac myosin heavy chain is more abundant than the α-isoform in these cells. Second, there is increased α-skeletal actin relative to α-cardiac actinin; myosin light chain 2v is also present. Third, both MEF2A and MEF2D isoforms replace MEF2C from early to late passages. Additionally, it was reported that these cells will beat spontaneously and synchronously, which is most likely due to the formation of intercalated discs, as has been shown when they are co-cultured with neonatal myocytes [52]. Finally, the differentiated cells will express competent α- and β-adrenergic and muscarinic receptors, as indicated by increased rates of contraction in response to isoproterenol and by decreased rates of contraction induced by β-adrenergic blockers [53]. Yet, it should be noted that other studies suggest that bone marrow stem cells cannot differentiate to cardiac myocytes [38, 39]. Whether or not MSCs can differentiate into functional cells of the other three lineages will require further investigation.

MSCs for Myocardial Repair

MSCs have several unique features that make them attractive candidates for cell transplantation. First, as they are easily accessible and expandable, MSCs could potentially become a so-called "off the shelf" allogeneic product, one which would be more cost-effective, easier to administer, and allow a greater number of cells to be transplanted. Additionally, they may also permit transplantation at the time of urgent interventions, e.g., to relieve ischemia and injury such as percutaneous or surgical revascularization procedures. Importantly, these cells appear to be hypoimmunogenic [54–56]. Additionally, these cells lack MHC-II and B-7 costimulatory molecule expression and thus limit T-cell responses [57, 58]. Yet, they are considered to directly inhibit inflammatory responses via paracrine mechanisms including production of transforming growth factor beta 1 and hepatocyte growth factor (HGF) [59, 60]. Importantly, all the above properties taken together make them attractive candidates for cell transplantation.

It should be noted that MSC transplantation was tested in a study in which isogenic adult rats were used as donors and recipients to simulate autologous transplantation clinically. MSC intracoronary delivery in these rat hearts following an experimentally induced MI showed that there was a milieu-dependent differentiation of these cells, a fibroblastic phenotype within the scar, and cardiomyocyte phenotype outside the infarction area [61]. However, direct intramyocardial injection of autologous MSCs into the region of the scar resulted in the focal differentiation of these cells into "cardiac-like" muscle cells within the scar tissue. There was also noted increased angiogenesis and improved myocardial function [62]. In a different approach, the delivery of MSCs

via direct left ventricular cavity infusion in a rat MI model resulted in the preferential migration and colonization of these cells in the ischemic myocardium (i.e., at 1 week) [63]. This MSC infusion also resulted in both increased vascularity and improved cardiac function 2 months following delivery in a canine model of chronic ischemic disease [64]. However, it should be noted that Kloner's laboratory, using a rat model of postinfarction LV remodeling, found that the beneficial effects on left ventricular function were short term and were absent after 6 months [65].

Importantly, MSC transplantation has been reported to result in functional improvement in large animal ischemic models. For example, the direct intramyocardial injection of 5-azacytidine-treated autologous MSCs was performed 4 weeks after MI in a swine model; these injected cells formed islands of cardiac-like tissue, induced angiogenesis, prevented thinning and dilatation of the infarct region, and ultimately improved regional and global contractile functions [66]. Similarly, allogeneic intramyocardial transplantation of MSCs in a porcine model of MI resulted in profound improvements in border zone energetics and regional contractile function [67]. These latter findings were hypothesized to be related to a paracrine mechanism, as evidenced by increased vascularity in the border zone and spared native cardiomyocytes in the infarct zone [67]. Finally, the percutaneous delivery of allogenic MSCs 3 days after MI in a porcine model resulted in long-term engraftments (detected at 8 weeks), profound reductions in scar sizes, and near normalization of cardiac function [68].

40.2.3.4 Endothelial Progenitor Cells

EPCs were first isolated from blood in 1997 [69]. They originate from a common hemangioblast precursor in the bone marrow [70]. However, many other cells including myeloid/monocyte (CD14+) cells and stem cells from adult organs can also differentiate into cells with EPC characteristics. Thus, circulating EPCs are a heterogeneous group of cells originating from multiple precursors within the bone marrow and can be isolated in different stages of endothelial differentiation within peripheral blood. Therefore, the characterization of these cells can be challenging because they share certain surface markers of hematopoietic cells and adult endothelial cells. Typically, they express CD34 (a hematopoietic cell characteristic), CD-133 (a more specific marker of EPCs), and KDR (kinase insert domain-containing receptor), which is the receptor for vascular endothelial growth factor (VEGF). Interestingly, in a study of sex-mismatched bone marrow transplant patients by Hebbel and coworkers, 95 % of circulating endothelial cells in the peripheral blood of transplant patients had the recipient genotype, but 5 % had the donor genotype [71]. It was found that the endothelial cells with the donor phenotype had delayed growth in culture, but had a high proliferative capacity with more than a 1000-fold expansion within 1 month; these were termed *endothelial outgrowth cells*. It was concluded that the endothelial outgrowth cells were of bone marrow origin [71]. In contrast, the cells with the recipient phenotype only had a 17-fold expansion within the same time period; these circulating endothelial cells most likely originated from the vessel wall [71].

EPCs have been used for treatment in different animal models of cardiovascular disease. For example, the intravenous delivery of CD34+ cells into athymic nude rats following MI was shown to promote angiogenesis in the peri-infarct region, leading to decreased myocyte apoptosis, reduced interstitial fibrosis, and improvement of left ventricular function [72]. Similarly, intramyocardial implantation of CD34+ selected human peripheral blood mononuclear cells into nude rats after MI resulted in neovascularization and improved LV function [73].

40.2.3.5 UCB Stem Cells

Human UCB is rich in stem and progenitor cells, which have high proliferative capacities [74–76]. Human UCB also contains fibroblast-like cells termed *unrestricted somatic stem cells*, which adhere to culture dishes, are negative for c-kit, CD34, and CD45, and differentiate both in vitro and in vivo into a variety of tissue types, including cardiomyocytes [77]. Direct intramyocardial injection of these human unrestricted somatic cells into the infarcted hearts of immunosuppressed pigs resulted in: (1) improved perfusion and wall motion; (2) reduced infarct size; and (3) enhanced cardiac function [78]. Further, intravenous injection of human mononuclear UCB cells, a small fraction of which were CD34+, into NOD/SCID mice led to enhanced neovascularization with capillary endothelial cells of both human and mouse origin and reduced infarct sizes [79]. However, no myocytes of human origin were found, thus arguing against cardiomyogenic differentiation and regeneration of cardiomyocytes from donor cells. Finally, the direct intramyocardial injection of UCB CD34+ cells into the peri-infarct rim in a rat model resulted in improved cardiac function [80]. To date, there have been no reported clinical studies of UCB transplantation for cardiac repair.

40.2.3.6 Cardiac Progenitor Cells

The innate ability of the cardiomyocytes to replicate has been a highly controversial issue for a long time. Previous studies have established that increases in cardiac mass in mammals during fetal life occur mainly due to cardiomyocyte proliferation. However, during the perinatal period, mammalian cardiomyocytes withdraw from the cell cycle, thus limiting their ability to divide and increase in number [81–83]. Thus, normal postnatal growth and adaptive increases in cardiac mass in adults, as a result of hemodynamic burden, are achieved mainly through the increases in

cell sizes, known as *hypertrophy* [81–83]. This belief was supported by the inability to identify mitotic figures in myocytes, as well as the observation that regions of transmural infarction evolved into essentially avascular, thin collagenous scar. This paradigm of heart growth had been dominant over the past 50 years, i.e., the heart is a postmitotic organ, consisting of a predetermined number of parenchymal cells, that is defined at birth and preserved throughout life until the death of the organ and/or organism. However, recent studies have challenged this concept of the heart being a postmitotic organ, one being incapable of regeneration. For example, it has been shown that the human heart contains cycling myocytes undergoing mitosis and cytokinesis under normal and pathological conditions [84–87]. The occurrence of these mitotic events is considered to support the hypothesis that CPC populations reside in the adult heart and can contribute to limited growth, turnover, and/or regeneration. This notion further supports that the adult heart belongs to the group of renewable adult tissues, and that this capacity for renewal is provided by a population of stem cells (i.e., CPCs) that reside in the myocardium [88, 89].

Origins of CPCs

To date, the primary origins of CPCs remain unclear. It is feasible that the cycling cardiomyocytes might be derived from uncommitted stem-like population cells that reside in the heart which expand and differentiate into cardiomyocytes in response to signals and cues in response to growth and/or injury. Alternatively, these stem-like cells may reside in extracardiac tissues such as the bone marrow, and are capable of being recruited into the circulation and induced to home to the heart by signals emanating from the injured heart.

For example, Mouquet et al. demonstrated that cardiac SP cells are maintained by local progenitor cell proliferation under physiological conditions [90]. After MI, this cardiac SP is decreased by as much as 60 % in the infarct and to a lesser degree in the noninfarct regions within 1 day. Cardiac SP pools are subsequently reconstituted to baseline levels within 7 days after MI, through both proliferation of resident cardiac SP cells and by homing of bone marrow-derived stem cells to specific areas of myocardial injury. These cells then undergo immunophenotypic conversions and adopt a cardiac SP phenotype (CD45+ to CD45−) [90]. Interestingly, bone marrow-derived stem cells accounted for approximately 25 % of the SP cells in the heart under pathological conditions, as compared to <1 % under physiological conditions [90]. In addition to these CD45+ cells that Mouquet et al. reported, bone marrow also contains CD45−, CXCR4+, and Sca-1+ cells within the nonadherent, nonhematopoietic mononuclear fraction, which will express early cardiac markers such as Nkx2.5 and GATA-4 [91]. These cells can also mobilize into the blood after MI and eventually home to the infarcted myocardium in mice. Cerisoli et al. [92] also demonstrated that (at least in pathological conditions) a subpopulation of the c-kit+ CPC population may derive from cells that originate in the bone marrow which are capable of contributing to myocardial regeneration in a similar fashion as the CPCs that are resident in the adult heart.

Number of CPCs

In 2005, Anversa and coworkers proposed that CPCs are undifferentiated multipotent cells that express the stem cell-related antigens, c-kit, MDR-1 (another ABC transporter), and Sca-1, in variable combinations [93]. Quantitative data from mouse, rat, dog, and human hearts were provided that demonstrated there is approximately one CPC for every 30,000–40,000 myocardial cells. Interestingly, ~65 % of all CPCs possess the three stem cell antigens, ~20 % express two stem cell antigens, and ~15 % express only one; roughly 5 % each of these CPCs exclusively express c-kit, MDR1, or Sca-1 [93].

Importantly, none of the above-mentioned reports demonstrated a signature CPC phenotype; this cell population also has significant overlap in the expression of other surface markers. It remains to be determined whether these CPCs are actually the same stem cell type and that differing surface markers reflect differing developmental phases or qualitatively separate subpopulations. Nevertheless, it is believed that these CPCs may participate in myocyte turnover, the rate of which remains to be determined.

Isolation of CPCs

CPCs have been isolated based on their expression (or absence) of specific cell surface markers, proteins, and/or tissue culture methods using the following strategies:

1. Isolation based on expression of the cell surface stem cell marker c-kit;
2. Isolation based on expression of the cell surface stem cell marker Sca-1;
3. Isolation based on the ability to efflux Hoechst 33342 dye (SP cells);
4. Isolation based on the expression of the islet-1 transcription factor;
5. Tissue culture of cardiac explants resulting in the spontaneous shedding of CPCs in vitro.

Isolation Based on Expression of the Cell Surface Stem Cell Marker c-kit

Belrami et al. [94] isolated cells expressing the tyrosine kinase receptor for stem cell factor (also referred to as steel factor; c-kit) from the interstitial regions of the adult rat heart. The highest density of these lineage negative (lin-), c-kit+ stem cells was in the atria and ventricular apex.

These cells were identified to be self-renewing, clonogenic, and multipotent. Further, they had the ability to differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells. Moreover, the delivery of these c-kit-expressing clonogenic stem cells following myocardial injury resulted in both improved functional recovery and evidence of myocardial regeneration. Recently, the same laboratory expanded their analyses to include preclinical studies using large animal models. They have demonstrated that the canine model also harbors a c-kit-expressing stem cell population in the adult heart that is clonogenic, multipotent, and capable of activation following injury. In response to myocardial injury, these c-kit-expressing stem cells are activated by cytokines (including HGF and insulin-like growth factor 1), and also home to areas of injury to participate in repair and regeneration. These preclinical studies have been further extended to the study of the human heart. A similar c-kit-expressing stem cell population (c-kit-positive but negative for the expression of the hematopoietic and endothelial antigens including CD45, CD31, and CD34) has been isolated from the adult human heart that was identified to be multipotent (capable of forming myocyte, smooth muscle cell, and endothelial cell lineages) in vivo and in vitro [95, 96]. Moreover, studies have established that these human c-kit-expressing cardiac stem cells undergo both symmetrical and asymmetrical cell divisions [96]. Importantly, these studies are also currently being validated by other cardiac stem cell laboratories. For example, van Berlo et al., utilizing genetic labeling strategies, demonstrated that c-kit-expressing stem cells were able to daughter cardiomyocytes, yet this was an infrequent event [97]. Rather, these investigators showed that c-kit-expressing stem/progenitors give rise largely to the endothelial cell population within the adult mouse heart. These studies underscore the ongoing controversies associated with many therapeutic approaches being developed within this field [98].

Isolation Based on the Expression of the Cell Surface Stem Cell Marker Sca-1

Resident murine CPCs have also been isolated on the basis of stem cell antigen-1 (Sca-1) expression [98]. These Sca-1-expressing CPCs were small interstitial cells that lacked hematopoietic lineage markers such as CD45, B220, TER119, or Flk-1, and they lacked c-kit expression, supporting the notion that they are distinct from the c-kit stem cell population. Using RT-PCR analyses, the Sca-1-expressing CPC population expressed the vascular marker CD31 and the cardiogenic transcription factors including Gata4, Mef2c, and TEF-1 (but lacked expression of Nkx2-5). A small percentage of these Sca-1-expressing CPCs activated cardiac genes, but did not exhibit spontaneous contractile properties in response to DNA demethylation with 5-azacytidine [98]. This laboratory further examined the abilities of the Sca-1 CPCs to form cardiomyocytes independent of fusion to the differentiated host cardiomyocytes, using genetic mouse models (Cre/Lox and the R26R genetic mouse models) for cellular labeling. Genetically tagged Sca-1 CPCs isolated from the αMHC-Cre transgenic mouse model and delivered into the R26R (all host cells are labeled with LacZ) injured these hearts. Two weeks following injury, the animals were sacrificed and hearts were examined for Cre and LacZ expression. Interestingly, approximately half of the cells expressing αMHC-Cre did not express LacZ, suggesting that the Sca-1-expressing cells are capable of myocardial differentiation independent of fusion to existing (host) cardiomyocytes [98]. Additional studies from another laboratory (Matsura and coworkers) have also isolated Sca-1+ cells from adult murine hearts, and have demonstrated that they are capable of differentiation into beating cardiomyocytes in the presence of oxytocin but not 5-azacytidine [99]. These Sca-1 CPCs are heterogeneous, but a subpopulation is capable of effluxing Hoechst 33342 dye.

Isolation Based on the Ability to Efflux Hoechst 33342 Dye (SP Cells)

Other laboratories have utilized flow cytometry to identify an adult stem cell population that is capable of effluxing Hoechst 33342 dye. Due to their ability to efflux Hoechst 33342 dye, these cells were located as a side population using flow cytometry and were termed SP cells. Subsequently, these SP cells have been isolated from a number of lineages including: adult bone marrow, skeletal muscle, lung, brain, liver, and mouse ESCs. These respective SP cell populations are multipotent when placed in a permissive environment. The ability of the SP cells to efflux the Hoechst dye is due to the presence of multidrug resistance proteins. Studies have demonstrated that Abcg2 is a member of the ATP [100] binding cassette (ABC) transporters (also known as multidrug resistance proteins), and is the molecular determinant for the SP cell phenotype. Both specific (FTC) and nonspecific (calcium channel blockers such as verapamil) blockers of Abcg2 prevent Hoechst 33342 dye exclusion. Abcg2-expressing SP cells participate in cardiac development and reside in the adult mouse heart. Following injury, these Abcg2-expressing cardiac SP cells increase in number and form fetal cardiomyocytes. In addition to serving as a marker for the SP cell population, Abcg2 has a cytoprotective function in response to oxidative stress. Moreover, previous studies have demonstrated that Hif2α is a direct upstream regulator of the Abcg2 gene. These results support the notion that CPCs in the adult heart likely play a protective role that promotes survival following injury.

To date, whole genome analyses using microarray platforms have examined the molecular signature of adult cardiac SP cells, adult bone marrow SP cells, adult skeletal muscle SP cells, and SP cells isolated from ESCs. As expected, cardiac

SP cells express Abcg2, Sca-1, and c-kit. They also have induction of signaling pathways including the notch signaling pathway and the Wnt signaling pathway, which are characteristic of a number of other stem cell populations. Yet, the cardiac SP cells largely lack expression of hematopoietic markers (CD45 and TER119). Importantly, the cardiac SP cells appear to be a subpopulation of the Sca-1-expressing CPCs. Other groups have isolated the so-called SP cells from mouse hearts based on their ability to exclude Hoechst 33342 dye [100, 101]. These cells express Abcg2, an ATP-binding cassette (ABC) transporter, and they are Sca-1+ and c-kit low, and differentiate into cardiomyocytes after co-culture with rat cardiomyocytes.

Isolation Based on *Islet-1 Gene* Expression

Recent studies by Laugwitz et al. have identified Isl-1-expressing cells as an important stem cell population during cardiac development [102]. The heart is derived from a primary heart field and a secondary heart field which segregate from a common progenitor during gastrulation. The primary heart field (which gives rise to the cardiac crescent) contributes to the left ventricle and atria, while the secondary heart field (which is derived from the pharyngeal mesoderm) gives rise to the right ventricle and the outflow tract. Utilizing a gene disruption strategy, embryos lacking Isl-1 are lethal and lack a secondary heart field (i.e., right ventricle and outflow tract), supporting the notion that Isl-1 is a critical regulator for cardiac development. Additional studies have further uncovered an Isl-1-expressing multipotent cardiac stem cell population that expresses Nkx2-5 and Flk-1 and gives rise to all the cardiac lineages (cardiomyocyte, smooth muscle, and endothelial cells) during heart development. While Isl-1-expressing cells are resident in the neonatal heart, there is no evidence of an Isl-1-expressing CPC population in the unperturbed or injured adult heart. Future studies will be necessary to define distinct and common molecular pathways that govern stem cell populations during cardiac development and regeneration of the adult injured heart. For more information on cardiac development, refer to Chap. 3.

Tissue Culture of Cardiac Explants with Spontaneous Shedding of CPCs In Vitro

In 2004, Messina et al. isolated undifferentiated cells that grew as self-adherent clusters (termed *cardiospheres*) from subcultures of postnatal atrial or ventricular human biopsy specimens and also from murine hearts [103]. These cardiospheres varied in size (20–150 μm) and were observed to beat spontaneously in culture. The cardiosphere-forming cells had the properties of adult cardiac stem cells as they were clonogenic, they expressed stem and EPC antigens/markers (c-kit, Sca-1, CD31, and Flk-1), were capable of long-term self-renewal, and could differentiate in vitro and in vivo into myocytes and endothelial cells [103]. Importantly, the expansion of the cardiosphere-forming cells resulted in more than one million human cardiospheres within a 1-month period. These studies were confirmed and expanded as Marban's laboratory obtained ventricular tissue from percutaneous endomyocardial biopsies from both humans and pigs. These ventricular biopsy specimens were cultured to form cardiospheres, which were further plated to yield cardiosphere-derived cells [104]. Cardiospheres and cardiosphere-derived cells expressed antigenic characteristics of stem cells at each stage of processing, as well as proteins vital for cardiac contractile and electrical function [104]. Human and porcine cardiosphere-derived cells co-cultured with neonatal rat ventricular myocytes exhibited biophysical signatures characteristic of myocytes, including calcium transients synchronous with those of neighboring myocytes [104]. Moreover, the delivery of cardiosphere-derived cells following myocardial injury resulted in improved myocardial function compared to their respective controls.

Myocardial Regeneration from CPCs

Anversa and coworkers demonstrated that the direct intramyocardial injection of c-kit+ cells into an ischemic rat heart reconstituted well-differentiated myocardium, comprised of new blood-carrying vessels and cardiomyocytes with the characteristics of fetal cells; these cells were present in approximately 70 % of the ventricle [95]. Later, it was also shown that intracoronary delivery of these cardiac stem cells in an ischemia/reperfusion rat model resulted in myocardial regeneration, infarct size reduction of 29 %, and improvement of LV function [105]. Given intravenously after ischemia/reperfusion, Sca-1 cells also homed to injured myocardium and differentiated into cardiomyocytes [98]. The relative contributions of regenerated cardiomyocytes and preservation of injured native cardiomyocytes in these studies requires clarification.

Wang et al. recently reported that heart-derived Sca-1$^+$/CD31$^-$ cells possess stem cell characteristics and play an important role in cardiac repair [106]. In that study, immunofluorescent staining and fluorescence-activated cell sorter analysis indicated that endogenous Sca-1$^+$/CD31$^-$ cells significantly increased in the infarct and peri-infarct areas at 3 and 7 days after MI. Western blot analyses confirmed elevated Sca-1 protein expression 7 days after MI. Sca-1$^+$/CD31$^-$ cells cultured in vitro were induced to express both endothelial cell and cardiomyocyte markers. Transplantation of Sca-1$^+$/CD31$^-$cells into a murine model of MI led to functional preservation and decreased remodeling after MI [106]. Immunohistochemical data indicated a significant increase of neovascularization, but a low level of cardiomyocyte regeneration at the infarct border zone. Despite the absence of significant cardiomyocyte regeneration, cell transplantation

remarkably improved myocardial bioenergetics [106]. These findings provide evidence that Sca-1^{+}/CD31^{-} cells possess both endothelial cell and cardiomyocyte progenitor cell characteristics. However, this study also reported that the regeneration rates of cardiomyocytes and/or endothelial cells from the engrafted stem cells were very low. Hence, trophic effects associated with the transplanted cells were most likely the primary basis of the beneficial effects of these cells [106]. Nevertheless, the expansion of these progenitor cells may have therapeutic applicability for the treatment of MI.

CPCs and early committed cells have been shown to: (1) express c-Met and IGF-1 receptors; and (2) synthesize and secrete the corresponding ligands, such as HGF and IGF-1 [107]. HGF mobilizes cardiac stem cells-early committed cells, and IGF-1 promotes both their survival and proliferation [107]. Therefore, in a separate study, HGF and IGF-1 were injected in mice following MI and a growth factor gradient was introduced between the site of storage of primitive cells in the atria and the region bordering the infarct to facilitate homing. Importantly, the newly formed myocardium contained arterioles, capillaries, and functionally competent myocytes that increased in size over time. This regenerative response was associated with improved ventricular performance and overall increased survival. Surprisingly, this intervention rescued animals with infarcts that comprised as much as 86 % of ventricular mass, which implied the elicited low ejection fractions. Subsequently, the above findings were replicated in a dog model, where HGF and IGF-1 were also used to stimulate resident cardiac stem cells after MI; noteworthy growth factor therapy again resulted in improvement of myocardial function [108].

Before they can be used therapeutically, CPCs have to be isolated from fragments of the myocardium and subsequently expanded in vitro. This was achieved in a pig model [109] where c-kit+ cells were isolated and each cell was propagated to form approximately 400,000 cells. Another group performed autologous transplantation of CPCs in an ischemia/reperfusion swine model [110]. To accomplish this, each pig had an initial biopsy from the right ventricular septum at the time of injury. The biopsies weighed approximately 92 mg, and yielded mean cell counts of 14.2×10^{6} cells after isolation and expansion (after 2.8 cell passages over 23 days). Intracoronary delivery was then performed 4 weeks after injury; engraftment primarily occurred in the MI border zone and islands of engrafted cells were present within the scar 8 weeks after coronary delivery [110].

Human CPCs have also been isolated from the myocardium, expanded in vitro, and then used for transplantation in animal models of ischemic myocardium. For example, Hosoda et al. isolated human CPCs from surgical samples [111], then these c-kit+ human CPCs were injected into the hearts of immunodeficient mice and rats. Foci of myocardial regeneration were identified at 2–3 weeks which consisted of myocytes, resistance arterioles, and capillaries [111]. The presence of connexin 43 and N-cadherin in the developing human myocytes strongly suggested that the engrafted human cells were functionally competent. Two-photon microscopy was used to further demonstrate the functional integration of enhanced green fluorescent protein-positive human myocytes with the surrounding myocardium [111]. More recently, Torella et al. [112] also isolated human CPCs from myocardial samples from all four chambers of the human heart; these were c-kit+, MDR-1+, and CD133+. In these studies, one clone was shown to generate over 5×10^{9} cells and form functional myocardium after injection into infarcted rat hearts [112].

Altogether, these studies provide early evidence of the rationale for the use of human CPCs in patients with ischemic heart disease. These cells appear to be excellent candidates for exogenous stem cell therapy, yet they must be harvested from patients and expanded ex vivo to generate numbers sufficient for transplantation. To date, there have been no reported clinical trials of human CPC therapy.

40.3 Update of Clinical Trials of Stem Cell Treatment in Heart Disease

Skeletal myoblasts were the first cell type to be used in therapeutic clinical trials. These cells can be transplanted in an autologous fashion without immunosuppression, and have several advantages including a high proliferative potential that allows an initial biopsy to be easily expanded in vitro. They are also terminally differentiated, thus decreasing the chances of tumorigenesis, and are known to be resistant to ischemia, allowing them to survive in scar or peri-scar areas where there is minimal perfusion. To date, there have been six phase I safety and feasibility studies of skeletal myoblast transplantation in patients with severe LV dysfunction caused by MI. Four of these studies [113–116] were surgical and entailed myoblast implantation at the time of coronary artery bypass grafting or left ventricular assist device implantation, and two were catheter-based trials [117, 118] using an endoventricular or coronary sinus transvenous approach. Although not the primary outcome of these studies, modest left ventricular functional improvement was noted following transplantation. Engraftment of myoblasts has been documented in pathological specimens up to 18 months after transplantation [115, 119]. Yet, some concerns regarding the development of ventricular tachycardia have prompted the use of intracardiac defibrillators in protocols for skeletal myoblast transplantation. Future studies may utilize the use of skeletal muscle stem cells (i.e., satellite cells) as opposed to well-differentiated myoblasts.

Bone marrow cells have also received intense interest as a cell therapy for patients with cardiovascular disease.

Importantly, the Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) trial revealed significant improvement in LV ejection fraction as well as significantly enhanced myocardial viability and regional wall motion in the infarct regions following transplantation of bone marrow mononuclear cells or blood-derived progenitor cells [120, 121]. The BOOST (bone marrow cell transfer to enhance ST-elevation infarct regeneration) study [122] also resulted in an increase in LV ejection fraction at 6 months following cell transplantation, but surprisingly there was no statistical difference between the treated and placebo groups at 18 months. The Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial [123] randomized 204 patients with acute MI to receive either an intracoronary infusion of progenitor cells derived from bone marrow or a placebo medium into the infarct artery 3–7 days after successful reperfusion therapy. It was reported that the absolute increase in LV ejection fraction was significantly greater (2.5 %) in the bone marrow cell group than in the placebo group at 4 months, although this represents a modest improvement. However, other trials (i.e., ASTAMI—Autologous Stem Cell Transplantation in Acute Myocardial Infarction and STEMI—ST-elevation Acute Myocardial Infarction) [124, 125] have shown negative results with no improvement in ejection fraction with cell transplantation; differences in cell preparation [126] and numbers have been proposed as possible causes for these conflicting results. Bone marrow cells have also been used in the chronic heart failure setting. For example, in the Transplantation of Progenitor Cells and Recovery of LV Function in Patients with Chronic Ischemic Heart Disease (TOPCARE-CHD) trial [127], 75 patients with stable ischemic heart disease who had a MI at least 3 months previously were assigned to receive: (1) no cell infusion; (2) infusion of circulating progenitor cells; or (3) bone marrow cell into the patent coronary artery supplying the most dyskinetic LV area. It was reported that the transplantation of bone marrow cells was associated with a moderate (2.9 percentage points), but significant improvement in LV function 3 months post-transplantation.

MSC transplantation has been used therapeutically in patients with acute MI 18 days after primary percutaneous intervention, and this resulted in significant improvement in LV ejection fraction up to 6 months following delivery [128, 129]. Furthermore, this was associated with a significant reduction in the size of the perfusion defect measured by positron emission tomography at 3 months following delivery [127, 129]. More recently, it has been demonstrated in a randomized double-blind placebo controlled trial that intravenous delivery of allogenic human MSCs led to improved ventricular function after MI [130]. Yet, the degree of response to intravenous therapy occurred early after MI, and compared favorably with previous studies using intracoronary infusions of bone marrow cells [130].

The clinical application of EPCs is limited by the fact that it is difficult to expand them into sufficient numbers without either inducing a change in their phenotype or the development of cell senescence. Recently, Erb et al. randomized patients with chronically occluded coronary arteries to receive intracoronary progenitor cells or a placebo; they mobilized bone marrow cells using GCSF, harvested them from peripheral blood, and expanded them ex vivo. Subsequently, the intracoronary delivery of these cells led to improvement in coronary flow reserve and cardiac function at 3 months post-transplant [131]. Currently, clinical trials using CD34+ cells from bone marrow that are enriched in EPC content are underway.

Phase I clinical studies that deliver cardiosphere-derived cells (CADUCEUS or CArdiosphere-Derived aUtologous stem CELLs to reverse ventricular dysfunction) following MI have been evaluated at 6 months and 1 year postdelivery. As these were Phase I safety studies and were not associated with major complications, they also supported the notion that cell therapy was associated with reduced scar size and improved regional function of injured myocardium. Future studies will require larger patient numbers which will have the statistical power to determine functional improvement [132].

Similarly, Phase I clinical studies to assess the safety of autologous c-kit+ cells isolated from the patient's heart and delivered at the time of surgical revascularization supported the notion that this cell population also was safe, reduced scar formation and was associated with increased left ventricular viable mass compared to controls [133]. These studies will warrant further analysis to determine their impact as a putative therapeutic approach.

40.4 Cardiac Patches

Stem cells, and more frequently, fully differentiated cardiac cells can be used to engineer a patch of myocardial tissue by either (1) culturing the cells in a monolayer until confluence [26, 134] or (2) seeding the cells into a scaffold of biomaterial, and the biomaterial can be modified to contain factors that enhance neovascularization and cell survival. For example, when gelatin microspheres containing thymosin β4 (Tβ4), which promotes angiogenesis while impeding inflammation and apoptosis, were incorporated into an MSC-containing fibrin patch, the engraftment and survival of the transplanted cells increased, and more endogenous CPCs were recruited to the site of injury [135]. More mature and structurally aligned patches can be created by constructing a ring of cardiac-cell-containing fibrin and then rhythmically stretching the ring for several days before implantation over the injury site; when

tested in a rat model of myocardial injury, this approach was associated with a remarkable (>77 %) decline in infarct size and complete functional recovery. Note that this was only when the cells seeded into the rings included a population of cardiomyocytes [136]. A recent, and much more extensive, review of the various materials and methods used to create myocardial patches has been published elsewhere [137].

40.5 Mechanisms of Beneficial Effects of Stem Cell Treatment

Several studies using animal models have established that stem cell treatment leads to a functional benefit after MI. The initial results from the human clinical trials are also promising; however, the mechanisms underlying the beneficial effects of stem cell transplantation remain somewhat unclear. The proposed mechanisms are discussed below.

40.5.1 Primary Remuscularization

First, the replacement of infarcted tissue by new myocardium generated by the transplanted cells is one explanation for the beneficial effects. This was observed in the case of human cardiac stem cells, which are able to form functional myocardium after transplantation into mice with MI; the transplanted cardiomyocytes were structurally integrated with the host myocardium and led to improvement in ventricular function [96]. This was also observed after the delivery of cardiomyocytes derived from human ESCs which were transplanted into mice with induced MIs [17]. To date, the ability of bone marrow cells to transdifferentiate into cardiomyocytes remains controversial with some reports suggesting that these cells can transdifferentiate into cardiomyocytes [40], while other reports refute this claim [38]. Other adult stem cells such as skeletal myoblasts and EPCs are not able to form cardiomyocytes, but have been found to still exert a beneficial effect, thus suggesting other mechanisms for improvement in LV function.

40.5.2 Attenuation of Adverse Remodeling

The structural changes that occur after myocardial injury, such as ventricular dilatation, wall thinning, increased chamber volume, and hypertrophy of the surrounding myocardium [138], are accompanied by substantial hypoxia-induced changes in cellular ATP metabolism [139]. After the heart recovers from the initial infarction, these metabolic changes can persist and are believed to contribute to progressive declines in cardiac function that eventually lead to heart failure [140, 141]. The transplantation of stem cells, or especially a patch of hiPSC-derived cells [142], can reduce adverse structural changes and partially restore a more native-like metabolic profile in the myocardium, thereby preserving or perhaps improving myocardial performance during the chronic phase of heart disease. For more details on cardiac bioenergetics, the reader is referred to Chap. 21.

40.5.3 Improved Perfusion

Enhanced blood flow, as measured by microspheres, has been shown to increase after stem cell transplantation in a rat model following MI [143]. Increased blood flow can be due to new vessel formation (angiogenesis) or enlargement of preexisting collaterals (arteriogenesis). A number of the stem cell populations discussed in this chapter have been shown to promote or contribute to neovascularization after transplantation in the setting of MI. Yet, some groups have challenged this concept and have shown that stem cells home to the region of developing vascular collateralization, but do not anatomically incorporate into the vessel as either endothelial or smooth muscle cells [144]; however, the delivery of these cell populations still improve collateral flow.

40.5.4 Paracrine Effects

Stem cells may secrete factors that act through totally different repair pathways to ultimately promote cardioprotection. Evidence supporting such a hypothesis recently emerged from Dzau's laboratory; they showed that the injection of the conditioned medium from Akt-overexpressing MSCs alone can decrease the infarct size and lead to functional improvement in an animal model of MI [145, 146]. Hypoxic Akt-transduced MSCs showed increased release of VEGF, FGF-2, IGF-1, HGF, and thymosin β4. It is likely that various factors acting in concert will ultimately exert numerous beneficial effect, as anti-VEGF and anti-FGF antibodies only partially decrease the conditioned medium-induced proliferation of endothelial and smooth muscle cells [147, 148].

40.5.5 Immunomodulation of the Infarct Environment

The inflammatory response after a MI has been recognized as a potential target for improving functional outcome after acute MI. Some stem cells may act, in part, by modulating the immune environment within the recently infarcted heart. For example, MSCs have been shown to directly inhibit the

inflammatory responses via paracrine mechanisms including the production of transforming growth factor beta 1 and HGF [60, 61].

40.5.6 Modulation of Extracellular Matrix Homeostasis

Remodeling of the ventricle is also known to involve modifications in the extracellular matrix which are thought to contribute to myocardial dysfunction. As such, MSC implantation in a rat model of MI significantly attenuated the increased expression of collagen types I and III, TIMP-1, and TGF-β, but had no effects on MMP-1 levels [149, 150]. This was associated with reduced LV dilatation and improved global ventricular function.

40.5.7 Stimulation of Endogenous Cardiac Progenitors Cells

Stem cell treatment could also lead to increased mobilization, differentiation, survival, and function of endogenous CPCs that are associated with the paracrine effects. This possibility is receiving intense interest as cell therapy has uniformly been shown to improve cardiac function but has variable contributions to newly regenerated myocardium.

40.6 Techniques of Enhancing Efficacy of Stem Cell Therapy

Although stem cell transplantation improves LV function after MI, to date, the observed stem cell engraftment is still found to be minimal. Furthermore, the majority of transplanted cells that do engraft remain as spindle-shaped stem cells and do not fully differentiate into the host cardiac cell phenotypes. Therefore, other techniques are considered necessary to enhance the efficacy of stem cell transplantation.

40.6.1 Mobilization

Granulocyte colony stimulating factor, VEGF, stromal cell-derived factor-1 (SDF-1), angiopoietin-1, placental growth factor, and erythropoietin are several factors that may be utilized as therapies to mobilize stem cells from the bone marrow to the systemic circulation. Once these stem cells are mobilized, they may participate in endogenous repair or alternatively be collected and expanded in vitro for future cell therapy uses. As an example, intracoronary infusion of peripheral blood stem cells mobilized by granulocyte colony stimulating factor resulted in the improvement of LV function in patients with MI [151].

40.6.2 Homing

An important goal is to enhance the homing of stem cells to the injured region of the heart. It is known that factors that contribute to the homing of stem cells include stromal-derived growth factor (SDF-1) [152, 153], high mobility group box protein 1 [154], and integrins. It is also known that the microenvironment after acute MI is more favorable to cell homing as compared to the chronically infarcted myocardium. For example, Lu et al. [155] examined the local conditions requisite for cell homing and migration using a rat model of permanent coronary artery ligation, and concluded that the optimal time period for cell homing and migration is within the 2-week period following an MI.

40.6.3 Function and Survival

Assuming that the number of transplanted cells that survive is critical to therapeutic benefit, multiple research groups are exploring new methods to increase the survival of transplanted cells As such, apoptosis can be decreased by the constitutive expression of Akt (a serine threonine kinase with potent prosurvival activity) or by heat shock prior to transplantation [156]. Furthermore, rat MSCs transduced to overexpress Akt1 (encoding the Akt protein) transplanted into ischemic myocardium were found to inhibit cardiac remodeling by reducing inflammation, collagen deposition, and myocyte hypertrophy in a dose-dependent fashion [157]. Similarly, MSCs transduced to express Akt were also studied in an ischemic porcine model, which showed an improvement in ejection fraction as compared to nontransduced MSCs. Recently, in order to determine the exact mechanisms of these beneficial effects, the effects of the apoptotic stimulus, H_2O_2, on MSCs transduced with Akt was studied in vitro. Specifically, Akt-MSCs were found to be more resistant to apoptosis and were related to higher levels of extracellular signal-regulated protein kinase activation and VEGF expression [158]. Yet, a significant concern also exists regarding the potential tumorigenicity of Akt-transduced cells, particularly when Akt is constitutively expressed because Akt has been shown to be sufficient to induce oncogenic transformation of cells and tumor formation; therapeutic efforts are underway to target the Akt pathway for the treatment of malignancies [159]. Additional strategies that have been widely tested involve those which increase vasculogenesis with VEGF; transfection with VEGF and IGF-1 improved survival of transplanted bone marrow cells in a rat model of MI [160]. Furthermore, it was observed that the delivery of cells which had undergone adenoviral transduction and overexpressed VEGF also resulted in improved LV function and neovascularization [161], but the addition of VEGF protein alone to cells did not show any benefit in a rat model of fetal cardiomyocyte transplantation [162].

Enhanced expression of other gene products has also been examined and found to be effective, including cardiotrophin-1, heme oxygenase-1, an IL-1 inhibitor, and CuZn-superoxide dismutase. It was also shown that MSCs transfected with a hypoxia-regulated heme oxygenase-1 vector were found to be more tolerant to hypoxia-reoxygen injury in vitro and result in improved viability in ischemic hearts [163]. Likewise, treatment with CuZn-superoxide dismutase has been shown to attenuate the initial rapid cell death following transplantation, leaving a twofold increase in the total number of engrafted cells at 72 h compared with controls [164].

To date, the use of viruses for gene expression cannot be translated into clinical studies due to the risk of mutagenesis, carcinogenesis, and induction of an immune response. Yet recently, Jo et al. [165] developed a nonviral carrier of cationized polysaccharide for the genetic engineering of MSCs. When genetically engineered by a spermine-dextran complex with plasmid DNA of adrenomedullin, MSCs secreted a large amount of adrenomedullin, an anti-apoptotic and angiogenic peptide. Transplantation of these adrenomedullin gene-engineered MSCs improved cardiac function after MI significantly more than did nontransduced MSCs. Thus, this genetic engineering technology using the nonviral spermine-dextran (and other promising new methods) is an emerging strategy to improve MSC therapy for ischemic heart disease.

40.6.4 Use of Biomaterials to Design Microenvironment

The microenvironment in which the cells are injected is of extreme importance for their survival and subsequent beneficial effects. It has been shown that biomaterials can be designed to regulate quantitative timed release of factors, which direct cellular differentiation pathways such as angiogenesis and vascular maturation. Moreover, it is believed that smart biomaterials are capable of responding to the local environment, such as protease activity or mechanical forces, with controlled release or activation [166]. Recently, Davis et al. [167] designed self-assembling peptide nanofibers for the prolonged delivery of IGF-1, a cardiomyocyte growth and differentiation factor, to the myocardium using a "biotin sandwich" strategy. Specifically, biotinylated IGF-1 was complexed with tetravalent streptavidin and then bound to biotinylated self-assembling peptides. After injection into rat myocardium, biotinylated nanofibers provided sustained IGF-1 delivery for 28 days, and targeted delivery of IGF-1 in vivo increased the activation of Akt in the myocardium. Therefore, cell therapeutic strategies using IGF-1 delivery by biotinylated nanofibers improved systolic function after experimental MI, demonstrating the importance of engineering the local cellular microenvironment and the impact of these and future interventions to improve the outcomes of cell therapy.

Importantly, many of these new biomaterials provide improved flexibility for regenerating tissues ex vivo, but emerging technologies such as self-assembling nanofibers can now establish intramyocardial cellular microenvironments following injection. This may allow percutaneous cardiac regeneration and repair approaches, i.e., injectable tissue engineering. It has been shown that materials can be made to multifunction by providing sequential signals with the custom design of differential release kinetics for individual factors. Thus, new rationally designed biomaterials no longer simply coexist with tissues, but can provide precision bioactive control of the microenvironment that may be required for cardiac regeneration and repair.

40.7 Summary

Recent studies continue to support the notion that cardiomyocyte regeneration may occur both during normal physiological adaptation and during the expression of pathological states in the adult human heart. Such findings may indicate the possibility for myocardial regeneration to occur via cardiomyocyte proliferation and/or differentiation of putative cardiac stem cells. To date, various cell types have been used for cardiac repair, including: skeletal myoblasts, bone marrow-derived cells, MSCs, EPCs, UCB stem cells, cardiac stem cells, and ESCs. This chapter has reviewed the current knowledge relative to these different stem cell populations being utilized for the potential treatment of heart disease. Findings to date continue to be promising, but much work remains before these therapeutic approaches become commonplace. Furthermore, specific cardiac devices/technologies for the clinical delivery of such cellular therapies will be required and are being currently being developed.

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