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REVIEW

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Stem cells as therapy for cardiac disease — a review

Katarzyna Jezierska-Woźniak[1], Dorota Mystkowska[1], Anna Tutas[1], Marek Kajetan Jurkowski[2]

[1]Department of Neurobiology and Human Anatomy, Faculty of Medical Sciences, University of Warmia and Mazury, Olsztyn, Poland

[2]Nuclear Medicine and Endocrine Oncology Department, Cancer Center and Institute of Oncology, Gliwice, Poland

Abstract: Acute myocardial infarction (AMI) is one of the most significant causes of morbidity and mortality worldwide. Stem cells represent an enormous chance to rebuild damaged heart tissue. Correct definition of the cardiac progenitors is necessary to understand heart development, and would pave the way for the use of cardiac progenitors in the treatment of heart disease. Identifying, purifying and differentiating native cardiac progenitor cells are indispensable if we are to overcome congenital and adult cardiac diseases. To understand their functions, physiology and action, cells are tested in animal models, and then in clinical trials. But because clinical trials yield variable results, questions about proper cardiac stem cells remain unanswered. Transplanted stem cells release soluble factors, acting in a paracrine fashion, which contributes to cardiac regeneration. Cytokines and growth factors have cytoprotective and neovascularizing functions, and may activate resident cardiac stem cells. Understanding all these mechanisms is crucial to overcoming heart diseases. (*Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 1, pp. 13–25*)

Key words: cell therapy, stem cells, heart failure, paracrine signaling

Introduction

Despite major advances in the understanding and treatment of coronary artery disease, acute myocardial infarction (AMI) still represents a significant cause of morbidity and mortality worldwide. Obstruction of coronary arteries leads to the death of cardiomyocytes, and if blood supply is not quickly restored, the cardiac tissue undergoes necrosis or apoptosis, leading to chronic sequelae of ischemic cardiomyopathy and congestive heart failure. Dead cardiomyocytes are replaced by a fibrotic scar, which renders normal electromechanical work impossible. Experiments with animal models and recent clinical trials suggest that cardiac cell therapy (CST) can improve cardiac function. Recent studies have demonstrated the therapeutic potential of stem cells in damaged hearts, both in animal models and in clinical trials. Different cell types are likely to induce functional improvement, but through distinct mechanisms. Therefore, it is crucial to determine the proper cell type in each cardiac disease. The major goals of cell therapy for ischemic heart diseases are to improve vascularization, reduce detrimental remodeling, prevent cardiomyocyte apoptosis, and enhance electromechanical function.

We here review the current state of knowledge as to regeneration in the adult mammalian heart. We consider the various stem and progenitor cell types which might regenerate the myocardium, and review the major problems and challenges to such therapy.

Correspondence address: K. Jezierska-Woźniak,
Dept. of Neurobiology and Human Anatomy,
Faculty of Medical Sciences, Univeristy of Warmia and Mazury,
Aleja Warszawska 30, 10–083 Olsztyn, Poland;
e-mail: katarzyna.jezierska@uwm.edu.pl

Which cell types improve cardiac function?

Embryonic stem cells

The potential of using embryonic stem cells (ESC) has long been evident. As pluripotent stem cells, they have unlimited self renewal potential, with developmental ability for various cell fates [1, 2], including cardiomyocytes [3–9], vascular smooth muscle cells and endothelial cells [10]. The first evidence that transplantation of embryonic stem cell-derived cardiomyocytes (ESC-CMs) was technically feasible was delivered by Kolossov et al. [11]. After the application of ESC-CMs and fibroblasts into the injured hearts of syngenic mice, long-term engraftment, without teratoma formation, was observed. Cardiomyocytes derived from embryonic stem cells integrate into heart tissue, improve its function and create a new myocardium. These results have been observed both in rodents [10, 12] and sheep [13], where murine embryonic stem cell-derived cardiomyocytes were transplanted into the sheep ventricle after infarction, where they formed a new myocardium. Moreover, no immunosuppression was noted.

Studies led by Laflamme [14] and van Laake [15] have revealed that the potential source for replacing missing cardiomyocytes in rodents may be human embryonic stem cell-derived cardiomyocytes (hESC--CMs). To limit cardiomyocyte death after transplantation, Laflamme [14] used a cocktail of pro-survival factors, including: Matrigel to avoid anoikis; 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 to mimic ischemic preconditioning; IGF-1 to activate Akt pathways; and caspase ihhibitor ZVAD-fmk. The results obtained by van Laake et al. [15] featuring longer term experiments are significant. Like Laflamme et al. [14], they revealed that hESC-CMs survived and improved heart function at four weeks, but 12 weeks after myocardial infarction there was no improvement compared to control mice.

However, research studies with human ESC have encountered many difficulties. The biggest obstacle has been the vigorous ethical discussion about their origin, in that they require the destruction of human embryos [16, 17]. Embryonic stem cells have a propensity to form teratomas, tumors containing a wide array of cell types [18], also in the heart [19]. The solution to this problem seems to be differentiation *in vitro* into specialized cell types, followed by introduction *in vivo*. This means that the teratogenicity has been lost [6]. The teratogenicity was also suppressed in mice with cardiac-restricted overexpressing cytokine TNF-α [20]. Furthermore, a huge challenge in clinical application is the immunological incompatibility of embryonic stem cells because of allogeneic origin [21]. It is probable that the immunological properties of predifferentiation *in vitro* and purified ES cell-derived organotypic cells differ from undifferentiated cells [11]. Other obstacles are animal culture reagents, which may contain viruses or prions and may complicate clinical applications [22]. Zhang et al. [23] observed increased cell deaths caused by ischemia after grafting the ES cell-derived cardiomyocytes into a normal myocardium.

The major focus of embryonic stem cell research seems to be the discovery of direct ES-derived cardiac progenitor cells to culture specialized cell types before application [24–27]. This 'guided cardiopoiesis' assumes genomic and proteomic characterization of the cardioinductive signals, which is necessary to achieve proper differentiation, and mimic the natural embryonic milieu. These signals represent cardiac transcription factors Nkx2.5, the myocyte-specific enhancer factor 2C involved in cardiac morphogenesis and myogenesis and vascular development (MEF 2C), and GATA4 [20, 28–30]. The compounds responsible for cardiac differentiation are transforming growth factor β1 (TGF-β1), fibroblast growth factor (FGF-2/4), insulin-like growth factor 1 (IGF-1/2), epidermal growth factor (EGF), bone morphogenetic proteins (BMP-2/4), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF-A), and activin-A [20]. This process has been supported by mesenchymal stem cells obtained from bone marrow, with a cocktail of secreted proteins, to enhance the cardiogenic potential [27] (Figure 1).

Induced pluripotent stem cells (iPS)

Pluripotency was thought to be characteristic of inner cell mass of an embryo. Takahashi and Yamanaka [31] introduced genes expressed in ES cells into

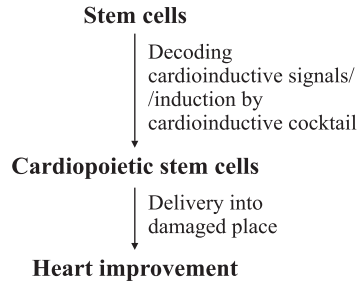

Figure 1. Assumption of guided cardipoiesis theory

mouse skin cells, which made them able to form all tissue cells. Reports of the possibility of generating ES-like cells suggested a new research field in medicine. This new development made use of reprogramming adult somatic cells [32–35] or spermatogonia from adult individuals [36], by expressing specific transcription factors. Induced pluripotent stem cells (iPS cells) can differentiate into tissues of the three germ layers, have similar morphology and express typical ESC markers. Using this technique, Freund et al. [37] derived iPS cell lines from healthy people and patients with the vascular disease hemorrhagic telangiectasia (HHT). Obtained iPS cell lines were also able to form cardiomyocytes. Transcription factors: Oct3/4, Sox2, Klf4, Nanog, Lin28 and c-Myc [31, 38] suppress the expression of genes responsible for forming somatic cells, block differentiation, and are sufficient to yield iPS [39]. Groups led by Martinez-Fernandez [40] and Mauritz [41] demonstrated that iPS cells express cardiac markers and form spontaneously contracting cardiomyocytes. However, other results showed that iPS-derived cardiomyocytes have impaired capacity to form differentiated, functional cells [42], and, like ES, can form tumors. The main reason for this phenomenon may be insertional mutations caused by usage of the retro- and lentiviral vectors. Therefore, it is important to replace this gene delivery system with a non-viral one [43, 44]. This would mean that adult stem cells could be used autologously, which could bypass the ethical issues pertaining to embryonic stem cells. In addition, iPS cells seem to be highly promising for drug screening.

Why not use adult stem cells?

In contrast to embryonic stem cells, these populations of stem cells have a limited capacity for differentiation.

Cardiac stem cells

Historically, the heart has been viewed as a terminally-differentiated organ without the capacity for self regeneration, caused by deficiency of endogenous stem cells. Diseased or damaged cardiomyocytes are removed by macrophages. In this heart region, scar tissue is formed, which leads to infarction. More recently, a number of groups have reported that the adult heart contains cell populations with stem cell characteristics and that senescent or apoptic cells are replenished, which enable the maintenance of cardiac homeostasis [45–49]. These cells have been named cardiac stem cells (CSC).

Bearzi et al. [50] reported that human cardiac stem cells have properties proper for stem cells, meaning they are self-renewing, clonogenic and multipotent. These cells can differentiate into cardiomyocytes, smooth muscles and endothelial cells. During ischemia, paracrine signals activate CSC to divide [51]. However, other studies have revealed that these divisions are insufficient to overcome losses caused by myocardial infarction [52]. Moreover, their numbers reduce with age and are only significant in fetal and neonatal mammalian organs. Bergmann et al. [53] reported that cardiomyocytes can renew (1% per year at age 25, 0.45% at age 75) by cell cycle reentry to resume mitosis and synthesis DNA. Thus, approximately 50% of these cells are exchanged during a normal lifespan. Urbanek et al. [54], on the basis of mitotic index, revealed that the number of CSCs increases during heart infarcts, but at a higher level in the acute than in the chronic phase. Moreover, their differentiation potential is also higher in an acute infarct.

These results suggest that dysfunction of the left ventricle in chronic ischemic cardiomyopathy may be the effect of a deficiency of functional cardiac stem cells. Other evidence for cardiomyocyte renewal has been provided in a study by Bergmann et al. [53]. Through incorporating the not-naturally occurring C^{14} into the DNA of human cardiomyocytes, they revealed that 50% of adult cells are exchanged during a normal lifespan.

To identify cardiac cell populations, several cell markers, morphology markers, pharmacology and isolation techniques have been used.

Side population cells

In 1996, Goodell et al. [55] named one of the populations of cardiac stem cells the 'side population' (SP). SP cells are identified on the basis of their ability to efflux Hoechst 33342 dye and rhodamine by ATP-binding cassette transporters, Absg2 and MDR1 [56]. Multipotent SP cells have been found in various organs [56], including the heart [57, 58]. These cells express cardiac specific markers when co-cultured with cardiomyocytes or under treatment with cardiogenic agents (oxytocin, histone deacetylase inhibitor trichostatin A) [59, 60]. The greatest cardiogenic potential has a subgroup of cells positive for stem cell antigen 1 ($Sca1^+$) and negative for endothelial marker ($CD31^-$) expression. An increased number of SP cells has been observed after a heart infarct. In the mouse model, it is connected both with proliferation of SP cells and homing of cells derived from bone marrow [61], but this can change with another mammalian model [60]. The regenerative potential of SP cells to functionally repair a damaged heart by differentiation into contracting myocytes requires evalua-

tion. Oyama et al. [60] revealed the capacity of side population cells to differentiate into cardiomyocytes, endothelial cells and smooth muscle cells, but Tomita et al. [62] found they could differentiate also into glia and neurons, which may suggest a neural crest origin.

c-Kit$^+$ progenitor cells

Other cardiac stem cells resident in the heart are c-kit$^+$ cells. These multipotent [50, 63, 64] and clonogenic cells are located within the atria and ventricles of the heart [60]. Beltrami et al. [63], using antigenic approaches, demonstrated the absence of markers typical of blood cells — CD8 (Lin$^-$), B-lymphocyte antigen (CD20), hematopoietic progenitor cell antigen (CD34), leukocyte antigen (CD45), leukocyte common antigen (CD45RO), erythroid cell marker (TER119), skeletal muscle — MyoD (the transcription factor that activates muscle-specific genes as myoblast precursors differentiate and fuse to form mature muscle fibers), myogenin, myogenic factor 5 (Myf5), and neural markers — microtubule-associated protein 1B (MAP1b), neurofilament 200, and snf glial fibrillary acidic protein (GFAP). These cells were positive for homeobox-containing genes that play critical roles in regulating tissue-specific gene expression essential for tissue differentiation (Nkx2.5), transcription factors that regulate genes involved in embryogenesis and in myocardial differentiation and function (GATA4), and myocyte enhancer factor-2 (Mef2). Although obtained cardiomyocytes, endothelial cells and smooth muscle cells have immature phenotypes in culture, and when placed in a differentiation medium only resemble mature cells, in rodent models these cells contribute to improved left ventricular ejection [60]. Li et al. [65] examined the function of this marker in cardiomyocyte terminal differentiation, and demonstrated its new function — promotion of SCS differentiation and regulation of terminal differentiation. Similarly, Tallini et al. [66] concluded that increased expression of c-kit could be caused by c-kit reexpression in committed myocytes, which is associated with fibrous and vascular infarct repair. Co-cultured c-kit$^+$ cardiac cell population with adult rat cardiomyocytes with insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF) caused increased cardiomyocyte survival [67].

Sca-1$^+$ progenitor cells

Next resident of progenitor population cells are positive for Sca-1, but negative for c-kit. These cells also express factors for early cardiogenesis, such as GATA4, Mef2 or dependent muscle-specific gene regulation factor (Tef1), but they are negative for Nkx2.5 and sarcomeric proteins. Stimulation with 5-azacytidine (5-Aza-C) or oxytocin results in expression of all genes of cardiac transcription factors, including cardiac troponin1, sarcomeric α-actin, myosin heavy chain and Nkx2.5 [52, 68]. Given intravenously into mouse hearts after ischemia/reperfusion, they home to the injured myocardium and express connexin 43, cardiac troponin-I (cTnI) and sarcomeric α-actin [52]. A few subgroups have been specified and described in this population. Sca-1$^+$/c-kit$^+$ population is unique, capable of differentiating into cardiomyocytes, after culture in the presence of oxytocin [68]. A combination of fibroblast growth factor (FGF), 5-Aza-C and Wnt antagonist Dkk-1 induces Sca-1$^+$/CD31$^-$ cells to differentiate into cardiac myocytes and endothelial cells. Transplantation of these cells enhances neovascularization and cardiac improvement, primarily through their paracrine effects [69]. Tateishi et al. [70] confirmed the potential of this line to upregulate the secreted paracrine effectors, which contribute to new vessel formation and limitation of cardiomyocyte apoptosis. Liang et al. [71] demonstrated that Sca1$^+$/CD31$^-$ cells have the capacity to migrate into a damaged myocardium and differentiate into both cardiomyocyte- and endothelial-like cells. An important role in this migration is played by the SDF-1α/CXCR4 system, a member of the CXC chemokine family, which influences normal development of the embryo and directs many processes in mature organisms.

isl-1$^+$ progenitor cells

Laugwitz et al. [72] isolated isl-1$^+$ but c-kit1$^-$ and Sca1$^-$ cardiac progenitors. They revealed that the presence of these cells in post-natal rat, mouse and human hearts decreases after birth. Isl-1$^+$ cardiac cells were located in atrias, in ventricles observed as single cells. This localization was conserved in analyzed species. This line has the capacity to differentiate into endothelial cells and smooth muscle cells of the aorta, pulmonary artery and proximal coronary tree [73]. Moretti et al. [74], using genetic fate-mapping, showed that expression of Isl1, Nkx2-5 and Kdr defines cardiovascular progenitor cells, which can give rise to mature cardiac, pacemaker, smooth muscle, and endothelial cell types. Moretti et al. [75] demonstrated that isl-1$^+$ cardiovascular progenitor generated from mouse iPS cells can differentiate into endothelial cells, smooth muscle cells and cardiomyocytes, without teratoma. Moreover, isl-1$^+$ and MDR-1 cardiac progenitors contribute to proepicardium during cardiac development [76].

Cardiosphere-derived progenitor cells

The cardiosphere (CS) is a cluster of self-adherent cells, expanded from human and murine biopsy specimens [48]. This structure is composed of clonal cells. At its core are c-kit$^+$ cells, while cells that exhibit endothelial and stem cell markers (Sca-1, CD34 and CD31) are on the periphery [49]. These cells, similarly to other cardiac progenitors, have the capacity to differentiate into cardiomyocytes, endothelial cells and smooth muscle cells. They demonstrate contractile activity in culture [49, 77] and expression of connexin-43, which is critical in forming connections between cells [48, 49]. Cardiosphere-derived cardiac progenitor cells contribute to improving ventricular function in mouse and swine models [49, 77, 78].

Neither the origin nor exact properties of cardiac stem cells are fully understood. Slack [79] suggested that cardiac stem cells resident in the adult heart are recruited from other sources, mainly the bone marrow. They may be remainder of embryonic stem cells or are only artifacts of isolation procedures. Mouquet et al. [61] showed that following myocardial injury in a mouse model, populations of cardiac progenitor cells are depleted and are restored to baseline levels by self-proliferation and by extracardiac source — selective homing of bone marrow-derived cells. Moreover, depletion of this source could contribute to decreased reparative capacity. Additionally, many c-Kit$^+$ cells were reported to co-express markers of mast cells and not express crucial markers of cardiac progenitors — Nkx2.5 and isl-1, critical in heart development [80]. Their presence could be part of a local innate immune response, after leaving bone marrow in search of pathogens in peripheral tissues [81].

Correct definition of the hierarchy of cardiac progenitors is necessary to understand heart development, which would pave the way for use of cardiac progenitors in the treatment of heart disease. Identifying, purifying and differentiating native cardiac progenitor cells are indispensable to overcoming congenital and adult cardiac diseases.

What about adult non-cardiac stem and progenitor cells?

Skeletal myoblast

Winitsky et al. [82] revealed skeletal myoblast that contain a population of non-satellite cells with the characteristics of cardiomyocytes, developing into usual cell culture with modest modifications. Because of their autological origin, being easy to isolate from muscle biopsies, their rate of amplification and resistance to ischemia, skeletal myoblast were the first cells injected into a failed myocardium [83]. Animal studies show improvement in infarct cardiac tissue [84–87], concerning recovery in left ventricular hemodynamics, increased wall thickness or decreased deleterious effects of post-infarction cardiac remodeling. However, these cells cannot form intercalated disks with resident cardiomyocytes, which causes arrhythmias [88, 89]. Moreover, some clinical trials have found episodes of ventricular tachycardia [90]. Menasché et al. [91] found that the improvement in left ventricular function is not sustained, and the cells are not electrically integrated, so that the benefits are unlikely to overcome the risk. Myoblast stem cells may act by paracrine effect on the surrounding myocardium, but not by generating new cardiomyocytes, because of their strict commitment to a myogenic lineage. Payne et al. [92] suggested that vascular endothelial growth factor (VEGF) is important to proper angiogenesis after muscle stem cells transplantation into an ischemic heart. The tools of genetic engineering allow the reprogramming of skeletal myoblast into cells capable of expressing connexin-43, the main gap junction protein, with the potential to reduce life-threatening post-infarct arrhythmias through the augmentation of intercellular coupling [93].

Mesenchymal stem cells

Bone marrow-derived stem cells

The bone marrow (BM) is home to a variety of cell populations, capable of migrating and transdifferentiating into diverse phenotyped cells. Major subsets of these cells are hematopoietic stem cells (HSC), mesenchymal stem cells (MSC) and endothelial progenitor cells. These cells can be sorted and categorized into subpopulations according to their cell-surface markers. However, the proper cardiac stem cells, and particularly their cell-surface markers, have not yet been determined among the abundance of bone marrow-derived stem cells. At present, stem cells from this source are firstly identified by absence of hematopoietic lineage markers, then by markers of mesenchymal stem cells, and finally by markers of endothelial stem cells. Such sorted analysis including cells exhibit markers c-Kit, Sca-1, VEGF2, AC133, CXCR4, CD34, but is not limited to them [94]. The existence of a huge number of possible combinations of surface markers contributes to variable results in different laboratories and it is critical to standardize the protocols of isolation.

Subpopulations of BM cells under specific conditions can differentiate into cardiomyocytes, endothelial cells and smooth muscle cells [95–97]. As early as 1997, Asahara et al. [98] demonstrated the potential of $CD34^+$ cells to promote neovascularization by differentiation into endothelial cells. Orlic Kajstura, Rota, and Zhao [99–103] have confirmed their vascular fate. An increase of mononuclear $CD34^+$ cells after acute myocardial infarction has been well documented [104, 105]. However, a study by Murry et al. [106] demonstrated a lack of transdifferentiation of the hematopoietic stem cells into cardiomyocytes. Mobilization of mononuclear bone marrow $CD34^+$ by granulocyte colony-stimulating factor (G-CSF), after percutaneous coronary intervention, offers a strategy for improving the cardiac function after acute myocardial infarction [107]. Leone et al. [108] demonstrated that the spontaneous mobilization of $CD34^+$ cells into the peripheral blood of patients with acute myocardial infarction is significantly correlated to endogenous G-CSF. It is worth mentioning that the beneficial effect of G-CSF is connected to the time of treatment. Delayed application reduces its cardioprotective effect [109, 110].

Endothelial progenitor cells (EPCs) have been identified both in blood and in bone marrow. EPCs maintain vascular homeostasis by secreting angiogenesis growth factors [111]. On the other hand, Murry et al. obtained results which indicated *in viv*o insignificant amounts of differentiated BM cells in the direction of cardiovascular types.

Kinnaird et al. [112] revealed that monocytic cells, which are the part of BM mononuclear cells, contribute to collateral vessel growth and vascularization. Mathieu et al. [113] compared autologous bone marrow derived cells and mesenchymal stem cells in a canine model of chronic myocardial infarction. After cell transfer contractility, regional systolic function and reduction in infarct size were improved in the mononuclear group mainly due to neovascularization. Based on these and other results, clinical trials using bone marrow mononuclear cells have been instigated.

Mesenchymal stem cells account for between 0.001% and 0.01% of nucleated cells in bone marrow [114], normally differentiated into muscle, cartilage, bone and fat, and can also transdifferentiate into other cell types, including cardiomyocytes [99, 115, 116], but with low efficiency [115, 117]. An important property of MSCs utilization is releasing the soluble factors that contribute to cardiac repair and regeneration, by inducing cytoprotection and neovascularization. Moreover, those paracrine factors may mediate endogenous regeneration via activation of resident cardiac stem cells [118]. Their low immunogenicity allows their use in allogeneic recipients [119, 120]. In many studies, MSCs after genetic modification, have been used to deliver factors necessary to improve infarct cardiac function — stromal cell derived factor 1 (SDF-1) [121], insulin-like growth factor 1 (IGF-1) [122], human heme oxygenase-1 (hHO-1) [123] or Wnt antagonist [124].

Despite successful results in animal models [115, 120, 125–127], and also using human MSC [128], these cells have the potential to form calcification and ossification islands in heart tissue [129, 130], which suggests their fate is not restricted to the tissue of destination.

Adipose tissue-derived stem cells

Another source of mesenchymal stem cells is adipose tissue, with perivascular niche. Adipose tissue has the ability to differentiate into several mesodermal lineages including bone, cartilage, muscle and fat. Recent research suggests that these cells can also form non-mesodermal tissues — neuron-like cells [131]. This cell fraction, known as stromal vascular fraction (SVF), contains adherent unit cells [132, 133], which can be isolated and cultured *in vitro*. Their ability to differentiate may be modified under adequate conditions to cardiomyocyte and vascular cell types [134–136]. Rangappa et al. [134] found adipose-derived stem cells from rabbits which differentiate into cardiomyocyte under 5-azacytidine factor. Moreover, these cells demonstrated spontaneous contraction and expressed markers of α-actinin, troponin-1 and myosin heavy chain. The first trials with human cells were made by Gaustad et al. [137] with extracts of rat cardiomyocytes. These studies allowed the delineation of cardiomyocyte lineage including morphology and biochemistry. An immunocompromised murine model was used to inject human adipose derived stem cells [138]. MRI was used to track labeled SPIO and 5-bromodeoxyuridine cell position and to quantify cardiac function. Transplanted cells did not demonstrate cardiac phenotype, but there was an observed improvement in cardiac function. Yamada et al. [139] reported that cardiac progenitor cells are present in brown adipose tissue (BAT) and are relevant to the regeneration of the injured myocardium. One year later [140], the same researchers revealed that $CD133^+$ cells in BAT differentiate into cardiomyocytes and can induce bone marrow cells in this direction. Two processes may explain this phenomenon — secreted protein (vascular endothelial growth factor VEGF, hepatocyte growth factor HGF, angiopoietin-1) and cell-to-cell contact proteins (platelet-derived growth factor PDGF expressed by $CD133^+$ cells and

platelet-derived growth factor receptor α PDGFR-α expressed by bone marrow cells). However, because BAT does not exist in adults, it might not have clinical application in adult patients. This beneficial effect appears to be related to the paracrine mechanism of action. Song, Nakagami, Rehman, and Cao [141–145] confirmed this hypothesis, demonstrating that VEGF, HGF, placental growth factor, FGF-2, TGF-β and angiopoietin-1 are critical in differentiating adipose-derived stem cells into cardiomyocytes. Bai et al. [146] compared the effects of freshly isolated adipose tissue-derived cells to that of cultured human adipose tissue-derived stem cells on cardiac function following myocardial infarction, and whether it is the result of differentiation or paracrine mechanism. Both cell types underwent differentiation in the cardiomyogenic direction, expressed connexin-43 and troponin1, and were found to be integrated with host cardiomyocytes or incorporated into new vessels. Additionally, mature adipocyte-derived de-differentiated fat cells have the ability to differentiate into cardiomyocyte-like cells, and their transplantation into an infarcted heart led to neovascularization [147].

Clinical trials

A wide variety of cell types have been considered for transplantation in humans, including bone marrow-derived cells, skeletal myoblast stem cells, cardiac stem cells, endothelial progenitor cells, adipose-derived stem cells, and embryonic stem cells [148]. A comprehensive list of clinical trials is available at http://www.the-scientist.com/supplementary/html/24104, [149] the web page of the American Library of Medicine. The list contains information about source of transplanted cells, allocation, means of delivery, patient age and condition and other information. However, many clinical trials yield variable results, leaving questions about proper cardiac stem cells unanswered [150]. Firstly, these results are difficult to interpret due to the wide range of delivery methods and lack of research methodologies. Neither the appropriate cardiac cell type, nor the appropriate means of application, has yet been found. Actual profit of stem cells therapy base on paracrine effect of transplanted cells (Table 1).

Methods of stem cells delivery and required cell doses

Currently available ways of delivering stem cells include intravenous infusion [151], intracoronary injection [152], and direct epicardial [153] or endocardial injection via a catheter [154, 155]. All these techniques have advantages and disadvantages. For example, Hofmann et al. [156] revealed that only 1.2––3.6% of bone marrow cells were found in the heart after infusion into the coronary arterial circulation. This method of delivery seems to be the most straightforward. Moreover, cells are conducted into regions where nutrition and oxygen are preserved. However, due to occlusion of the arteries of the ischemic regions, the survival of these cells is limited. Because of the injection being into the myocardium, we expect that cells are placed in the region of interest. Nevertheless, ischemic conditions and inflammation create a highly unfavorable environment. Additionally, the blood supply in the ischemic and scarred myocardium is insufficient, causing the formation of islands of cells [150]. And cells are mechanically lost during injection [157]. Similarly low retention of injected cells have been observed for bone marrow cells in sheep [158], endothelial progenitor cells in a rat [159] or mouse model, where only 7.4% of injected cells survived beyond 72 hours [160]. Hou et al. [161] concluded that 50–70% cells are lost at the time of delivery, and 90% if delivery is via arterial coronary infusion. This loss might be the result of programmed

Table 1. Stem cells therapy for cardiac disease

	The best source of cells	Way of delivery	Engraftment
What we know, what we can?	Embryonic stem cells iPS cells Heart Skeletal muscle Bone marrow Adipose tissue	Intravenous Intracoronary Intramyocardial	
What remains to be known?	Purity of cells Sufficient dose of cells Paracrine effect Necessity and method of differentiation into cardiomyocytes and vessels	Cells survive Cells retention Ischemic conditions Immune response Timing Homing	Effective differentiation into cardiomyocytes and vessels Mechanical coupling Electricaly integration Long-term engraftment Lack of arrhythmia

cell death, the toxic effect of an ischemic milieu, or allogenic origin of injected cells. Therefore, the dose required to achieve the therapeutic goal must take into consideration cell loss during the treatment protocol. Moreover, cells being cultured *in vitro* and administered in several doses at different times turned out to be indispensable [162]. A meta-analysis by Singh et al. [163] suggested that intracoronary infusion of bone marrow stem cells is effective in patients after acute myocardial infarction. But larger randomized trials are needed to validate these results.

Homing

For correct homing (the process of directing stem cells where they are needed for repair) of transplanted cells, an interaction between vascular wall and transendothelial migration is required, which involves a similar mechanism as in immune cells during their site of inflammation [164, 165]. Stem cell factor and granulocyte-colony stimulating factor (G-CSF) facilitate homing of BM cells to the infarct zone [100]. G-CSF also causes increased and better migration of resident cardiac stem cells [166].

Whereas Yoon et al. [167] showed the influence of vascular growth factor on increased angiogenesis and homing of endothelial progenitor cells from BM, Ryzhov et al. [168] demonstrated that adenosine causes increased adhesion of endothelial progenitor cells to the vascular wall. Delivered intracoronarily, adenosine does not cause any adverse events, has a short half-life in the bloodstream, and may recruit progenitor cells [169, 170].

It is very important to find a solution to the means of homing. Intravenous infusion results in improvement of cardiac function in a rat model, but histological examination revealed noncardiac and nonspecific homing, identifying labeled cells in the lung, liver, spleen, and bone marrow [151]. Swijnenburg et al. [171] compared the viability and effects of transplanted bone marrow mononuclear cells on cardiac function in acute and subacute phases of myocardial infarction. They demonstrated that the timing of cell delivery has minimal influence on the analyzed questions.

Conclusions

Stem cells comprise an enormous opportunity to rebuild damaged tissues. To understand their functions, physiology and action, cells are tested in animal models, and then in clinical trials. Results as yet do not allow the choice of the best source of cells for cardiac improvement. Autologous stem cells, despite their limited plasticity, are the safest in clinical trials. Embryonic stem cells, with their huge potential to differentiate, provoke ethical questions and carry risks of arrhythmias and teratoma. Obtaining pluripotent stem cells from adult tissues might be essential in clinical applications.

The evidence for a role in cardiac improvement for bone marrow-derived cells, endothelial progenitors, cardiac resident stem cells and others, might prove that during cardiac damage all available cells are mobilized, or that different cells have different functions, or both. Using various stem cell sources might be significant in clinical trials. Moreover, it is necessary to identify the optimal dose and the best method of administering cells.

It is important to note that transplanted stem cells releasing soluble factors, and acting in a paracrine fashion, contribute to cardiac regeneration. Cytokines and growth factors have functions of cytoprotection and neovascularization. Moreover, paracrine factors may activate resident cardiac stem cells. Paracrine factors may also influence cardiac remodeling, contractility, and metabolism.

Genetic modification of stem cells may overcome difficulties with cell viability, determining their function or ability to differentiate.

Recent research showing that genetically modified cells secrete therapeutic factors provides a potential strategy in cardiac therapy.

Analyzing the microenvironments in which cells act seems to be of critical importance. Bone marrow-derived cells assume that phenotypes depend on an extracellular matrix [172]. These results have significant implications for understanding the physical effects of the *in vivo* microenvironment, including for ischemia, inflammatory or toxic products, and for therapeutic uses in cell therapy.

References

1. Keller G. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev.* 2005;19:1129–155.
2. Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell.* 2008;132:661–680.
3. Klug MG, Soonpaa MH, Koh GY, Field LJ. Genetically selected cardiomyocytes from differentiating embryonic stem cells from stable intracardiac grafts. *J Clin Invest.* 1996;98:216–224.
4. Kehat I, Khimovich L, Caspi O et al. Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol.* 2004;22:1282–1289.
5. Behfar A, Zingman LV, Hodgson DM et al. Stem cell differentiation requires a paracrine pathway in the heart. *The FASEB Journal.* 2002;16:1558–1566.
6. Laflamme MA, Gold J, Xu C et al. Formation of human myocardium in the rat heart from human embryonic stem cells. *Am J Pathol.* 2005;167:663–671.

7. Yan P, Nagasawa A, Uosaki H et al. Cyclosporin-A potently induces highly cardiogenic progenitors from embryonic stem cells. *Biochem Biophys Res Commun.* 2009;379:115–120.
8. Yang L, Soonpaa MH, Adler ED et al. Human cardiovascular progenitor cells develop from a KDR$^+$ embryonic-stem-cell--derived population. *Nature.* 2008;453:524–528.
9. Wu SM, Fujiwara Y, Cibulsky SM et al. Developmental origin of a bipotential myocardial and smooth muscle cell precursor in the mammalian heart. *Cell.* 2006;127:1137–1150.
10. Singla DK, Hacker TA, Ma L et al. Transplantation of embryonic stem cells into the infarcted mouse heart: formation of multiple cell types. *J Mol Cell Cardiol.* 2006;40:195–200.
11. Kolossov E, Bostani T, Roell W et al. Engraftment of engineered ES cell-derived cardiomyocytes but not BM cells restores contractile function to the infarcted myocardium. *J Exp Med.* 2006;203:2315–2327.
12. Hodgson DM, Behfar A, Zingman LV et al. Stable benefit of embryonic stem cell therapy in myocardial infarction. *Am J Physiol Heart Circ Physiol.* 2004;287:H471–H479.
13. Menard C, Hagege AA, Agbulut O et al. Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study. *Lancet.* 2005;366:1005–1012.
14. Laflamme MA, Chen KY, Naumova AV et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol.* 2007;25:1015–1024.
15. van Laake LW, Passier R, Doevendans PA, Mummery CL. Human embryonic stem cell-derived cardiomyocytes and cardiac repair in rodents. *Circ Res.* 2008;102:1008–1010.
16. Bahadur G. Ethics of testicular stem cell medicine *Hum. Reprod.* 2004;19:2702–2710.
17. de Wert G, Mummery C. Human embryonic stem cells: research, ethics and policy *Hum Reprod.n* 2003;18:672–682.
18. Nussbaum J, Minami E, Laflamme MA et al. Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. *FASEB J.* 2007;21:1345–1357.
19. Liu YP, Dovzhenko OV, Garthwaite MA et al. Maintenance of pluripotency in human embryonic stem cells stably overexpressing enhanced green fluorescent protein. *Stem Cells Dev.* 2004;13:636–645.
20. Behfar A, Perez-Terzic C, Faustino RS et al. Cardiopoietic programming of embryonic stem cells for tumor-free heart repair. *J Exp Med.* 2007;204:405–420.
21. Sarić T, Frenzel LP, Hescheler J. Immunological barriers to embryonic stem cell-derived therapies. *Cells Tissues Organs*. 2008;188:78–90.
22. Passier R, van Laake LW, Mummery CL. Stem-cell-based therapy and lessons from the heart. *Nature.* 2008;453:15.
23. Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE. Cardiomyocyte grafting for cardiac repair: graft cell death and antideath strategies. *J Mol Cell Cardiol.* 2001;33:907–921.
24. Behfar A, Terzic A. Cardioprotective repair through stem cell--based cardiopoiesis. *J Appl Physiol*. 2007;103:1438–1440.
25. Behfar A, Faustino RS, Arrell DK, Dzeja PP, Perez-Terzic C, Terzic A. Guided stem cell cardiopoiesis: discovery and translation. *J Mol Cell Cardiol*. 2008;45:523–529.
26. Behfar A, Perez-Terzic C, Faustino RS et al. Cardiopoietic programming of embryonic stem cells for tumor-free heart repair. *J Exp Med*. 2007;204:405–420.
27. Behfar A, Terzic A. Optimizing adult mesenchymal stem cells for heart repair [editorial]. *J Mol Cell Cardiol*. 2007;42:283–284.
28. Faustino RS, Behfar A, Perez-Terzic C, Terzic A. Genomic chart guiding embryonic stem cell cardiopoiesis. *Genome Biol.* 2008;9:R6.
29. Arrell DK, Niederländer NJ, Faustino RS, Behfar A, Terzic A. Cardioinductive network guiding stem cell differentiation revealed by proteomic cartography of TNF α-primed endodermal secretome. *Stem Cells.* 2008;26:387–400.
30. Perez-Terzic C, Faustino RS, Boorsma BJ et al. Stem cells transform into a cardiac phenotype with remodeling of the nuclear transport machinery. *Nat Clin Pract Cardiovasc Med.* 2007; 4:S68–S76.
31. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006;126:663.
32. Takahashi K**,** Tanabe K, Ohnuki M et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007;131:861–872.
33. Wernig M, Meissner A, Foreman R et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature.* 2007;448:318–324.
34. Okita K, Ichisaka T, Yamanaka S. Generation of germ-line competent induced pluripotent stem cells. *Nature.* 2007; 448:313–317.
35. Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell*. 2007;1:39–49.
36. Guan K, Nayernia K, Maier LS et al. Pluripotency of spermatogonial stem cells from adult mouse testis. *Nature.* 2006;440:1199–1203.
37. Freund C, Davis RP, Gkatzis K, Ward-van Oostwaard D, Mummery CL. The first reported generation of human induced pluripotent stem cells (iPS cells) and iPS cellderived cardiomyocytes in the Netherlands. *Neth Heart J.* 2010;18:51––54.
38. Yu J, Vodyanik MA, Smuga-Otto K et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science.* 2007;318:1917–1920.
39. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell.* 2008; 132:567–582.
40. Martinez-Fernandez A, Nelson TJ, Yamada S et al. iPS programmed without c-MYC yield proficient cardiogenesis for functional heart chimerism. *Circ Res*. 2009;105:648–656.
41. Mauritz C, Schwanke K, Reppel M et al. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. *Circulation.* 2008;118:507–517.
42. Liu YH, Karra R, Wu SM. Cardiovascular stem cells in regenerative medicine: ready for prime time? *Drug Discov Today Ther Strateg.* 2008;5:201–207.
43. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science*. 2008;7;322:949–953.
44. Stadtfeld M., Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science.* 2008;322:945–949.
45. Ellison GM, Torella D, Karakikes I, Nadal-Ginard B. Myocyte death and renewal: modern concepts of cardiac cellular homeostasis. *Nat Clin Pract Cardiovasc.* 2007;4:S52–S59.
46. Kajstura J, Urbanek K, Rota M et al. Cardiac stem cells and myocardial disease. *J Mol Cell Cardiol.* 2008;45:505–513.
47. Beltrami AP, Barlucchi L, Torella D et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*. 2003;114:763–776.

48. Messina E, De Angelis L, Frati G et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res*. 2004;95:911–921.
49. Barile L, Messina E, Giacomello A, Marbαn E. Endogenous cardiac stem cells. *Prog Cardiovasc Dis*. 2007;50:31–48.
50. Bearzi C, Rota M, Hosoda T et al. Human cardiac stem cells. *Proc Natl Acad Sci USA*. 2007;104:14068–14073.
51. Linke A, ller PM, Nurzynska D et al. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. *Proc Natl Acad Sci USA*. 2005;102:8966–8971.
52. Oh H, Bradfute SB, Gallardo TD et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci USA*. 2003;100: 12313–12318.
53. Bergmann O, Bhardwaj RD, Bernard S et al. Evidence for cardiomyocyte renewal in humans *Science.* 2009:324:98–102.
54. Urbanek K, Torella D, Sheikh F et al. Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. *Proc Natl Acad Sci USA*. 2005;102: 8692–8697.
55. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med* 1996;183: 1797–1806.
56. Challen GA, Little MH. A side order of stem cells: the SP phenotype. *Stem Cells.* 2006;24:3–12.
57. Hierlihy AM, Seale P, Lobe CG et al. The post-natal heart contains a myocardial stem cell population. *FEBS Lett.* 2002;530:239–243.
58. Martin CM, Meeson AP, Robertson SM et al. Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. *Dev Biol*. 2004;265:262–275.
59. Pfister O, Mouquet F, Jain M et al. $CD31^-$ but not $CD31^+$ cardiac side population cells exhibit functional cardiomyogenic differentiation. *Circ Res.* 2005;97:52–61.
60. Oyama T, Nagai T, Wada H et al. Cardiac side population cells have a potential to migrate and differentiate into cardiomyocytes in vitro and in vivo. *J Cell Biol.* 2007;176:329–341.
61. Mouquet F, Pfister O, Jain M et al. Restoration of cardiac progenitor cells after myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. *Circ Res.* 2005;97:1090–1092.
62. Tomita Y, Matsumura K, Wakamatsu Y et al. Cardiac neural crest cells contribute to the dormant multipotent stem cell in the mammalian heart. *J. Cell Biol.* 2005;170:1135–1146.
63. Beltrami AP, Barlucchi L, Torella D et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell.* 2003;114:763–776.
64. Rota M, Padin-Iruegas ME, Misao Y et al. Local activation or implantation of cardiac progenitor cells rescues scarred infarcted myocardium improving cardiac function. *Circ Res.* 2008;103:107–116.
65. Li M, Naqvi N, Yahiro E et al. c-kit is required for cardiomyocyte terminal differentiation. *Circ Res.* 2008;102:677–685.
66. Tallini YN, Greenea KS, Cravena M et al. c-kit expression identifies cardiovascular precursors in the neonatal heart. *Proc Natl Acad Sci USA*. 2009;106:1808–1013.
67. Miyamoto S, Kawaguchi N, Ellison GM, Matsuoka R, Shinoka T, Kurosawa H. Characterization of long-term cultured $c\text{-}kit^+$ cardiac stem cells derived from adult rat hearts. *Stem Cells Dev*. 2010;19:105–116.
68. Matsuura K, Nagai T, Nishigaki N et al. Adult cardiac Sca-1--positive cells differentiate into beating cardiomyocytes. *J Biol Chem.* 2004;279:11384–11391.
69. Wang X, Hu Q, Nakamura Y et al. The role of the $sca\text{-}1^+$/ /$CD31^-$ cardiac progenitor cell population in postinfarction left ventricular remodeling. *Stem Cells.* 2006;24:1779–1788.
70. Tateishi K, Ashihara E, Takehara N et al. Clonally amplified cardiac stem cells are regulated by Sca-1 signaling for efficient cardiovascular regeneration. *J Cell Sci.* 2007;120:1791–1800.
71. Liang SX, Tan TYL, Gaudry L, Chong B. Differentiation and migration of $Sca1^+/CD31^-$ cardiac side population cells in a murine myocardial ischemic model. *Int J Cardiol.* 2010; 138:40–49.
72. Laugwitz KL, Moretti A, Lam J et al. Postnatal $isl1^+$ cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature*. 2005;433:647–653.
73. Wu SM, Chien KR, Mummery C. Origins and fates of cardiovascular progenitor cells. *Cell.* 2008;132:537–543.
74. Moretti A, Caron L, Nakano A. Postnatal $isl1^+$ cardioblasts enter fully differentiated cardiomyocyte lineages. Multipotent embryonic $isl1^+$ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. *Cell.* 2006;127:1151–1165.
75. Moretti A, Bellin M, Jung CB. Postnatal $isl1^+$ cardioblasts enter fully differentiated cardiomyocyte lineages. Multipotent embryonic $isl1^+$ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification Mouse and human induced pluripotent stem cells as a source for multipotent $Isl1^+$ cardiovascular progenitors. *FASEB J.* 2010;24:700–711.
76. Zhou B, Gise A, Ma Q, Rivera-Feliciano J, Pu WT. Nkx2-5--and Isl1-expressing cardiac progenitors contribute to proepicardium. *Biochem Biophys Res Commun.* 2008;375:450–453.
77. Smith RR, Barile L, Cho HC et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation.* 2007; 115:896–908.
78. Takehara N, Tsutsumi Y, Tateishi K et al. Controlled delivery of basic fibroblast growth factor promotes human cardiosphere-derived cell engraftment to enhance cardiac repair for chronic myocardial infarction. *J Am Coll Cardiol.* 2008;52: 185–1865.
79. Slack JM. Origin of stem cells in organogenesis. *Science.* 2008;322:1498–1501.
80. Pouly J, Bruneval P, Mandet C et al. Cardiac stem cells in the real world. *J Thorac Cardiovasc Surg.* 2007;135:673–678.
81. Massberg S, Schaerli P, Knezevic-Maramica I et al. Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. *Cell.* 2007; 131:994–1008.
82. Winitsky SO, Gopal TV, Hassanzadeh S. et al. Adult murine skeletal muscle contains cells that can differentiate into beating cardiomyocytes in vitro. *PLoS Biol* 2005;3:e87.
83. Menasché P. Skeletal myoblasts as a therapeutic agent. *Prog Cardiovasc Dis*. 2007;50:7–17.
84. Murry CE, Wiseman RW, Schwartz SM, Hauschka SD. Skeletal myoblast transplantation for repair of myocardial necrosis. *J Clin Invest.* 1996;98:2512–2523.
85. Taylor DA, Atkins BZ, Hungspreugs P et al. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med.* 1998;4:929–933.
86. He KL, Yi GH, Sherman W et al. Autologous skeletal myoblast transplantation improved hemodynamics and left ventricular function in chronic heart failure dogs. *J Heart Lung Transplant*. 2005;24:1940–1949.

87. van den Bos EJ, Thompson RB, Wagner A et al. Functional assessment of myoblast transplantation for cardiac repair with magnetic resonance imaging. *Eur J Heart Fail*. 2005;7:435–443.
88. Reinecke H, Poppa V, Murry CE. Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J Mol Cell Cardiol*. 2002;34:241–249.
89. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci USA*. 2003;100:7808–7811.
90. Siminiak T, Kalawski R, Fiszer D et al. Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: phase I clinical study with 12 months of follow-up. *Am Heart J*. 2004;148:531–537.
91. Menasché P, Alfieri O, Janssens S et al. The myoblast autologous grafting in ischemic cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation*. 2008;117:1189–1200.
92. Payne TR, Oshima H, Okada M et al. Relationship between vascular endothelial growth factor, angiogenesis, and cardiac repair after muscle stem cell transplantation into ischemic hearts. *JACC*. 2007;50:1677–1684.
93. Roell W, Lewalter T, Sasse P et al. Engraftment of connexin 43- expressing cells prevents post-infarct arrhythmia. *Nature*. 2007;450:819–824.
94. Young PP, Vaughan DE, Hatzopoulos AK. Biologic properties of endothelial progenitor cells and their potential for cell therapy. *Prog Cardiovasc Dis*. 2007;49:421–429.
95. Badorff C, Brandes RP, Popp R et al. Transdifferentiation of blood derived human adult endothelial progenitor cells into functionally active cardiomyocytes. *Circulation*. 2003;107:1024–1032.
96. Koyanagi M, Bushoven P, Iwasaki M, Urbich C, Zeiher AM, Dimmeler S. Notch signaling contributes to the expression of cardiac markers in human circulating progenitor cells. *Circ Res*. 2007;101:1139–1145.
97. Flaherty MP, Abdel-Latif A, Li Q et al. Noncanonical Wnt11 signaling is sufficient to induce cardiomyogenic differentiation in unfractionated bone marrow mononuclear cells. *Circulation*. 2008;117:2241–2252.
98. Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275:964–967.
99. Orlic D, Kajstura J, Chimenti S et al. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;410:701–705.
100. Orlic D, Kajstura J, Chimenti S et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci USA*. 2001;98:10344–10349.
101. Kajstura J, Rota M, Whang B et al. Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell fusion. *Circ Res*. 2005;96:127–137.
102. Rota M, Kajstura J, Hosoda T et al. Bone marrow cells adopt the cardiomyogenic fate in vivo. *Proc Natl Acad Sci USA*. 2007;104:17783–17788.
103. Zhao TC, Tseng A, Yano N et al. Targeting human $CD34^+$ hematopoietic stem cells with anti- CD45 x anti-myosin light chain bispecific antibody preserves cardiac function in myocardial infarction. *J Appl Physiol*. 2008;104:1793––1800.
104. Shintani S, Murohara T, Ikeda H et al. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation*. 2001;103:2776–2779.
105. Wojakowski W, Tendera M, Michalowska A et al. Mobilization of CD34/CXCR4ţ, CD34/CD117ţ, c-metţ stem cells, and mononuclear cells expressing early cardiac, muscle, and endothelial markers into peripheral blood in patients with acute myocardial infarction. *Circulation*. 2004;110:3213–3220.
106. Murry CE, Soonpaa MH, Reinecke H et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*. 2004;428:664–668.
107. Ince H, Petzsch M, Kleine HD et al. Prevention of left ventricular remodeling with G-CSF after acute myocardial infarction: final one-year results of the FIRSTLINE-AMI trial (front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction by granulocyte colony-stimulating factor). *Circulation*. 2005;112(9 Suppl):I73–80.
108. Leone AM, Rutella S, Bonanno G et al. Endogenous G-CSF and CD34ţ cell mobilization after acute myocardial infarction. *Int J Cardiol*. 2005;111:202–208.
109. Harada M, Qin Y, Takano H et al. G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak--Stat pathway in cardiomyocytes. *Nat Med*. 2005;11:305–311.
110. Beohar N, Flaherty JD, Davidson CJ et al. Granulocyte-colony stimulating factor administration after myocardial infarction in a porcine ischemia-reperfusion model: functional and pathological effects of dose timing. *Catheter Cardiovasc Interv*. 2007;69:257–266.
111. Rehman J, Li J, Orschell CM, March KL. Peripheral blood 'endothelial progenitor cells' are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation*. 2003;107:1164–1169.
112. Kinnaird T, Stabile E, Burnett MS, Epstein SE. Bone-marrow derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. *Circ Res*. 2004;95:354–363.
113. Mathieu M, Bartunek J, Oumeiri E et al. Cell therapy with autologous bone marrow mononuclear stem cells is associated with superior cardiac recovery compared with use of nonmodified mesenchymal stem cells in a canine model of chronic myocardial infarction. *J Thorac Cardiovasc Surg*. 2009;138:646–653.
114. Pittenger MF, Martin BJ. Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res*. 2004;95:9–20.
115. Tomita S, Li RK, Weisel RD et al. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation*. 1999;100(suppl):II247–II256.
116. Jackson KA, Majka SM, Wang H et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest*. 2001;107:1395–1402.
117. Shiota M, Heike T, Haruyama M et al. Isolation and characterization of bone marrow-derived mesenchymal progenitor cells with myogenic and neuronal properties. *Exp Cell Res*. 2007;313:1008–1023.
118. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res*. 2008;103:1204–1219.
119. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*. 2005;105:1815–1822.
120. Amado LC, Saliaris AP, Schuleri KH et al. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proc Natl Acad Sci USA*. 2005;102:11474–11479.
121. Zhang M, Mal N, Kiedrowski M et al. SDF-1 expression by mesenchymal stem cells results in trophic support of cardiac myocytes after myocardial infarction. *FASEB J*. 2007;21:3197–3207.

122. Haider HK, Jiang S, Idris NM, Ashraf M. IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1alpha/CXCR4 signaling to promote myocardial repair. *Circ Res.* 2008; 103:1300–1308.
123. Zeng B, Chen H, Zhu C, Ren X, Lin G, Cao F. Effects of combined mesenchymal stem cells and heme oxygenase-1 therapy on cardiac performance. *Eur J Cardio-thorac.* 2008;34:850––856.
124. Alfaro MP, Pagni M, Vincent A et al. The Wnt modulator sFRP2 enhances mesenchymal stem cell engraftment, granulation tissue formation and myocardial repair. *Proc Natl Acad Sci USA.* 2008;105:18366–18371.
125. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*. 2002;105:93–98.
126. Schuleri KH, Amado LC, Boyle AJ et al. Early improvement in cardiac tissue perfusion due to mesenchymal stem cells. *Am J Physiol Heart Circ Physiol*. 2008;294:2002–2011.
127. Quevedo HC, Hatzistergosa KE, Oskoueia BN et al. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. *PNAS* 2009;106:14022–14027.
128. Potapova IA, Doronin SV, Kelly DJ et al. Enhanced recovery of mechanical function in the canine heart by seeding an extracellular matrix patch with mesenchymal stem cells committed to a cardiac lineage. *Am J Physiol Heart Circ Physiol.* 2008; 259:2257–2263.
129. Breitbach M, Bostani T, Roell W et al. Potential risks of bone marrow cell transplantation into infarcted hearts. *Blood.* 2007;110:1362–1369.
130. Yoon YS, Park JS, Tkebuchava T, Luedeman C, Losordo DW. Unexpected severe calcification after transplantation of bone marrow cells in acute myocardial infarction. *Circulation.* 2004;109:3154–3157.
131. Jezierska-Woźniak K, Nosarzewska D, Tutas A, Mikołajczyk A, Okliński M, Jurkowski MK. Use of adipose tissue as a source of mesenchymal stem cells. *Postepy Hig Med Dosw.* 2010; 64:326–32.
132. Robdell M. Metabolism of isolated fat cells. Effect of hormone on fat metabolism and lipolysis. *J Biol Chem*. 1964;223:375–380.
133. Robdell M, Jones AB. Metabolism of isolated fat cells. The similar inhibitory action of phospholipase c (clostridium perfringens alpha toxin) and of insulin on lipolysis stimulated by lipolytic hormones and theophylline. *J Biol Chem.* 1966; 241:140–142.
134. Rangappa S, Entwistle JW, Wechsler AS, Kresh JY. Cardiomyocyte — mediated contact programa human mesenchymal stem cells to express cardiogenic phenotype. *J Thorac Cardiovasc Surg.* 2003;126:124–132.
135. Psaltis PJ, Zannettino AC, Worthley SG, Gronthos S. Concise review: mesenchymal stromal cells: potential for cardiovascular repair *Stem Cells.* 2008;26:2201–2210.
136. Planat-Bénard V, Menard C, André M et al. Spontaneous cardiomyocyte differentiation from adipose tissue stromal cells. *Circ Res.* 2004;94:223–229.
137. Gaustad K.G, Boquest AC, Anderson BE, Gerdes AM, Collas P. Differentiation of human adipose tissue stem cells using extracts of rat cardiomyocytes. *Biochem Biophys Res Commun.* 2004;314:420–427.
138. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res*. 2007;11;100:1249–1260.
139. Yamada Y, Wang XD, Yokoyama S, Fukuda N, Takakura N. Cardiac progenitor cells in brown adipose tissue repaired damaged myocardium. *Biochem Biophys Res Commun.* 2006; 342:662–670.
140. Yamada Y, Yokoyama S, Wang XD, Fukuda N, Takakura N. Cardiac stem cells in brown adipose tissue express CD133 and induce bone marrow nonfematopoetic cells to differentiate into cardiomyocytes. *Stem Cells.* 2007;25:1326–1333.
141. Song YH, Gehmert S, Sadat P et al. VEGF is critical for spontaneous differentiation of stem cells into cardiomyocytes. *Biochem Biophys Res Commun.* 2007;354:999–1003.
142. Nakagami H, Morishita R, Maeda K, Kikuchi Y, Ogihara T, Kaneda Y. Adipose tissue-derived stromal cells as a novel option for regenerative cell therapy. *J Atheroscler Thromb.* 2006;13:77–81.
143. Nakagami H, Morishita R, Maeda K et al. Novel autologous cell therapy in ischemic limb disease through growth factor secretion by cultured adipose tissue-derived stromal cells. *Arterioscler Thromb Vasc Biol*. 2005;25:2542–2547.
144. Rehman J, Traktuev D, Li J et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation.* 2004;109:1292–1298.
145. Cao Y, Sun Z, Liao L et al. Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. *Biochem Biophys Res Commun.* 2005;332:370–379.
146. Bai X, Yan Y, Song YH et al. Both cultured and freshly isolated adipose tissue-derived stem cells enhance cardiac function after acute myocardial infarction. *Eur Heart J.* 2009;31:489–501.
147. Jumabay M, Matsumoto T, Yokoyama S et al. Dedifferentiated fat cells convert to cardiomyocyte phenotype and repair infracted cardiac tissue in rats. *J Mol Cell Cardiol*. 2009;47:565–575.
148. Mazhari R, Hare JM. Advances in cell-based therapy for structural heart disease [Review]. *Prog Cardiovasc Dis.* 2007;49: 387–395.
149. The Scientist Magazine of the Life Science. Trials of the heart. Available at: http://www.the-scientist.com/supplementary/ /html/24104/ Accessed May 10, 2010.
150. Tousoulis D, Briasoulis A, Antoniades C, Stefanadi E, Stefanadis C. Heart regeneration: what cells to use and how? *Curr Opin Pharmacol.* 2008;8:211–218.
151. Barbash IM, Chouraqui P, Baron J et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation*. 2003;108:863–868.
152. Janssens S, Dubois C, Bogaert J et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet*. 2006;367:113–121.
153. Perin EC, López J. Methods of stem cell delivery in cardiac diseases. *Nat Clin Pract Cardiovasc Med*. 2006;3(suppl 1):S110––S113.
154. Gyöngyösi M, Lang I, Dettke M et al. Combined delivery approach of bone marrow mononuclear stem cells early and late after myocardial infarction: the MYSTAR prospective, randomized study. *Nat Clin Pract Cardiovasc Med*. 2009;6:70–81.
155. Sherman W, Martens TP, Viles-Gonzalez JF, Siminiak T. Catheter-based delivery of cells to the heart. *Nat Clin Pract Cardiovasc Med*. 2006;3(suppl 1):S57–S64.
156. Hofmann M, Wollert KC, Meyer GP et al. Monitoring of bone marrow cell homing into the infarcted human myocardium. *Circulation.* 2005;111:2198–2202.

157. Teng CJ, Luo J, Chiu RC, Shum-Tim D. Massive mechanical loss of microspheres with direct intramyocardial injection in the beating heart: implications for cellular cardiomyoplasty. *J. Thorac. Cardiovasc. Surg.* 2006;132:628–632.
158. Bel A, Messas E, Agbulut O, et al. Transplantation of autologous fresh bone marrow into infarcted myocardium: a word of caution. *Circulation.* 2003;108:II247–II252.
159. Aicher A, Brenner W, Zuhayra M et al. Assessment of the tissue distribution of transplanted human endothelial progenitor cells by radioactive labeling. *Circulation.* 2003;107:2134–2139.
160. Suzuki K, Murtuza B, Beauchamp JR et al. Dynamics and mediators of acute graft attrition after myoblast transplantation to the heart. *FASEB J*. 2004;10:1153–1155.
161. Hou D, Youssef EA-S, Brinton TJ et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation.* 2005;112:I–150.
162. Lyon A, Harding S. The potential of cardiac stem cell therapy for heart failure. *Curr Opin Pharmacol.* 2007;7:164–170.
163. Singh S, Arora R, Handa K et al. Stem cells improve left ventricular function in acute myocardial infarction. *Clin Cardiol.* 2009;32:176–180.
164. Chavakis E, Aicher A, Heeschen C et al. Role of beta2- integrins for homing and neovascularization capacity of endothelial progenitor cells. *J Exp Med.* 2005;201:63–72.
165. Chavakis E, Urbich C, Dimmeler S. Homing and engraftment of progenitor cells: a prerequisite for cell therapy. *J Mol Cell Cardiol.* 2008;45:514–522.
166. Brunner S, Huber BC, Fischer R et al. G-CSF treatment after myocardial infarction: impact on bone marrow-derived vs cardiac progenitor cells. *Exp Hematol*. 2008;36:695–702.
167. Yoon YS**,** Johnson IA, Park JS, Diaz L, Losordo DW. Therapeutic myocardial angiogenesis with vascular endothelial growth factors. *Mol Cell Biochem* 2004;264:63–74.
168. Ryzhov S, Solenkova NV, Goldstein AE et al. Adenosine receptor-mediated adhesion of endothelial progenitors to cardiac microvascular endothelial cells. *Circ Res.* 2008;102: 356–363.
169. Shen J, DiCorleto PE. Adenosine prompts the heart to recruit endothelial progenitors. *Circ Res.* 2008;102:280–282.
170. Leesar MA, Stoddard M, Ahmed M, Broadbent J, Bolli R. Preconditioning of human myocardium with adenosine during coronary angioplasty. *Circulation*. 1997;95:2500––2507.
171. Swijnenburg RJ, Govaert JA, van der Bogt KEA et al. Timing of bone marrow cell delivery has minimal effects on cell viability and cardiac recovery after myocardial infarction. *Circ Cardiovasc Imaging*. 2010;3:77–85.
172. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell.* 2006;126:677–689.

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