Published in final edited form as:

Basic Res Cardiol. 2011 May ; 106(3): 317–324. doi:10.1007/s00395-011-0168-x.

Stem cell-based therapies in ischemic heart diseases: a focus on aspects of microcirculation and inflammation

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Abstract

Stem cells possessing the potential to replace damaged myocardium with functional myocytes have drawn increasing attention in the past decade in treating ischemic heart diseases; these diseases are the leading cause of morbidity and mortality in the world. The adult heart has recently been shown to contain a few cardiac stem cells (CSCs) that, in theory, suggest cardiac repair following acute myocardial infarction is possible if the CSC titer could be increased. Stem cell-based therapies, including hematopoietic stem cells and mesenchymal stem cells, were proven to be marginal and transitional. Multiple factors and mechanisms, rather than direct cardiac regeneration are involved in stem cell-mediated cardiac functional improvement. This review will focus on (1) the interaction between inflammation and stem cells; (2) the fate of stem cells at the microcirculatory level, and their subsequent influences on stem cell-based therapies.

Keywords

Stem cell; Inflammation; Microcirculation; Ischemic heart disease; Cardiac repair; Cell-based therapy

Introduction

Acute myocardial infarction is the leading cause of morbidity and mortality in the world. Reperfusion is the only way to rescue the ischemic heart by restoration of blood flow and oxygen supply. Early restoration of blood supply in the ischemic myocardium restricts the infarction size and improves clinical outcomes [13]. However, reperfusion itself is typically accompanied by acute inflammation and pulses of oxidative stress, resulting in further tissue damage, and referred to as reperfusion injury [5, 80]. Stem cells have increasingly attracted the attention of the bench scientist and the clinician interested in treating ischemic heart disease, due to their potential for repairing damaged cardiac tissue. However, the improvement of cardiac function has been proven to be marginal and transitional in both preclinical and clinical studies. The actual regeneration of damaged cardiac tissue was minimal, and below the level needed to achieve clinical benefits. This review will focus on the influence of inflammation, a major factor implicated in reperfusion injury, and microcirculation, the primary site where reperfusion injury is initially manifested, to further explore how these factors affect stem cell-based therapies.

Inflammation and ischemia/reperfusion injury

Inflammation mediated ischemia/reperfusion (I/R) injury has been extensively studied. In the acute phase of reperfusion injury, neutrophils are one of the earliest responders and the major mechanism for acute reperfusion injury. Activation of neutrophils is triggered by a variety of factors induced by ischemia, such as complement fragment C5a, reactive oxygen species (ROS), and pro-inflammatory cytokines (e.g. TNF- α , IL-1 and IL-6). Upon reperfusion, all the vascular beds downstream of the infarct inevitably interact with the activated neutrophils. (1) Post-capillary venues are the classical location for neutrophil recruitment. Activated neutrophils upregulate the adhesion molecules and interact with endothelial cells via the well-known mechanism of rolling-adhesion-diapedesis [6]. Significant adhesion and emigration of neutrophils were observed as early as 1 h after the onset of reperfusion, starting from the border zone and extending deeper into the infarct by 3 h [26]. Myeloperoxidase and NADPH oxidase are the major contributors to the neutrophil oxidative burst, and cause direct injury to the endothelial cells and cardiomyocytes [18, 92]. (2) At the capillary level, activated neutrophils are trapped in the microvessels due to their increased stiffness and decreased deformability, which contributes to the no-flow phenomena observed in reperfusion that prolongs the ischemia period in the downstream tissue [19, 86]. (3) Increased neutrophil adhesion to the arterial endothelium, associated with impaired endothelium-dependent vasodilation, was also evidenced after reperfusion [92]. Moreover, both clinical study and basic research highlighted C-reactive protein as a critical pathophysiological factor in myocardial microembolization and impaired reperfusion [69], which will pose detrimental effects on the migration, differentiation and survival of stem cells in the ischemic heart.

TNF- α is one of the important up-stream signals that initiate the cytokine cascade, and exerts ambivalent effect in the infarct or reperfused myocardium [34, 66]. Gao et al. revealed that TNF- α -induced coronary microvascular dysfunction following I/R was independent of neutrophils. In neutropenic mice or in mice treated with a neutrophil NAD(P)H oxidase inhibitor, I/R induced similar endothelial dysfunction when compared with the control group [22]. Our work suggested that TNF- α might be the initiator of coronary vascular dysfunction by two lines of evidence: (a) TNF- α expression was induced or up-regulated in coronary arteriole smooth muscle cells, macrophages and mast cells, both at the mRNA and protein levels [22], (b) Pre-treatment with TNF-neutralizing antibody before I/R attenuated both endothelial dysfunction in coronary arterioles [22] and infarct size [39]. Using TNF- α over-expression mice (TNF^{+/+}), TNF knockout mice (TNF^{-/-}) and their heterozygous cross mice (TNF^{+/-}), a direct relationship was demonstrated between levels of TNF- α expression and endothelial dysfunction in reperfusion injury [89]. In consistent, recent clinical data revealed that TNF- α may contribute to coronary dysfunction by potentiating the effects of vaso-constrictors in vein graft bypassed patients [33].

Endogenous stem cells in ischemic heart diseases

Cardiomyocytes early on came to be considered terminally differentiated and to offer little to no hope for repopulation of damaged cardiac tissue by adjacent cardiomyocytes. Alternative efforts by dedicated cardiovascular scientists also have yet to find a satisfactory treatment for rescuing cardiac function after I/R injury. Despite skepticism regarding the regenerative capacity of the adult heart accumulating evidence has emerged to show that even the healthy adult heart keeps renewing itself. In the adult mouse heart, the proliferating cells comprised from 0.25 to 1% of the entire cardiac cell population [67]. However, the proliferation rate of cardiomyocytes, which represents 20% of the total cardiac cells, was only 0.0005% [70]. Anversa et al. [4] pointed out that the size of proliferating cells in the

heart is much smaller than the mature cardiomyocytes, and suggested the renewal of cardiomyocytes may be achieved by stem cells. The concept of cardiac stem cells is now widely accepted and the presence is in evidence [38, 43, 59]. However, due to their extremely low abundance in the heart (about one in 30,000–40,000 myocardial cells) [38], the role of cardiac stem cells in cardiac repair following acute myocardial infarction is doubtful. Furthermore, the markedly inability for differentiation of cardiac stem cells in the infarct area was also reported as compared to those in the viable myocardium [38].

In addition to cardiac stem cells, stem cells outside of the heart may also contribute to cardiac repair. In adults, bone marrow is the major source of stem cells. There are two major groups of stem cells, the hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). HSCs give rise to red blood cells, platelets, monocytes and lymphocytes, while MSCs are generally regarded as bone marrow driven non-HSCs that are able to adhere to the plastic culture dish in vitro. MSCs are easy to multiply in culture and readily differentiated into mesenchymal lineages, such as osteoblasts, chondrocytes and adipocytes [60]. There are two lines of evidence in support of the argument that external stem cells may migrate to the heart and differentiate into cardiomyocytes as well as other cardiac cells. (1) In vitro studies demonstrated that bone marrow derived cells have the potential to differentiate into cardiomyocytes [45]. (2) Sex-mismatched cardiac transplantation in humans confirmed the presence of host derived-cardiomyocytes in the transplanted heart [28, 36, 50, 62].

Following myocardial infarction, mobilization of both HSCs and MSCs from bone marrow has been reported [15, 25, 40, 47, 77, 85]. The mobilization of stem cells is usually defined as increased circulating stem cells bearing various cell surface markers, such as CD34, CXCR4, CD117 (also known as c-kit), c-met, CD133, etc. Although multiple disease status, physical exercises, and cytokines expression have been shown to correlate with increases in circulating stem cell numbers [53, 55, 78, 90], direct evidence for regeneration of infarcted cardiomyocytes by bone marrow-derived stem cells is lacking. In fact, scar formation is the common fate of the infarcted myocardium.

Stem cell-based therapies

Whereas the extremely low abundance of stem cells in our body may minimize their utility for cardiac self-regeneration, stem cell-based therapies attempt to promote cardiac repair by direct delivery of a large number of exogenous stem cells to the infarct area. In 2001, Orlic et al. [56] first reported that the injection of bone marrow-derived Lin⁻ c-kit⁺ stem cells, a subset of HSCs, into the infarcted heart drove cardiac regeneration by differentiation into cardiac cells, including cardiomyocytes, muscle cells and endothelial cells. However, these observations were seriously challenged by other groups based on very similar protocols [8, 51]. The diverse fates of the administrated HSCs were thought to be due to different tracking methods used by these authors [14]. We also noticed that these studies were based on very different animal strains. Orlic et al. [56] adopted C57BL/6 mice in their experiments, which is a widely used mouse strain and often regarded as a standard strain. The other two groups used MHC-nLAC and C57BL/Ka strains, respectively. The MHC-nLAC mouse is of DBA/2J background [70], which often contrasts with C57BL/6J and shows a low susceptibility to atherosclerosis. The C57BL/Ka mouse is reported as a model for the human idiopathic paraproteinaemia and multiple myeloma [63, 64]. These distinct animal strains may have significant influence on the actual population of HSCs and/or their ability for cardiac differentiation. The outcome of infarct healing in mice also strongly depends on genetic background [79]. Recently, a similar set of progenitor cells (c-kit⁺ CD45⁻) was identified in the adult human heart, containing potential of endothelial but not cardiac differentiation [68]. Wnt signaling, a key regulator of stem cell growth, differentiation, and proliferation,

was suggested to be involved in resident Sc progenitor cells and endothelial cells after myocardial infarction [54].

In addition to these original observations, a wide range of stem cells, such as embryonic stem cells, skeletal myoblasts, bone marrow mononuclear cells, endothelial progenitor cells, mesenchymal cells, etc., were reported to be beneficial to cardiac function following infarction, [48]. However, the overall benefits of stem cell-based therapies are marginal and transitional [83], which indicate that the regeneration of infarct myocardium is not the predominant mechanism for stem cell-based therapy. Emerging evidence suggests multiple mechanisms could be involved, including paracrine, angiogenesis, cell fusion, and passive mechanical effects, and influence of endogenous cardiac stem cells [83]. Unfortunately, without substantial regeneration of new myocardium to replace the infarct tissue, scar formation and adverse remodeling in the left ventricle often lead to heart failure.

Fate of exogenous stem cells

Following implantation, the intravital engraftment and differentiation of stem cells into cardiomyocytes are not guaranteed. In a healthy animal model, intra-artery or intravenous infusion of ex vivo expanded MSCs were trapped primarily in the lung and then secondarily in the liver and other organs. Pretreatment with sodium nitro-prusside (a vessel dilator) increased the lung clearance resulting in a larger proportion of infused cells detected in the liver [21]. In a rat model of myocardial infarction, Barbash et al. [9] found that the majority of the stem cells were trapped in the lung following systemic intravenous infusion. Less than 1% of the infused stem cells were detected in the injured heart. However, direct left ventricular cavity infusion improved stem cell engraftment to the ischemic myocardium. This evidence indicates the micro-vasculature might function as a physical barrier that blocks the trafficking of exogenous stem cells. Toma et al. further tested this hypothesis by tracking the fate of intra-artery infused ex vivo with expanded MSCs. The authors found that 92% of the infused stem cells were arrested in the pre-capillary level during the first pass, with a 27% reduction of blood flow in the feeding arterioles. As a result, only 14% of the stem cells survived the microembolization-induced ischemia by 72 h, and extravasated the micro-vascular wall [7, 76]. Although the deformability of the ex vivo expanded MSCs were comparable to the white blood cells, the actual size of these cells are much bigger than normal white blood cells as well as the capillaries. Thus, intra-vascular delivery of stem cells might cause further ischemia in the targeted tissue and/or result in a loss of the majority of the infused cells [7, 76].

These concerns that infusion of ex vivo expanded stem cells may exacerbate myocardial infarction were confirmed in healthy animal studies. Intra-coronary infusion of MSCs into healthy dogs [81] or sheep [24] resulted in immediate elevation of the ST segment in electrocardiograms (a marker of regional myocardial ischemia) and histological evidence of myocardial infarction was shown 7 days later. The size of the ex vivo expanded MSCs was larger than the freshly isolated bone marrow cells and thought to be the main reason causing the microcirculatory obstruction [81]. The severity of the injury also occurred in a dose-dependent manner [24].

Direct intracardiac injection of stem cells offers an alternative route for cell delivery, which may avoid microcirculatory problems. Unfortunately, low retention efficiency is still significant. Mechanical leakage following direct intracardiac injection is 33 and 89% in non-beating and beating porcine hearts, respectively [75]. Only 3% of endothelial progenitor cells were detected in the infarcted rat heart right after injection [3], while approximately 7% of myoblasts survived by 72 h following intracardiac injection into the infarcted mouse heart [73].

Long term follow-up study also confirmed the low survival rate of exogenous stem cells in the ischemic heart. In a mouse coronary ligation model, less than 2% of the injected embryonic stem cells survived 8 weeks after myocardial infarction [41]. Interestingly, instead of turning into cardiomyocytes, the surviving stem cells differentiated into endothelial cells, forming blood-carrying microvessels [41]. Of note, stem cells from different sources may differ in the ability to incorporate into or form new capillaries and/or arterioles [29]. Clinical study also confirmed that progenitor cell transplantation promoted restoration of microvascular function in the infarct-related artery [20]. On the other hand, cardiac function was deteriorated by elimination of endothelial-committed cells following transplantation of bone marrow mononuclear cells into the infarcted heart, but not affected by deletion of cardiac-committed cells [87]. Thus, stem cell-mediated angiogenesis and improvement of cardiac perfusion in the infarct or border zone is more likely via a mechanism involving cardiac functional improvement rather than through direct cardiac regeneration.

Interaction between inflammation and stem cells

The interaction between system/local inflammation and stem cells significantly influences the survival and function of stem cells. The SDF-1/CXCR4 axis is a well-known signaling pathway that directs the homing of stem cells to the infarct area or border zone in the setting of ischemic cardiomyopathy [57, 88]. However, the specificity of this pathway in stem cell recruitment has to be interpreted carefully, as SDF-1 was also reported as a potent chemotactic factor for a variety of cells, such as T cells [30], B cells [16], dendritic cells [71], mast cells [42] and eosinophils [52]. In fact, SDF-1 plays a critical role in regulating the trafficking of all the CXCR4+ haemato/lymphopoietic cells in inflammation [35]. In the absence of injury, even forced over-expression of SDF-1 is not sufficient to recruit stem cells [2, 58]. Malek et al. [46] found that the severity of acute inflammation is critical for stem cell recruitment in a murine myocarditis model. Delivery of embryonic stem cells at the peak of inflammation (the time point with highest cytokine production) following induction of myocarditis resulted in the highest level of stem cell engraftment in the heart and greatest functional improvement 3 months later. Pretreatment of stem cells with TNF- α , a well-known proinflammatory cytokine, enhanced MSC engraftment into infarcted myocardium [32]. Recently evidence demonstrated that TNF- α has diverse effects on stem cell function via different subtypes of TNF receptors. Ablation of TNF receptor type 1 (TNFR1), but not TNF receptor type 2 (TNFR2) or both receptors, improved MSC-mediated cardiac protection. Infusion of TNFR1 knockout MSCs was associated with decreased proinflammatory cytokines and increased protective vascular endothelial growth factor (VEGF) [31, 74]. TNFR2 expressed in bone marrow-derived stem cells was required for the ischemia-induced endothelial progenitor cell-mediated neovascularization [23]. Interestingly, TNF- α is also required for the activation of MSCs and the production of VEGF, which is mediated by Toll-like receptor 2 (TLR2) [1]. In addition to their regenerative ability, the antiinflammation and immunomodulation effects of stem cells are attracting investigative attention in various diseases [10, 37, 44, 49, 84, 91]. In the setting of acute myocardial infarction, continuous secretion of the anti-inflammatory cytokine IL-10 by transplanted stem cells was reported to improve cardiac function without affecting the infarct size, but associated with reduced T cell infiltration as well as T cell-mediated inflammation [12]. The positive immunomodulatory effect by IL-10 secreting stem cells was considered as a new mechanism of stem cell-based therapy that is distinct from both tissue regeneration and paracrine effects [11]. Salem et al. [66] summarized the immunosuppressive effects of MSCs on T cells, natural killer cells and dendritic cells. Recent evidence suggests that the inhibitory effect on T cell proliferation and secretion of interferon- γ is mediated by stem cell-secreted prostaglandin E2 (PGE2), while CD14+ monocyte promoted the immunosuppressive effects of stem cells via IL-1 β dependent

pathways [82]. Also, stem cell-secreted PGE2 was reported to play a central role in inhibition of monocyte-derived dendritic cell maturation [71]. These observations matched with the beneficial role of PGE2 in myocardial I/R injury or acute myocardial infarction [17, 27]. Intriguingly, selective deletion of PGE2 receptor EP4 in cardiomyocytes resulted in reduced cardiac function with less fibrosis following myocardial infarction [61], which indicated stem cells/PGE2 may have diverse effects on inflammatory cells and cardiomyocytes.

Summary

The last decade has witnessed a boost of interest in stem cell-based therapies by both basic scientists and clinicians. In spite of the widespread studies in this area, stem cells-based therapy for ischemic heart diseases is still in its infancy. An effective treatment of patients with stem cells is currently not in sight, and the mechanisms of stem cell-mediated effects are not yet fully understood. In particular, the outcomes desired for stem cell-based therapies may be complicated by the diverse effects of stem cells on inflammatory cells and cardiac cells, as well as the behavior and fate of stem cells at the microvascular level. Further investigations are required on the interaction between stem cells and inflammatory cells and the subsequent effects on the fate of cardiomyocytes in the microvasculature and beyond to improve on delivery of these therapies.

References

1. Abarbanell AM, Wang Y, Herrmann JL, Weil BR, Poynter JA, Manukyan MC, Meldrum DR. Toll-like receptor 2 mediates mesenchymal stem cell-associated myocardial recovery and VEGF production following acute ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2010; 298:H1529–H1536.10.1152/ajpheart.01087.2009 [PubMed: 20173040]
2. Abbott JD, Huang Y, Liu D, Hickey R, Krause DS, Giordano FJ. Stromal cell-derived factor-1 alpha plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. *Circulation*. 2004; 110:3300–3305.10.1161/01.CIR.0000147780.30124.CF [PubMed: 15533866]
3. Aicher A, Brenner W, Zuhayra M, Badorff C, Massoudi S, Assmus B, Eckey T, Henze E, Zeiher AM, Dimmeler S. Assessment of the tissue distribution of transplanted human endothelial progenitor cells by radioactive labeling. *Circulation*. 2003; 107:2134–2139.10.1161/01.CIR.0000062649.63838.C9 [PubMed: 12695305]
4. Anversa P, Kajstura J, Leri A, Bolli R. Life and death of cardiac stem cells: a paradigm shift in cardiac biology. *Circulation*. 2006; 113:1451–1463.10.1161/CIRCULATIONAHA.105.595181 [PubMed: 16549650]
5. Ar'Rajab A, Dawidson I, Fabia R. Reperfusion injury. *New Horizons*. 1996; 4:224–234. [PubMed: 8774798]
6. Arslan F, de Kleijn DP, Timmers L, Doevendans PA, Pasterkamp G. Bridging innate immunity and myocardial ischemia/reperfusion injury: the search for therapeutic targets. *Curr Pharm Des*. 2008; 14:1205–1216. [PubMed: 18473868]
7. Bagi Z, Kaley G. Where have all the stem cells gone? *Circ Res*. 2009; 104:280–281.10.1161/CIRCRESAHA.108.192641 [PubMed: 19213961]
8. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature*. 2004; 428:668–673.10.1038/nature02460 [PubMed: 15034594]
9. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation*. 2003; 108:863–868.10.1161/01.CIR.0000084828.50310.6A [PubMed: 12900340]

10. Bian F, Qi H, Ma P, Zhang L, Yoon KC, Pflugfelder SC, Li DQ. An immunoprotective privilege of corneal epithelial stem cells against TH17 inflammatory stress by producing glial cell-derived neurotrophic factor. *Stem Cells*. 2010;10.1002/stem.539
11. Bishopric NH. Mesenchymal stem cell-derived IL-10 and recovery from infarction: a third pitch for the chord. *Circ Res*. 2008; 103:125–127.10.1161/CIRCRESAHA.108.180596 [PubMed: 18635828]
12. Burchfield JS, Iwasaki M, Koyanagi M, Urbich C, Rosenthal N, Zeiher AM, Dimmeler S. Interleukin-10 from transplanted bone marrow mononuclear cells contributes to cardiac protection after myocardial infarction. *Circ Res*. 2008; 103:203–211.10.1161/CIRCRESAHA.108.178475 [PubMed: 18566343]
13. Cannon CP, Gibson CM, Lambrew CT, Shoultz DA, Levy D, French WJ, Gore JM, Weaver WD, Rogers WJ, Tiefenbrunn AJ. Relationship of symptom-onset-to-balloon time and door-to-balloon time with mortality in patients undergoing angioplasty for acute myocardial infarction. *JAMA*. 2000; 283:2941–2947.10.1001/jama.283.22.2941 [PubMed: 10865271]
14. Chien KR. Stem cells: lost in translation. *Nature*. 2004; 428:607–608.10.1038/nature02500 [PubMed: 15034595]
15. Corallini F, Secchiero P, Beltrami AP, Cesselli D, Puppato E, Ferrari R, Beltrami CA, Zauli G. TNF-alpha modulates the migratory response of mesenchymal stem cells to TRAIL. *Cell Mol Life Sci*. 2010; 67:1307–1314.10.1007/s00018-009-0246-5 [PubMed: 20063037]
16. Corcione A, Ottonello L, Tortolina G, Facchetti P, Airolidi I, Guglielmino R, Dadati P, Truini M, Sozzani S, Dallegri F, Pistoia V. Stromal cell-derived factor-1 as a chemoattractant for follicular center lymphoma B cells. *J Natl Cancer Inst*. 2000; 92:628–635. [PubMed: 10772680]
17. Degousee N, Fazel S, Angoulvant D, Stefanski E, Pawelzik SC, Korotkova M, Arab S, Liu P, Lindsay TF, Zhuo S, Butany J, Li RK, Audoly L, Schmidt R, Angioni C, Geisslinger G, Jakobsson PJ, Rubin BB. Microsomal prostaglandin E2 synthase-1 deletion leads to adverse left ventricular remodeling after myocardial infarction. *Circulation*. 2008; 117:1701–1710.10.1161/CIRCULATIONAHA.107.749739 [PubMed: 18347209]
18. Duilio C, Ambrosio G, Kuppusamy P, DiPaula A, Becker LC, Zweier JL. Neutrophils are primary source of O₂ radicals during reperfusion after prolonged myocardial ischemia. *Am J Physiol Heart Circ Physiol*. 2001; 280:H2649–H2657. [PubMed: 11356621]
19. Engler RL, Dahlgren MD, Peterson MA, Dobbs A, Schmid-Schonbein GW. Accumulation of polymorphonuclear leukocytes during 3-h experimental myocardial ischemia. *Am J Physiol*. 1986; 251:H93–H100. [PubMed: 3089032]
20. Erbs S, Linke A, Schachinger V, Assmus B, Thiele H, Diederich KW, Hoffmann C, Dimmeler S, Tonn T, Hambrecht R, Zeiher AM, Schuler G. Restoration of microvascular function in the infarct-related artery by intracoronary transplantation of bone marrow progenitor cells in patients with acute myocardial infarction: the Doppler Substudy of the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial. *Circulation*. 2007; 116:366–374.10.1161/CIRCULATIONAHA.106.671545 [PubMed: 17620510]
21. Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs*. 2001; 169:12–20. [PubMed: 11340257]
22. Gao X, Zhang H, Belmadani S, Wu J, Xu X, Elford H, Potter BJ, Zhang C. Role of TNF-alpha-induced reactive oxygen species in endothelial dysfunction during reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2008; 295:H2242–H2249.10.1152/ajpheart.00587.2008 [PubMed: 18849334]
23. Goukassian DA, Qin G, Dolan C, Murayama T, Silver M, Curry C, Eaton E, Luedemann C, Ma H, Asahara T, Zak V, Mehta S, Burg A, Thorne T, Kishore R, Losordo DW. Tumor necrosis factor-alpha receptor p75 is required in ischemia-induced neovascularization. *Circulation*. 2007; 115:752–762.10.1161/CIRCULATIONAHA.106.647255 [PubMed: 17261656]
24. Grieve SM, Bhindi R, Seow J, Doyle A, Turner AJ, Tomka J, Lay W, Gill A, Hunyor SN, Figtree GA. Microvascular obstruction by intracoronary delivery of mesenchymal stem cells and quantification of resulting myocardial infarction by cardiac magnetic resonance. *Circ Heart Fail*. 2010; 3:e5–e6.10.1161/CIRCHEARTFAILURE.109.931360 [PubMed: 20484192]

25. Grundmann F, Scheid C, Braun D, Zobel C, Reuter H, Schwinger RH, Muller-Ehmsen J. Differential increase of CD34, KDR/CD34, CD133/CD34 and CD117/CD34 positive cells in peripheral blood of patients with acute myocardial infarction. *Clin Res Cardiol.* 2007; 96:621–627.10.1007/s00392-007-0543-7 [PubMed: 17676354]
26. Hawkins HK, Entman ML, Zhu JY, Youker KA, Berens K, Dore M, Smith CW. Acute inflammatory reaction after myocardial ischemic injury and reperfusion. Development and use of a neutrophil-specific antibody. *Am J Pathol.* 1996; 148:1957–1969. [PubMed: 8669481]
27. Hishikari K, Suzuki J, Ogawa M, Isobe K, Takahashi T, Onishi M, Takayama K, Isobe M. Pharmacological activation of the prostaglandin E2 receptor EP4 improves cardiac function after myocardial ischaemia/reperfusion injury. *Cardiovasc Res.* 2009; 81:123–132.10.1093/cvr/cvn254 [PubMed: 18805784]
28. Hruban RH, Long PP, Perlman EJ, Hutchins GM, Baumgartner WA, Baughman KL, Griffin CA. Fluorescence in situ hybridization for the Y-chromosome can be used to detect cells of recipient origin in allografted hearts following cardiac transplantation. *Am J Pathol.* 1993; 142:975–980. [PubMed: 7682765]
29. Iop L, Chiavegato A, Callegari A, Bollini S, Piccoli M, Pozzobon M, Rossi CA, Calamelli S, Chiavegato D, Gerosa G, DeCoppi P, Sartore S. Different cardiovascular potential of adult- and fetal-type mesenchymal stem cells in a rat model of heart cryoinjury. *Cell Transplant.* 2008; 17:679–694.10.3727/096368908786092739 [PubMed: 18819256]
30. Kantele JM, Kurk S, Jutila MA. Effects of continuous exposure to stromal cell-derived factor-1 alpha on T cell rolling and tight adhesion to monolayers of activated endothelial cells. *J Immunol.* 2000; 164:5035–5040. [PubMed: 10799858]
31. Kelly ML, Wang M, Crisostomo PR, Abarbanell AM, Herrmann JL, Weil BR, Meldrum DR. TNF receptor 2, NOT tnfr receptor 1, enhances mesenchymal stem cell-mediated cardiac protection following acute ischemia. *Shock.* 2010; 33:602–607.10.1097/SHK.0b013e3181cc0913 [PubMed: 19953003]
32. Kim YS, Park HJ, Hong MH, Kang PM, Morgan JP, Jeong MH, Cho JG, Park JC, Ahn Y. TNF-alpha enhances engraftment of mesenchymal stem cells into infarcted myocardium. *Front Biosci.* 2009; 14:2845–2856. [PubMed: 19273239]
33. Kleinbongard P, Bose D, Baars T, Mohlenkamp S, Konorza T, Schoner S, Elter-Schulz M, Eggebrecht H, Degen H, Haude M, Levkau B, Schulz R, Erbel R, Heusch G. Vasoconstrictor potential of coronary aspirate from patients undergoing stenting of saphenous vein aortocoronary bypass grafts and its pharmacological attenuation. *Circ Res.* 2011; 108:344–352.10.1161/CIRCRESAHA.110.235713 [PubMed: 21183739]
34. Kleinbongard P, Heusch G, Schulz R. TNFalpha in atherosclerosis, myocardial ischemia/reperfusion and heart failure. *Pharmacol Ther.* 2010; 127:295–314.10.1016/j.pharmthera.2010.05.002 [PubMed: 20621692]
35. Kucia M, Jankowski K, Reza R, Wysoczynski M, Bandura L, Allendorf DJ, Zhang J, Ratajczak J, Ratajczak MZ. CXCR4-SDF-1 signalling, locomotion, chemotaxis and adhesion. *J Mol Histol.* 2004; 35:233–245. [PubMed: 15339043]
36. Laflamme MA, Myerson D, Saffitz JE, Murry CE. Evidence for cardiomyocyte repopulation by extra cardiac progenitors in transplanted human hearts. *Circ Res.* 2002; 90:634–640.10.1161/01.RES.0000014822.62629.EB [PubMed: 11934829]
37. Lee SH, Jang AS, Kim YE, Cha JY, Kim TH, Jung S, Park SK, Lee YK, Won JH, Kim YH, Park CS. Modulation of cytokine and nitric oxide by mesenchymal stem cell transfer in lung injury/fibrosis. *Respir Res.* 2010; 11:16.10.1186/1465-9921-11-16 [PubMed: 20137099]
38. Leri A, Kajstura J, Anversa P. Cardiac stem cells and mechanisms of myocardial regeneration. *Physiol Rev.* 2005; 85:1373–1416.10.1152/physrev.00013.2005 [PubMed: 16183916]
39. Li D, Zhao L, Liu M, Du X, Ding W, Zhang J, Mehta JL. Kinetics of tumor necrosis factor alpha in plasma and the cardio-protective effect of a monoclonal antibody to tumor necrosis factor alpha in acute myocardial infarction. *Am Heart J.* 1999; 137:1145–1152. [PubMed: 10347344]
40. Li SC, Wang L, Jiang H, Acevedo J, Chang AC, Loudon WG. Stem cell engineering for treatment of heart diseases: potentials and challenges. *Cell Biol Int.* 2009; 33:255–267.10.1016/j.cellbi.2008.11.009 [PubMed: 19084605]

41. Li Z, Wu JC, Sheikh AY, Kraft D, Cao F, Xie X, Patel M, Gambhir SS, Robbins RC, Cooke JP. Differentiation, survival, and function of embryonic stem cell derived endothelial cells for ischemic heart disease. *Circulation*. 2007; 116:146–154.10.1161/CIRCULATIONAHA.106.680561 [PubMed: 17846325]
42. Lin TJ, Issekutz TB, Marshall JS. Human mast cells transmigrate through human umbilical vein endothelial mono-layers and selectively produce IL-8 in response to stromal cell-derived factor-1 alpha. *J Immunol*. 2000; 165:211–220. [PubMed: 10861054]
43. Madonna R, Rokosh G, De Caterina R, Bolli R. Hepatocyte growth factor/Met gene transfer in cardiac stem cells— potential for cardiac repair. *Basic Res Cardiol*. 2010; 105:443–452.10.1007/s00395-010-0102-7 [PubMed: 20393738]
44. Maggini J, Mirkin G, Bognanni I, Holmberg J, Piazzon IM, Nepomnaschy I, Costa H, Canones C, Raiden S, Vermeulen M, Geffner JR. Mouse bone marrow-derived mesenchymal stromal cells turn activated macrophages into a regulatory-like profile. *PLoS One*. 2010; 5:e9252.10.1371/journal.pone.0009252 [PubMed: 20169081]
45. Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H, Hata J, Umezawa A, Ogawa S. Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest*. 1999; 103:697–705.10.1172/JCI5298 [PubMed: 10074487]
46. Malek S, Kaplan E, Wang JF, Ke Q, Rana JS, Chen Y, Rahim BG, Li M, Huang Q, Xiao YF, Verheugt FW, Morgan JP, Min JY. Successful implantation of intravenously administered stem cells correlates with severity of inflammation in murine myocarditis. *Pflugers Arch*. 2006; 452:268–275.10.1007/s00424-005-0035-4 [PubMed: 16715295]
47. Massa M, Rosti V, Ferrario M, Campanelli R, Ramajoli I, Rosso R, De Ferrari GM, Ferlini M, Goffredo L, Bertoletti A, Klersy C, Pecci A, Moratti R, Tavazzi L. Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. *Blood*. 2005; 105:199–206.10.1182/blood-2004-05-1831 [PubMed: 15345590]
48. Mazhari R, Hare JM. Advances in cell-based therapy for structural heart disease. *Prog Cardiovasc Dis*. 2007; 49:387–395.10.1016/j.pcad.2007.03.004 [PubMed: 17498519]
49. Mei SH, Haitzma JJ, Dos Santos CC, Deng Y, Lai PF, Slutsky AS, Liles WC, Stewart DJ. Mesenchymal stem cells reduce inflammation while enhancing bacterial clearance and improving survival in sepsis. *Am J Respir Crit Care Med*. 2010; 182:1047–1057.10.1164/rccm.201001-0010OC [PubMed: 20558630]
50. Müller P, Pfeiffer P, Koglin J, Schäfers HJ, Seeland U, Janzen I, Urbschat S, Böhm M. Cardiomyocytes of noncardiac origin in myocardial biopsies of human transplanted hearts. *Circulation*. 2002; 106:31–35.10.1161/01.CIR.0000022405.68464.CA [PubMed: 12093766]
51. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JJ, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*. 2004; 428:664–668.10.1038/nature02446 [PubMed: 15034593]
52. Nagase H, Miyamasu M, Yamaguchi M, Fujisawa T, Kawasaki H, Ohta K, Yamamoto K, Morita Y, Hirai K. Regulation of chemokine receptor expression in eosinophils. *Int Arch Allergy Immunol*. 2001; 125(Suppl 1):29–32. [PubMed: 11408769]
53. Nervi B, Link DC, DiPersio JF. Cytokines and hematopoietic stem cell mobilization. *J Cell Biochem*. 2006; 99:690–705.10.1002/jcb.21043 [PubMed: 16888804]
54. Oerlemans MI, Goumans MJ, van Middelaar B, Clevers H, Doevendans PA, Sluijter JP. ActiveWnt signaling in response to cardiac injury. *Basic Res Cardiol*. 2010; 105:631–641.10.1007/s00395-010-0100-9 [PubMed: 20373104]
55. Orlandi A, Chavakis E, Seeger F, Tjwa M, Zeiher AM, Dimmeler S. Long-term diabetes impairs repopulation of hematopoietic progenitor cells and dysregulates the cytokine expression in the bone marrow microenvironment in mice. *Basic Res Cardiol*. 2010; 105:703–712.10.1007/s00395-010-0109-0 [PubMed: 20652278]
56. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. *Nature*. 2001; 410:701–705.10.1038/35070587 [PubMed: 11287958]

57. Penn MS. Importance of the SDF-1:CXCR4 axis in myocardial repair. *Circ Res.* 2009; 104:1133–1135.10.1161/CIRCRESAHA.109.198929 [PubMed: 19461103]
58. Penn MS. SDF-1:CXCR4 axis is fundamental for tissue preservation and repair. *Am J Pathol.* 2010; 177:2166–2168.10.2353/ajpath.2010.100803 [PubMed: 20889567]
59. Popescu LM, Gherghiceanu M, Manole CG, Fausone-Pellegrini MS. Cardiac renewing: interstitial cajal-like cells nurse cardiomyocyte progenitors in epicardial stem cell niches. *J Cell Mol Med.* 2009; 13:866–886.10.1111/j.1582-4934.2009.00758.x [PubMed: 19382895]
60. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science.* 1997; 276:71–74.10.1126/science.276.5309.71 [PubMed: 9082988]
61. Qian JY, Harding P, Liu Y, Shesely E, Yang XP, LaPointe MC. Reduced cardiac remodeling and function in cardiac-specific EP4 receptor knockout mice with myocardial infarction. *Hypertension.* 2008; 51:560–566.10.1161/HYPERTENSIONAHA.107.102590 [PubMed: 18180401]
62. Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P. Chimerism of the transplanted heart. *N Engl J Med.* 2002; 346:5–15.10.1056/NEJMoa012081 [PubMed: 11777997]
63. Radl J, Croese JW, Zurcher C, Van den Enden-Vieveen MH, de Leeuw AM. Animal model of human disease. Multiple myeloma. *Am J Pathol.* 1988; 132:593–597. [PubMed: 3414786]
64. Radl J, Hollander CF, van den Berg P, de Glopper E. Idiopathic paraproteinaemia I. Studies in an animal model—the ageing C57BL/KaLwRij mouse. *Clin Exp Immunol.* 1978; 33:395–402. [PubMed: 367647]
65. Ren G, Dewald O, Frangogiannis NG. Inflammatory mechanisms in myocardial infarction. *Curr Drug Targets Inflamm Allergy.* 2003; 2:242–256. [PubMed: 14561159]
66. Salem HK, Thiemermann C. Mesenchymal stromal cells: current understanding and clinical status. *Stem Cells.* 2010; 28:585–596.10.1002/stem.269 [PubMed: 19967788]
67. Sanchez A, Garcia-Sancho J. Cardiac repair by stem cells. *Cell Death Differ.* 2007; 14:1258–1261.10.1038/sj.cdd.4402146 [PubMed: 17431420]
68. Sandstedt J, Jonsson M, Lindahl A, Jeppsson A, Asp J. C-kit+ CD45-cells found in the adult human heart represent a population of endothelial progenitor cells. *Basic Res Cardiol.* 2010; 105:545–556.10.1007/s00395-010-0088-1 [PubMed: 20119835]
69. Schulz R, Heusch G. C-reactive protein: just a biomarker of inflammation or a pathophysiological player in myocardial function and morphology? *Hypertension.* 2011; 57:151–153.10.1161/HYPERTENSIONAHA.110.165837 [PubMed: 21220709]
70. Soonpaa MH, Field LJ. Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts. *Am J Physiol.* 1997; 272:H220–H226. [PubMed: 9038941]
71. Sozzani S, Luini W, Borsatti A, Polentarutti N, Zhou D, Piemonti L, D'Amico G, Power CA, Wells TN, Gobbi M, Allavena P, Mantovani A. Receptor expression and responsiveness of human dendritic cells to a defined set of CC and CXC chemokines. *J Immunol.* 1997; 159:1993–2000. [PubMed: 9257866]
72. Spaggiari GM, Abdelrazik H, Becchetti F, Moretta L. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. *Blood.* 2009; 113:6576–6583.10.1182/blood-2009-02-203943 [PubMed: 19398717]
73. Suzuki K, Murtuza B, Beauchamp JR, Smolenski RT, Varela-Carver A, Fukushima S, Coppen SR, Partridge TA, Yacoub MH. Dynamics and mediators of acute graft attrition after myoblast transplantation to the heart. *FASEB J.* 2004; 18:1153–1155.10.1096/fj.03-1308fje [PubMed: 15155562]
74. Tan J, Weil BR, Abarbanell AM, Wang Y, Herrmann JL, Dake ML, Meldrum DR. Ablation of TNF-alpha receptors influences mesenchymal stem cell-mediated cardiac protection against ischemia. *Shock.* 2010; 34:236–242.10.1097/SHK.0b013e3181d75ae3 [PubMed: 20160664]
75. 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; 132:628–632.10.1016/j.jtcvs.2006.05.034 [PubMed: 16935119]

76. Toma C, Wagner WR, Bowry S, Schwartz A, Villanueva F. Fate of culture-expanded mesenchymal stem cells in the micro-vasculature: in vivo observations of cell kinetics. *Circ Res*. 2009; 104:398–402.10.1161/CIRCRESAHA.108.187724 [PubMed: 19096027]
77. Turan RG, Brehm M, Koesterling M, Tobias Z, Bartsch T, Steiner S, Picard F, Ebner P, Schannwell CM, Strauer BE. Factors influencing spontaneous mobilization of CD34+ and CD133+ progenitor cells after myocardial infarction. *Eur J Clin Invest*. 2007; 37:842–851.10.1111/j.1365-2362.2007.01876.x [PubMed: 17973780]
78. Van Craenenbroeck EM, Hoymans VY, Beckers PJ, Possemiers NM, Wuyts K, Paelinck BP, Vrints CJ, Conraads VM. Exercise training improves function of circulating angiogenic cells in patients with chronic heart failure. *Basic Res Cardiol*. 2010; 105:665–676.10.1007/s00395-010-0105-4 [PubMed: 20508941]
79. van den Borne SW, van de Schans VA, Strzelecka AE, Vervoort-Peters HT, Lijnen PM, Cleutjens JP, Smits JF, Daemen MJ, Janssen BJ, Blankesteyn WM. Mouse strain determines the outcome of wound healing after myocardial infarction. *Cardiovasc Res*. 2009; 84:273–282.10.1093/cvr/cvp207 [PubMed: 19542177]
80. Vinten-Johansen J, Jiang R, Reeves JG, Mykytenko J, Deneve J, Jobe LJ. Inflammation, proinflammatory mediators and myocardial ischemia–reperfusion injury. *Hematol Oncol Clin North Am*. 2007; 21:123–145.10.1016/j.hoc.2006.11.010 [PubMed: 17258123]
81. Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. *Lancet*. 2004; 363:783–784.10.1016/S0140-6736(04)15695-X [PubMed: 15016490]
82. Wang D, Chen K, Du WT, Han ZB, Ren H, Chi Y, Yang SG, Bayard F, Zhu D, Han ZC. CD14+ monocytes promote the immunosuppressive effect of human umbilical cord matrix stem cells. *Exp Cell Res*. 2010; 316:2414–2423.10.1016/j.yexcr.2010.04.018 [PubMed: 20420825]
83. Wei HM, Wong P, Hsu LF, Shim W. Human bone mar-row-derived adult stem cells for post-myocardial infarction cardiac repair: current status and future directions. *Singapore Med J*. 2009; 50:935–942. [PubMed: 19907881]
84. Weil BR, Manukyan MC, Herrmann JL, Wang Y, Abarbanell AM, Poynter JA, Meldrum DR. Mesenchymal stem cells attenuate myocardial functional depression and reduce systemic and myocardial inflammation during endotoxemia. *Surgery*. 2010; 148:444–452.10.1016/j.surg.2010.03.010 [PubMed: 20434747]
85. Wojakowski W, Tendera M, Michalowska A, Majka M, Kucia M, Maslankiewicz K, Wyderka R, Ochala A, Ratajczak MZ. Mobilization of CD34/CXCR4+, CD34/CD117+, c-met+ stem cells, and mononuclear cells expressing early cardiac, muscle, and endothelial markers into peripheral blood in patients with acute myocardial infarction. *Circulation*. 2004; 110:3213–3220.10.1161/01.CIR.0000147609.39780.02 [PubMed: 15533859]
86. Worthen GS, Schwab B 3rd, Elson EL, Downey GP. Mechanics of stimulated neutrophils: cell stiffening induces retention in capillaries. *Science*. 1989; 245:183–186.10.1126/science.2749255 [PubMed: 2749255]
87. Yoon CH, Koyanagi M, Iekushi K, Seeger F, Urbich C, Zeiher AM, Dimmeler S. Mechanism of improved cardiac function after bone marrow mononuclear cell therapy: role of cardiovascular lineage commitment. *Circulation*. 2010; 121:2001–2011.10.1161/CIRCULATIONAHA.109.909291 [PubMed: 20421519]
88. Zaruba MM, Franz WM. Role of the SDF-1-CXCR4 axis in stem cell-based therapies for ischemic cardiomyopathy. *Expert Opin Biol Ther*. 2010; 10:321–335.10.1517/14712590903460286 [PubMed: 20132055]
89. Zhang C, Wu J, Xu X, Potter BJ, Gao X. Direct relationship between levels of TNF-alpha expression and endothelial dysfunction in reperfusion injury. *Basic Res Cardiol*. 2010; 105:453–464.10.1007/s00395-010-0083-6 [PubMed: 20091314]
90. Zhang CC, Lodish HF. Cytokines regulating hematopoietic stem cell function. *Curr Opin Hematol*. 2008; 15:307–311.10.1097/MOH.0b013e3283007db5 [PubMed: 18536567]
91. Zhang QZ, Su WR, Shi SH, Wilder-Smith P, Xiang AP, Wong A, Nguyen AL, Kwon B, Le AD. Human gingiva-derived mesenchymal stem cells elicit polarization of M2 macrophages and enhance cutaneous wound healing. *Stem Cells*. 2010; 1002/stem.503

92. Zhao ZQ, Nakamura M, Wang NP, Velez DA, Hewan-Lowe KO, Guyton RA, Vinten-Johansen J. Dynamic progression of contractile and endothelial dysfunction and infarct extension in the late phase of reperfusion. *J Surg Res.* 2000; 94:133–144.10.1006/jsre.2000.6029 [PubMed: 11104653]