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Mechanisms for Progenitor Cell-Mediated Repair for Ischemic Heart Injury

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Abstract

Recent studies have shown that treatments involving injection of stem cells into animals with damaged cardiac tissue result in improved cardiac functionality. Clinical trials have reported conflicting results concerning the recellularization of post-infarct collagen scars. No clear mechanism has so far emerged to fully explain how injected stem cells, specifically the commonly used mesenchymal stem cells (MSC) and endothelial precursor cells (EPC), help heal a damaged heart. Clearly, these injected stem cells must survive and thrive in the hypoxic environment that results after injury for any significant repair to occur. Here we discuss how ischemic preconditioning may lead to increased tolerance of stem cells to these harsh conditions and increase their survival and clinical potential after injection. As injected cells must reach the site in numbers large enough for repair to be functionally significant, homing mechanisms involved in stem cell migration are also discussed. We review the mechanisms of action stem cells may employ once they arrive at their target destination. These possible mechanisms include that the injected stem cells (1) secrete growth factors, (2) differentiate into cardiomyocytes to recellularize damaged tissue and strengthen the post-infarct scar, (3) transdifferentiate the host cells into cardiomyocytes, and (4) induce neovascularization. Finally, we discuss that tissue engineering may provide a standardized platform technology to produce clinically applicable stem cell products with these desired mechanistic capacities.

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Keywords

Stem cells; precursor cells; cardiac infarction; heart injury; cell-based cardiac therapy

INTRODUCTION

Although pluripotent stem cells have been used in therapies because their intrinsic capacity to differentiate into multiple lineages, there is a risk that these pluripotent stem cells implanted systemically or locally might promote teratoma formation and cause inflammation [1, 2]. Nevertheless, mesenchymal stem cells (MSC) and endothelial precursor cells (EPCs) injected intramyocardially have led to improved right ventricular systolic and diastolic function [3–5]. These stem cell-based cardiac therapies extracted from animal studies [6] (Table 1) offer potential treatments for myocardiac infarction as well as other heart diseases [7]. In practice, however, currently used methods have ignored the fact that MSCs are sensitive to the concurrent serum and O_2 deprivation to which they are exposed when transplanted *in vivo* in the hypoxic condition of the ischemic heart [8]. As such, majority of these transplanted MSCs are death so the clinical trials conducted showed no consistent results (Table 2) [4, 9, 10]. Limited understanding of the mechanisms involved in repair hinders the development of new methods of stem cell preparation for therapies. Here, we review the available data and discuss the possible mechanisms for beneficial transplantation of stem cells [11]. We have also explored the possible methods to manage the molecular signaling pathways responsible for stem cell migration, engraftment, and neovascularization upon the transplantation into an ischemic heart.

APOPTOTIC SIGNALS IN HEART INJURY

Understanding of some pathways involved in damaged tissue has begun to reveal how heart tissue is prompted to undergo apoptosis. Apoptosis triggered by hypoxia or injury is regulated either by direct targeting of mitochondria or TNFα signaling. Given the mitochondria's primary role in cellular respiration for producing ATP, interruption of blood flow into tissues results in low oxygen concentration (i.e., hypoxia) within a cell and leads to stop ATP production. Subsequently, ATP-dependent physiological events are halted [12]. Energy conservation during hypoxia is partially achieved by a decrease in RNA Polymerase III (Pol III) activity, leading to reduce the macromolecule synthesis that is not immediately required for a cell. The Pol III recruitment involved in gene transcription is controlled by Pol III-specific transcription factor IIIB (TFIIIB) association with c-Myc, ERK, and RB. Under the hypoxic condition, TFIIIB dissociates from c-Myc and ERK, preventing Pol III from targeting its gene template while increasing its association with its negative regulator RB [13]. Furthermore, phospholipase C (PLC) and IP3 receptor bind to Na^+/K^+ ATPases to form a complex that regulates calcium release from the intra-cellular stores *via* the activation of IP3 receptors, leading to that only roughly 40% of a cell's ATP is consumed [14, 15]. The central loop of the Na^+/K^+ ATPase-1 subunit is bound by PLC-1 while the N-terminus binds to IP3R2 and IP3R3 along with caveolin-1 and src, both of which respond to ouabain signals [15]. Cardiac glycosides like ouabain are normally used in high concentrations to inhibit the Na^+/K^+ pump, causing increased Na^+ levels and preventing Ca^+ extrusion through the Na

$^{+}/Ca^{2+}$ pump as well as inducing hypertrophy. In lower doses, cardiac glycosides like ouabain have been reported to have positive inotropic effects *via* Erk1/2 activation [16]. Also, mitochondria regulate apoptosis by the activation of caspases. Metallothionein (MT), an anti-apoptotic compound, helps protect cells from oxidative cardiac injury by inhibiting a cell's mitochondria from releasing cytochrome C, preventing caspase-3 activation and protecting it against ROS damage [17].

In addition, ischemia-associated cell death triggers immune responses that attract circulating cells including stem cells through secretion of various cytokines, such as SDF-1 (Fig. 1), interleukin-6 (IL-6) (Fig. 2), interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNFα) (Fig. 2). TNFα signaling follows a pathway that activates caspase 8, resulting in apoptosis [18].

However, all of these are also known to be responsible for reperfusion injury [19, 20]. Reperfusion injury occurs when spillage of cellular components signals immune cells as well as endothelial cells to accumulate at a site where they release reactive oxygen species (ROS) and block small capillaries, resulting in cell death even after blood flow is restored [21]. The release of ROS after oxidative stress activates Ras. In addition, oxygen deprivation or stress has been shown to lead to hypertrophy which results after Erk ½ [22], MAPK [23, 24], and c-Jun-N terminal kinases (JNK) activation [25–29], while activation of p38-MAPKs leads to apoptosis [30, 31]. Signaling for repair, if not properly regulated or controlled, can lead to increased injury. Yet further research in this area is necessary to pinpoint exact mechanisms that can be targeted to prevent or alleviate damage caused by ischemia.

ISCHEMIC PRECONDITIONING FOR INDUCED TOLERANCE OF STEM CELLS

Acute cardiac ischemia results in a hypoxic microenvironment (i.e., low oxygen) which leads to cell death (apoptosis). This low oxygen environment makes it extremely difficult for the injured area to be functionally repaired and usually leads to formation of a collagen scar [32–34]. Consequently, for future stem cell treatments to be effective, the injected cells will need to be tolerant of hypoxic conditions [35–38]. Currently, many studies have focused on protecting injected cells against ischemia by increasing tolerance to hypoxic conditions through ischemic preconditioning of the heart [39, 40]. Ischemic preconditioning is achieved by stopping the heart's blood supply several times for a few minutes [41]. During hypoxia, lack of ATP prevents ion pumps from working, resulting in a rise of intracellular calcium that accumulates inside mitochondria. When the tissue is reoxygenated, the rise of ATP, damaging to the electron transport chain, increases mitochondrial generation of reactive oxygen species (ROS). Thus, cardiac ischemic preconditioning activates signaling pathways that reduce calcium production [42]. Hypoxic tolerance also occurs naturally following ischemia because of the release of IL-6 and TNFα (Fig. 2). Cardioprotection from the functional and biochemical damage produced by ischemia can be induced by activators of cardiac ATP-sensitive K+ channels, a class of drugs that includes, in particular, aprikalim and nicorandil [43]. It is not, however, known whether these drugs can be used for preconditioning stem cells.

Recent clinical trials have sought to find an association between inflammatory signals, Matrix Metalloproteinases (MMPs), and heart injury. Serum levels of IL-6, MMP9 and high sensitivity C-reactive protein (hs-CRP) in 134 patients were measured [44] and it was found that the levels of these factors were higher in injured patients than controls. IL-6 is responsible for delayed preconditioning *via* JAK-STAT and conveys cell protection through PI3K/ Akt, nitric oxide synthase (iNOS) and Ca^{2+} handling [45] (Fig. 2).

Additionally, both IL-6 and TNFα activate TNFα receptor 1 [46] and p38MAPK [47]. Signaling *via* TNFα and its receptor 1 leads to apoptosis while forming a heterodimeric complex with TNFα receptor 2 results in NF-κB activation (Fig. 2). TNFα-receptor 2 is a protein that directly interacts with the TNFα receptor to form a heterodimeric complex with TRAF1 to activate MAPK8/JNK and NF-κB [41, 46]. Hydrogen sulfide preconditioning also protects the heart from ischemia and results in decrease in infarct site and improved contractile function. This is mediated by the activation of $K_{(ATP)}$/ PKC/ERK ½ and PI3k/ Akt pathways [48]. Preconditioning of MSCs used in transplantation, rather than the heart, could be performed at 1–3% O_2 concentrations. Maintaining MSCs in this hypoxic environment activates Akt which maintains viability and cell cycle rate, suggesting that preculturing MSC under hypoxic conditions prior to transplantation might improve their tissue regenerative potential by protecting them in the harsh infarct environment [49].

MESENCHYMAL AND ENDOTHELIAL STEM CELLS

Therapeutic injection of stem cells into a host requires accurate cell selection based on differentiation potential, relative ease of isolation, availability in large quantities, *in vitro* expansion, timing of injection (i.e., a therapeutic window) [50], site of injection, and long-term survival of implanted cells [11]. Based on these criteria, bone marrow-derived mesenchymal stem cells (BM-MSCs) and endothelial progenitor cells (EPCs) are the best candidates [51, 52] [53, 54]. MSCs and EPCs have demonstrated cardiomyogenic differentiation capacity in multiple *in vitro* and *in vivo* studies [55, 56]. However, these MSC and EPC therapies may not be compatible with current pharmacological treatments. For examples, treatment with the potassium channel blockers tetraethylammonium and clofilium inhibited MSC proliferation while treatment with 5-azacytidine caused profound changes in current density, which is the electrophysiological property required for functional cardiomyocytes [57–59]. These data suggest that the pharmacological intervention may interfere with stem cell therapies. A two-step treatment paradigm may be used to address the issue: First, the pharmacological treatment to stabilize the condition; second, the stem cell therapy to regenerate the damaged tissue.

BM-MSCs secrete VEGF, IGF-1, EGF, angiopoeitin-1, stromal derived factor 1 (SDF-1), macrophage inflammatory protein-1 a/b, and erythropoietin in wound repair [60, 61]. Besides the commonly used EPCs, other EPC subpopulations have also been tested including early EPCs and late outgrowth endothelial cells (ECs). These cells were cultured with human fibroblasts to test whether their angiogenic properties were equivalent to standard EPCs [62]. Their ability to form tube like structures, integrate into existing networks, and undergoes paracrine-induced angiogenesis were assessed. It was found that only ECs were able to differentiate into tubules but not EPCs. EPCs did induce angiogenesis

via paracrine secretion while ECs did not [62]. A recent study sought to optimize MSC expansion by replacing fetus calf serum (FCS) with platelet lysate (PL), since cells cultured with 10% FCS may pose a xenogeneic risk to the patient after transplantation [63]. MSCs were isolated *via* gravity sedimentation and grown in media supplemented with 5% PL fewer than 5% O_2 conditions. This method increased the yield and proliferation for expansion compared with standard protocols [63]. The functional studies showed that decreased levels of pro-apoptotic signals IL-1, IL-6, TNFα, bax, bak and p38 as well as matrix remodeling molecules such as MMP-3, MMP-6, MMP-9, TIMP-1 and TIMP-3, helped decrease inflammation [64–66].

HOMING MECHANISM BY WHICH INFLAMMATION SIGNALS FOR STEM CELL MIGRATION

As body injury-driven inflammation triggers the recruitment of immune cells and endogenous stem cells, to a site of injury for repair, thus, these cells have the potential benefit for clinical applications to treat heart diseases [67]. Multiple lines of evidence show that both MSCs and EPCs can migrate to damaged tissues [68–73]. It has been showed that the homing mechanism is based on their expression of the CXCR4 receptor that recognizes the chemokine stromal derived factor-1 (SDF-1), which is produced by an injured tissue (Fig. 1). The CXCR4 receptor is present intracellular and integrates into the plasma membrane upon activation of signal transduction in response to hypoxia, cytokines, and sheer forces [74–81]. Expression of CXCR4 in MSCs can be induced within a few hours by employing a cocktail of cytokines. Flt-3 ligand, SCF, IL-6, HGF and IL-3 all can upregulate cell surface and intracellular CXCR4 expression [82]. However, a recent study found that homing *via* the CXCR4 receptor and SDF-1 ligand decreases with age due to age-related decrease in cytokine/ chemokine expression. One obstacle in the future clinical application of systemically-injected MSCs is that long-term *in vitro* expansion of these cells decreases CXCR4 expression. Thus, cultured MSCs might not home into injured tissue in sufficient numbers to have a functional impact on damaged myocardium [83]. Tissue homing of MSCs can be increased by forced expression of CXCR4. However, this may also result in a loss of regulation of MSC migration because of a loss of CXCR4-internalization triggered “stop signal” [84]. Our data showed that both CXCR4 and PI-3K are essential for EPCs to home to areas of myocardial infarction because inhibitors of CXCR4 and PI3K reduced homing (Fig. 3).

Additionally, EPCs and MSCs have other migration mechanisms. Akt activation induces intercellular adhesion molecule 1 (ICAM-1) which is important in EPC homing to ischemic tissues [85]. Three stages believed to be involved in stem cell homing to an injury are endothelial cell adhesion, capillary incorporation, and transendothelial migration in which cells reach the extravascular space. Upregulation of the Akt gene results in increased levels of the effecter molecules VEGF and SDF-1 in EPCs [85]. Another receptor responsible for homing in EPCs is the leptin receptor. In a mouse model, EPCs cultured with leptin before injection into a host increased re-endothelialization, showing that leptin increases adhesive properties and homing potential of EPCs *in vivo* [86]. When the leptin receptor is internalized it increases STAT3 phosphorylation. It was reported that after seven days culture

EPCs bound to vitronectin and fibronectin in a receptor-specific manner. These cells were also able to integrate into a monolayer of existing endothelial cells. Granulocyte colony stimulating factor (G-CSF) treatment was reported to increase MSC migration into peripheral blood. Treating both donor and recipient BM stem cells with G-CSF has been shown to improve cardiac function [87]. In this study, immunosuppressed mice pretreated with G-CSF showed increased cell survival in heart along with proliferation and angiogenesis. After 30 days of transplantation, re-endothelialization was observed [87]. Another study found that G-CSF alone failed to induce recruited MSCs to promote differentiation into cardiomyocytes or aid in repair [88]. These contradictory and inconclusive data are an indicator of why it is so difficult to pinpoint the exact mechanisms involved. Standardization of study protocols may lead to a set of consensus criteria for successful comparison of data between laboratories (e.g., EPCs by the European Society of Cardiology) [3].

SIGNALING EVENTS REGULATED BY CYTOKINES AND PARACRINES

Cytokines are signaling molecules involved in cellular communication. They are immunomodulating agents that act *via* autocrine, paracrine, and endocrine signals to activate the immune system. Chemokines are usually involved in chemotaxis while growth factors stimulate cell growth, proliferation and differentiation. Injured or dying cells release apoptotic signals that promote cell death in a nearby surrounding tissue. Secreted factors released by implanted MSCs, including VEGFα, IGF-1, EGF, keratinocyte growth factor, angiopoietin-1, stromal derived factor-1, macrophage inflammatory protein-1α/β (MIP-1α/β, erythropoietin, and PDGF, recruit macrophages and endothelial cells to a site of injury [60, 89]. Culturing MSCs in conditioned media from normal or hypoxic preconditioned cardiomyocytes revealed soluble paracrine factors involved in differentiation [90]. Further studies revealed that this activation of signaling *via* the Akt pathway provides paracrine protection for ischemic tissues. Indeed, MSCs overexpressing survival gene Akt release secreted frizzled related protein 2 (Sfrp2) that inhibits apoptotic signals received by Wnt signal ligands during injury due to post-hypoxia reoxygenation,

Sfrp2 exerts anti-apoptotic effect by antagonizing pro-apoptotic properties of specific Wnt ligands Wnt3a. It was shown that Sfrp2 binds directly to Wnt3a, leading to significantly inhibit the Wnt3a-activated caspase and transcriptional activities [91]. It seems contradictory, in the ischemic heart, Wnt signaling induces secreted frizzled related proteins (sFRPs), the endogenous Wnt-antagonists, leading to apoptosis [92]. Nevertheless, MSCs overexpressing both angiopoietin-1 (Ang-1) and Akt also provide long-term therapeutic benefits for preventing apoptosis in an ischemic heart after intramyocardial transplantation up to three months after initial transplantation [93]. In support of this observation, imaging showed myogenic differentiation, increased blood vessel density, and overall improved heart function. Jagged-1 activation of Notch signaling increases Wnt5a expression in EPCs, leading to cardiac differentiation [94].

Angiotensin II is responsible for the secretion of the cardioprotective factors IGF-1and HGF [95]. MSCs overexpressing angiotensin II induce VEGF production and secretion *via* the AT_1 receptor through Akt/ ERK ½ pathway [96]. Evidence suggests that paracrine signaling

is in part responsible for the beneficial effects seen after initial transplantation. Medium from BM-MSCs overexpressing Akt (Akt-MSC) cells cultured under hypoxic conditions showed increased antiapoptotic molecules and triggered increased contraction in cultured rat cardiomyocytes [97]. The Akt-MSC-conditioned medium, containing VEGF, FGF-2, HGF, IGF-1, and TB4, injected into rat infarction models showed improved ventricular function. HGF, upon binding to its receptor c-met, regulates cell matrix interactions. A complex formed by paxillin, Raf, and MEK, in response to HGF, binds and activates ERK leading to epithelial morphogenesis [98]. Erythropoietin (EPO) has been shown to “attenuate” Ang II-caused hypertrophy by activating the PI3K/Akt-eNOS-NO pathway and downregulating TGF-β1 [99]. As downstream effectors, MEK1/2 (mitogen activated protein kinase) activates ERK1/2 (extracellular regulated kinase) on the TEY motif. The Raf-MEK-ERK complex is formed after TEY motif phosphorylation, binding of G-protein subunits, and dimerization of ERK1/2 which leads to Thr188 auto-phosphorylation and results in hyperthropic growth of cardiomyocytes [100].

Angiogenin is responsible for inducing vascularization in tissues. It acts as a tRNA-specific ribonuclease which binds to actin on the EC surface where it is endocytosed and transported into the nucleus for transcriptional regulation to promote invasiveness. Angiogenin-modified MSCs help improve viability in ischemic tissue [101]. The evidence showed that angiogenin overexpressing MSCs, six weeks after injection into infarcted rat heart, resulted in increased tolerance to low oxygen environments. These cells improved the left ventricular systolic and diastolic function through increased vasculogenesis. This is quite significant when compared to previous studies that showed almost complete cell death within four days of transplantation [101].

Many studies suggest that heart injury results in activation of the immune response, leading to the recruitment of immune cells to the injured site. For example, mononuclear cells (MNCs) are essential components of the immune system and can be used as a “Trojan Horse” for gene therapy [102]. MNCs overexpressing VEGF, when transplanted into peripheral circulation, have been implicated in increased angiogenesis and improved heart function in a pig model [103]. In this study, the human VEGF65 was transfected *via* a plasmid into MNCs isolated from pig peripheral blood, and these VEGF-expressed MNCs were injected into a pig with acute myocardial infarction (AMI) induced by occlusion of the mid portion of the left anterior descending coronary artery (LAD). They observed that the transplantation of these modified MNCs improved left ventricular (LV) function, collateral vessels, capillary density and increased wall thickness of the scar in the heart after AMI. These findings indicate that MNCs can be used as a gene therapy vehicle for the targeted therapy of ischemic heart disease.

Other important effectors including IL-6 and TNFα promote circulating stem cells to be recruited to a site of injury [104]. These factors may act on the Akt signaling that is important for cell growth and survival [105–108]. Current studies showed that stem cell survival rate after injection into ischemic cardiac tissue is critical to have directed clinical effects [109–114]. Since MAPKs are involved in both cell survival and apoptotic signaling, pretreatment for inhibition of MAPKs phosphorylation by specific inhibitors revealed the

antiapoptotic role of MAPKs in stem cells and increased their survival in the injured tissue environment as nitric oxide (NO) induced apoptosis [115].

MSC AND EPC DIFFERENTIATION INTO CARDIAC TISSUE

Other possible mechanisms for the beneficial effects seen after stem cell injection in cardiac injury include the potential for MSCs and EPCs to differentiate into cardiomyocytes or transdifferentiate into cardiomyocytes [116–122]. Unfractioned bone marrow cells (BMC) mixed with either MSCs or EPCs can be injected to improve recruitment of progenitor cells into the damaged heart area [123]. Angiogenesis and survival of the transplanted cells near the scar are promoted by both EPCs and MSCs, while only MSCs invade the scar. Long-term co-culture of human MSCs with neonatal rat cardiomyocytes resulted in differentiated cells exhibiting a cardiomyogenic phenotype. It was observed that NKX2.5 and GATA4 transcription factors were translocated into the nucleus which coincided with sarcomeric actinin expression [124]. GLUT4 translocation to sarcolemma by activation of PI3K (stimulated by insulin) depended on activation of the PKC-zeta isoform but these levels were not above basal (only permissive, no strong correlation) [125]. Stable improvement in heart tissue for a period of eight months after implantation along with EPCs was observed with increased levels of VEGF-and bFGF-mediated angiogenesis [126].

Rat MSCs co-cultured with mature cardiomyocytes showed that some MSCs express cardiogenic markers under standard culturing methods. Under hypoxic conditions (less than 1% oxygen concentration), deprivation of glucose and serum further increased to 7% cardiac committed MSCs in the presence of difluoromethylornithine (DFMO), an inhibitor of polyamine synthesis and cell proliferation, while only 5% MSCs showed cardiac markers positive without DFMO [127]. Although pluripotent stem cells have been proposed for cardiac therapy, the propensity of these cells to differentiate into multiple lineages may lead to heterogeneous tumor formation, cardiogenic specification using TNFα, however, has been shown to lead to cardiac differentiation *in vitro* and *in vivo* without neoplastic outgrowth, suggesting that injecting cardiac progenitors instead of pluripotent stem cells are safest [128].

More importantly, co-cultures of EPCs and cardiomyocytes promote forming intercellular connections commonly referred to as nanotubes, sized from 50–800 nm with a length of 5–120 nm. These nanotubes are responsible for organelle and cytoplasmic molecule exchange and increased after longer culture times [129]. Different culturing protocols showed mixed results for transdifferentiation, where some studies showed evidence while others did not [130]. Transplanted MSCs and host myocardium are able to communicate *via* nanotubes *in vitro* and *in vivo*. These cells communicate by cytosolic molecule exchange as well as transfer of mitochondria resulting from fusion [122, 131–134]. Transfer of mitochondria from MSCs to myocardium, although not observed within 24 hours after transplantation in this study, might in part explain the beneficial effects seen after MSC transplantation to help repair damaged mitochondria [122]. TGFβ1 has been shown to promote cardiomyogenic differentiation in rat adipose-derived stromal cells when cultured for 2 weeks *in vitro* [135].

MOBILIZATION OF CARDIAC PROGENITORS

It had been widely accepted that the heart loses its ability to regenerate after birth, however, recent studies have shown the post-natal heart is, in fact, able to regenerate and heal itself [136]. Resident cardiac progenitor cells are capable of minor repair of the heart yet these are not available in sufficient quantities to repair large damaged areas [137–139]. Radioactive labeling (pulse-labeling) to measure rates of cell growth has been used in animals but not in humans, because of ethical concerns. However, researchers have found that radioactive material from nuclear weapon testifying from 1963, have essentially labeled the entire world population indirectly *via* atmospheric contamination. Thus, they measured carbon-14 levels in human heart cells and found that at age 25 about 1% of cells are replaced each year, decreasing to around half of one percent by age 75 [140]. It has been found in chick embryos that cardiomyocytes are able to re-enter the cell cycle and proliferate after differentiation. LIM homeodomain transcription factor Islet1 (Isl1) is present in cardiac progenitor cells. It was found that Isl1 is expressed in response to β-catenin regulation, leading to proliferation and survival [16]. Isl1-expressed precursors did not proliferate before becoming cardiomyocytes but did do so after, even when contractility was observed. It was observed that almost 83% of differentiated cardiomyocytes proliferated [141]. The existence of resident heart cells (cardiac stem cells), as opposed to cardiac progenitors, was assessed using cells obtained by digesting adult mouse heart. Rounded adherent cells were expanded and cultured 2–5 days after plating. Further culturing leads to self-beating patches that displayed many cardiomyocyte markers. Patch-clamp measurements showed that they were able to create spontaneous action potentials, a self-beating property due to HCN4 channels which lead to depolarization [142].

Controlled regulation of ion channels is essential for regeneration of cardiac pacemakers. Hyperpolarization-activated cyclic nucleotide-gated channels (HCN 1–4) and T-type calcium channels are responsible for the pacemaker property of human embryonic stem cell (hESC)-derived cardiomyocytes [143]. Data from mouse embryos suggest that all three layers present in the heart, cardiomyocytes, endothelial, and smooth muscle, originate from a Flk1 (a.k.a., KDR, VEGF-R2) positive progenitor. To test whether this is also the case in humans, they studied hESC differentiation. After hESCs were cultured with a cocktail of many growth factors, embryoid bodies were made which resulted in KDR low/c-kit-(CD117) negative population that displayed cardiac, endothelial and vascular smooth muscle properties *in vitro* and *in vivo* [144]. Over 50% of these progenitors differentiated into beating cardiomyocytes in monolayer culture. Evidence supports a common precursor responsible for all cardiac lineages [145]. A cardiac-specific Nkx2.5 subpopulation isolated from mouse embryos showed that about 28% of these cells express c-kit, which in turn give rise to both cardiomyocytes and smooth muscle cells [146]. The second heart field formed during cardiac development is responsible for the supply of most cardiac progenitors that express Nkx2–5, which is a homeodomain factor that is a repressor of Bmp2/ Smad1 signaling [147]. Smad1-dependent negative feedback loop is responsible for cardiac differentiation [148]. The cardiomyogenic potential of cardiac stem cells and bone marrow-derived stem cells is associated with c-kit expression, leading to the possibility that these cells may be part of the same reservoir, connected by the systemic circulation.

Cardiomyocytes differentiated from bone marrow stem cells through the use of VEGF and FGFβ, express Flk1 and FGFR1, which are observed as parallel differentiation of ES cells into cardiomyocytes [89]. Resident cardiac stem cells are believed to have the ability to migrate to a site of injury *via* the SCF/ c-kit signaling [149–155]. This is specifically *via* phosphorylated p38 MAPK to induce SCF-mediated migration of cardiac stem cells.

TISSUE ENGINEERING

Research has rapidly moved towards three-dimensional (3-D) tissue engineering of left ventricular allograft, aortic valve allografts, and whole hearts, in mouse, rat, pig, and bovine models [156–167]. A cardiac patch made from MSCs shows the formation of neomuscle and neomicrovessels. Microvessels are essential, since they allow MSCs to undergo cell division or recruit other cells to make the patch thicker than it was originally [95]. Cell sheets are commonly made using temperature-responsive plates which allows for the intact removal from culture plates that contain cardiomyocyte cell sheets vascularized by microvesslesmicrovessels. These have been shown to have an improvement on the heart *via* secretion of paracrine factors and by providing mechanical support [168]. Decellularized heart scaffolds offer an exciting alternative for those whose heart injury is too severe for stem cell injection [157]. A bioartificial heart can be made using a donor heart since ECM without cells does not appear to elicit an immune response. These donor hearts would be treated with detergents that remove all cells but leave the ECM structure intact [169–172]. MSCs and EPCs seeded into the scaffold are able to recellularize it within 4 days, generate pump function at day 8, and undergo coronary perfusion by day 28 [173, 174]. A scaffold, serving to promote stem cell differentiation, is dependent, in part, on physical stimuli as well as soluble growth factors. Many studies have shown that the microenvironment significantly shapes cell differentiation [175]. *In vivo*, poor viability at the transplant site often hinders the therapeutic potential of MSCs. To overcome this barrier, cationized polysaccharide spermine-dextran is used as a carrier for protection of these cells from damage [168].

Tissue engineering may play a major role for future clinical applications because the heterogeneity of stem cells remains a main issue, especially for pluripotent stem cells. A clinically-applicable population of stem cells should be capable of being expanded and differentiated to result in a sufficient number of cells with consistent quality (e.g., a “pure” population of precursors). Cardiac precursors be derived from the embryoid bodies which are heterogeneous aggregates of cells, but these contain limited numbers of cells, making large-scale production difficult. It was reported that a degree of homogeneity of a stem cell product was obtained by using a microprinting strategy and a bioreactor methodology, with a large yield of cardiac precursors being obtained when applying hypoxic conditions [176]. All of these experiments suggest that an engineer design method can be used to develop a specific stem cell product with a desired specification for personalized treatment of ischemic heart injury.

CONCLUSION

MSCs and EPCs after systemic injection or local injection to a site of injury may be involved in direct tissue replacement or tissue protection by release of cytokines and

chemokines, or both. This may strengthen the post-infarct scar or mobilize resident cardiac stem cells to recellularize the damaged tissue. Studies show that key effectors and modulators are responsible for some of the beneficial effects, including IL-6 and TNFα, which direct circulating stem cells to go to the site of injury. Akt signaling, which is important for early heart development and growth, is activated after transplantation. Currently, stem cell survival rates after injection into ischemic cardiac tissue are not high enough to give rise to functional improvement. Engineered tissue could serve as a platform to test the effectiveness of stem cell treatment by mimicking the injured heart microenvironment. It may be advantageous to engineer these cells to have stronger homing mechanisms by upregulating their responsiveness to cytokines and chemokines, allowing them to reach injured tissue more effectively and in higher numbers. Because the exact mechanisms by which injected stem cells repair an injury site are still unclear, it is essential to employ a standardized platform technology like tissue engineering to understand the mechanisms of stem cell repair in different preclinical and clinical models [177].

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REFERENCES

[1]. He Q, Trindade PT, Stumm M, et al. Fate of undifferentiated mouse embryonic stem cells within the rat heart: role of myocardial infarction and immune suppression. J Cell Mol Med 2009; 13(1): 188–201. [PubMed: 18373734]

[2]. 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(7): 1345–57. [PubMed: 17284483]

[3]. Bartunek J, Dimmeler S, Drexler H, et al. The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. Eur Heart J 2006; 27(11): 1338–40. [PubMed: 16543252]

[4]. Odorfer KI, Walter I, Kleiter M, Sandgren EP, Erben RG. Role of endogenous bone marrow cells in long-term repair mechanisms after myocardial infarction. J Cell Mol Med 2008; 12(6B): 2867–74. [PubMed: 19210759]

[5]. Trop S, Tremblay ML, Bourdeau A. Modulation of bone marrow-derived endothelial progenitor cell activity by protein tyrosine phosphatases. Trends Cardiovasc Med 2008; 18(5): 180–6. [PubMed: 18790388]

[6]. Kudo M, Wang Y, Wani MA, Xu M, Ayub A, Ashraf M. Implantation of bone marrow stem cells reduces the infarction and fibrosis in ischemic mouse heart. J Mol Cell Cardiol 2003; 35(9): 1113–9. [PubMed: 12967634]

[7]. Lasala GP, Minguell JJ. Bone marrow-derived stem/progenitor cells: their use in clinical studies for the treatment of myocardial infarction. Heart Lung Circ 2009; 18(3): 171–80. [PubMed: 19081302]

[8]. Potier E, Ferreira E, Meunier A, Sedel L, Logeart-Avramoglou D, Petite H. Prolonged hypoxia concomitant with serum deprivation induces massive human mesenchymal stem cell death. Tissue Eng 2007; 13(6): 1325–31. [PubMed: 17518749]

[9]. Berry MF, Engler AJ, Woo YJ, et al. Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. Am J Physiol Heart Circ Physiol 2006; 290(6): H2196–203. [PubMed: 16473959]

[10]. Nagaya N, Kangawa K, Itoh T, et al. Transplantation of mesenchymal stem cells improves cardiac function in a rat model of dilated cardiomyopathy. Circulation 2005; 112(8): 1128–35. [PubMed: 16103243]

[11]. 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(3): 255–67. [PubMed: 19084605]

[12]. Guzun R, Timohhina N, Tepp K, et al. Regulation of respiration controlled by mitochondrial creatine kinase in permeabilized cardiac cells in situ. Importance of system level properties. Biochim Biophys Acta 2009; 1787(9): 1089–105. [PubMed: 19362066]

[13]. Ernens I, Goodfellow SJ, Innes F, et al. Hypoxic stress suppresses RNA polymerase III recruitment and tRNA gene transcription in cardiomyocytes. Nucleic Acids Res 2006; 34(1): 286–94. [PubMed: 16407335]

[14]. Bekedam MA, van Beek-Harmsen BJ, van Mechelen W, Boonstra A, Visser FC, van der Laarse WJ. Sarcoplasmic reticulum ATPase activity in type I and II skeletal muscle fibres of chronic heart failure patients. Int J Cardiol 2009; 133(2): 185–90. [PubMed: 18279980]

[15]. Yuan Z, Cai T, Tian J, Ivanov AV, Giovannucci DR, Xie Z. Na/K-ATPase tethers phospholipase C and IP3 receptor into a calcium-regulatory complex. Mol Biol Cell 2005; 16(9): 4034–45. [PubMed: 15975899]

[16]. Lin L, Cui L, Zhou W, et al. Beta-catenin directly regulates Islet1 expression in cardiovascular progenitors and is required for multiple aspects of cardiogenesis. Proc Natl Acad Sci USA 2007; 104(22): 9313–8. [PubMed: 17519333]

[17]. Wang GW, Klein JB, Kang YJ. Metallothionein inhibits doxorubicin-induced mitochondrial cytochrome c release and caspase-3 activation in cardiomyocytes. J Pharmacol Exp Ther 2001; 298(2): 461–8. [PubMed: 11454906]

[18]. Waetzig GH, Rosenstiel P, Arlt A, et al. Soluble tumor necrosis factor (TNF) receptor-1 induces apoptosis via reverse TNF signaling and autocrine transforming growth factor-beta1. Faseb J 2005; 19(1): 91–3. [PubMed: 15514103]

[19]. Bonetti A, Marchini M, Ortolani F. Immunolocalization of interleukin-1 receptor antagonist in healthy and infarcted myocardium. Histol Histopathol 2008; 23(9): 1093–102. [PubMed: 18581280]

[20]. Harder Y, Amon M, Schramm R, et al. Erythropoietin reduces necrosis in critically ischemic myocutaneous tissue by protecting nutritive perfusion in a dose-dependent manner. Surgery 2009; 145(4): 372–83. [PubMed: 19303985]

[21]. Bakker W, Eringa EC, Sipkema P, van Hinsbergh VW. Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. Cell Tissue Res 2009; 335(1): 165–89. [PubMed: 18941783]

[22]. Lv X, Wan J, Yang J, et al. Cytochrome P450 omega-hydroxylase inhibition reduces cardiomyocyte apoptosis via activation of ERK1/2 signaling in rat myocardial ischemia-reperfusion. Eur J Pharmacol 2008; 596(1–3): 118–26. [PubMed: 18771665]

[23]. Bueno OF, De Windt LJ, Tymitz KM, et al. The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. Embo J 2000; 19(23): 6341–50. [PubMed: 11101507]

[24]. Talmor D, Applebaum A, Rudich A, Shapira Y, Tirosh A. Activation of mitogen-activated protein kinases in human heart during cardiopulmonary bypass. Circ Res 2000; 86(9): 1004–7. [PubMed: 10807874]

[25]. Duplain H Salvage of ischemic myocardium: a focus on JNK. Adv Exp Med Biol 2006; 588: 157–64. [PubMed: 17089887]

[26]. Ferrandi C, Ballerio R, Gaillard P, et al. Inhibition of c-Jun N-terminal kinase decreases cardiomyocyte apoptosis and infarct size after myocardial ischemia and reperfusion in anaesthetized rats. Br J Pharmacol 2004; 142(6): 953–60. [PubMed: 15210584]

[27]. He H, Li HL, Lin A, Gottlieb RA. Activation of the JNK pathway is important for cardiomyocyte death in response to simulated ischemia. Cell Death Differ 1999; 6(10): 987–91. [PubMed: 10556976]

[28]. Shao Z, Bhattacharya K, Hsich E, et al. c-Jun N-terminal kinases mediate reactivation of Akt and cardiomyocyte survival after hypoxic injury in vitro and in vivo. Circ Res 2006; 98(1): 111–8. [PubMed: 16306447]

[29]. Yang LM, Xiao YL, Ou-Yang JH. [Inhibition of magnesium lithospermate B on the c-Jun N-terminal kinase 3 mRNA expression in cardiomyocytes encountered ischemia/reperfusion injury]. Yao Xue Xue Bao 2003; 38(7): 487–91. [PubMed: 14515790]

[30]. Yoshinari D, Takeyoshi I, Kobayashi M, et al. Effects of a p38 mitogen-activated protein kinase inhibitor as an additive to university of wisconsin solution on reperfusion injury in liver transplantation. Transplantation 2001; 72(1): 22–7. [PubMed: 11468529]

[31]. Iacobas DA, Fan C, Iacobas S, Haddad GG. Integrated transcriptomic response to cardiac chronic hypoxia: translation regulators and response to stress in cell survival. Funct Integr Genomics 2008; 8(3): 265–75. [PubMed: 18446526]

[32]. Brehm M, Stanske B, Strauer BE. Therapeutic potential of stem cells in elderly patients with cardiovascular disease. Exp Gerontol 2008; 43(11): 1024–32. [PubMed: 18845234]

[33]. Krupnick AS, Kreisel D, Riha M, Balsara KR, Rosengard BR. Myocardial tissue engineering and regeneration as a therapeutic alternative to transplantation. Curr Top Microbiol Immunol 2004; 280: 139–64. [PubMed: 14594210]

[34]. Yu Y, Fukuda N, Yao EH, et al. Effects of an ARB on endothelial progenitor cell function and cardiovascular oxidation in hypertension. Am J Hypertens 2008; 21(1): 72–7. [PubMed: 18091747]

[35]. Boudoulas KD, Hatzopoulos AK. Cardiac repair and regeneration: the Rubik's cube of cell therapy for heart disease. Dis Model Mech 2009; 2(7–8): 344–58. [PubMed: 19553696]

[36]. LaPar DJ, Kron IL, Yang Z. Stem cell therapy for ischemic heart disease: where are we? Curr Opin Organ Transplant 2009; 14(1): 79–84. [PubMed: 19337151]

[37]. Seidel M, Borczynnska A, Rozwadowska N, Kurpisz M. Cell-based therapy for heart failure: skeletal myoblasts. Cell Transplant 2009; 18(7): 695–707. [PubMed: 19500482]

[38]. Yamada S, Nelson TJ, Behfar A, Crespo-Diaz RJ, Fraidenraich D, Terzic A. Stem cell transplant into preimplantation embryo yields myocardial infarction-resistant adult phenotype. Stem Cells 2009; 27(7): 1697–705. [PubMed: 19544428]

[39]. Kim HW, Haider HK, Jiang S, Ashraf M. Ischemic preconditioning augments survival of stem cells via MIR-210 expression by targeting caspase-8 associated protein 2. J Biol Chem 2009; 284(48): 33161–8. [PubMed: 19721136]

[40]. Wisel S, Khan M, Kuppusamy ML, et al. Pharmacological preconditioning of mesenchymal stem cells with trimetazidine (1-[2,3,4-trimethoxybenzyl]piperazine) protects hypoxic cells against oxidative stress and enhances recovery of myocardial function in infarcted heart through Bcl-2 expression. J Pharmacol Exp Ther 2009; 329(2): 543–50. [PubMed: 19218529]

[41]. Valen G Extracardiac approaches to protecting the heart. Eur J Cardiothorac Surg 2009; 35(4): 651–7. [PubMed: 19216086]

[42]. Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiol Rev 2008; 88(2): 581–609. [PubMed: 18391174]

[43]. Cavero I, Djellas Y, Guillon JM. Ischemic myocardial cell protection conferred by the opening of ATP-sensitive potassium channels. Cardiovasc Drugs Ther 1995; 9 (Suppl 2): 245–55. [PubMed: 7647029]

[44]. Shu J, Ren N, Du JB, Zhang M, Cong HL, Huang TG. Increased levels of interleukin-6 and matrix metalloproteinase-9 are of cardiac origin in acute coronary syndrome. Scand Cardiovasc J 2007; 41(3): 149–54. [PubMed: 17487763]

[45]. Vinten-Johansen J, Zhao ZQ, Nakamura M, et al. Nitric oxide and the vascular endothelium in myocardial ischemia-reperfusion injury. Ann N Y Acad Sci 1999; 874: 354–70. [PubMed: 10415547]

[46]. Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. Science 2002; 296(5573): 1634–5. [PubMed: 12040173]

[47]. Yang YY, Liu H, Nam SW, Kunos G, Lee SS. Mechanisms of TNFalpha-induced cardiac dysfunction in cholestatic bile ductligated mice: interaction between TNFalpha and endocannabinoids. J Hepatol 2011; 53(2): 298–306.

[48]. Hu Y, Chen X, Pan TT, et al. Cardioprotection induced by hydrogen sulfide preconditioning involves activation of ERK and PI3K/Akt pathways. Pflugers Arch 2008; 455(4): 607–16. [PubMed: 17674030]

[49]. Rosova I, Dao M, Capoccia B, Link D, Nolta JA. Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. Stem Cells 2008; 26(8): 2173–82. [PubMed: 18511601]

[50]. Li SC, Han YP, Dethlefs BA, Loudon WG. Therapeutic Window, a Critical Developmental Stage for Stem Cell Therapies. Curr Stem Cell Res Ther 2010; 5(4): 297–3. [PubMed: 20528752]

[51]. Zhang FB, Yang HT. [Plasticity of bone marrow mesenchymal stem cells differentiating into cardiomyocytes and the potential of cardiac therapeutics]. Sheng Li Ke Xue Jin Zhan 2006; 37(3): 199–204. [PubMed: 17009725]

[52]. Zhang S, Ge J, Sun A, et al. Comparison of various kinds of bone marrow stem cells for the repair of infarcted myocardium: single clonally purified non-hematopoietic mesenchymal stem cells serve as a superior source. J Cell Biochem 2006; 99(4): 1132–47. [PubMed: 16795039]

[53]. Bartunek J, Dimmeler S, Drexler H, et al. The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. Eur Heart J 2006; 27(11): 1338–40. [PubMed: 16543252]

[54]. Papathanasopoulos A, Giannoudis PV. Biological considerations of mesenchymal stem cells and endothelial progenitor cells. Injury 2008; 39 (Suppl 2): S21–32. [PubMed: 18804570]

[55]. Roche S, Provansal M, Tiers L, Jorgensen C, Lehmann S. Proteomics of primary mesenchymal stem cells. Regen Med 2006; 1(4): 511–7. [PubMed: 17465845]

[56]. Iop L, Chiavegato A, Callegari A, et al. Different cardiovascular potential of adult-and fetal-type mesenchymal stem cells in a rat model of heart cryoinjury. Cell Transplant 2008; 17(6): 679–94. [PubMed: 18819256]

[57]. Balana B, Nicoletti C, Zahanich I, et al. 5-Azacytidine induces changes in electrophysiological properties of human mesenchymal stem cells. Cell Res 2006; 16(12): 949–60. [PubMed: 17160070]

[58]. Heubach JF, Graf EM, Leutheuser J, et al. Electrophysiological properties of human mesenchymal stem cells. J Physiol 2004; 554(Pt 3): 659–72. [PubMed: 14578475]

[59]. Bonnet P, Awede B, Rochefort GY, et al. Electrophysiological maturation of rat mesenchymal stem cells after induction of vascular smooth muscle cell differentiation in vitro. Stem Cells Dev 2008; 17(6): 1131–40. [PubMed: 19006452]

[60]. Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One 2008; 3(4): e1886. [PubMed: 18382669]

[61]. Luttun A, Carmeliet P. De novo vasculogenesis in the heart. Cardiovasc Res 2003; 58(2): 378–89. [PubMed: 12757872]

[62]. Sieveking DP, Buckle A, Celermajer DS, Ng MK. Strikingly different angiogenic properties of endothelial progenitor cell subpopulations: insights from a novel human angiogenesis assay. J Am Coll Cardiol 2008; 51(6): 660–8. [PubMed: 18261686]

[63]. Carrancio S, Lopez-Holgado N, Sanchez-Guijo FM, et al. Optimization of mesenchymal stem cell expansion procedures by cell separation and culture conditions modification. Exp Hematol 2008; 36(8): 1014–21. [PubMed: 18468767]

[64]. Molina EJ, Gupta D, Palma J, Gaughan JP, Macha M. Right ventricular beneficial effects of beta adrenergic receptor kinase inhibitor (betaARKct) gene transfer in a rat model of severe pressure overload. Biomed Pharmacother 2009; 63(5): 331–6. [PubMed: 18801641]

[65]. Molina EJ, Palma J, Gupta D, Gaughan JP, Houser S, Macha M. Right ventricular effects of intracoronary delivery of mesenchymal stem cells (MSC) in an animal model of pressure overload heart failure. Biomed Pharmacother 2009; 63(10): 767–72 [PubMed: 18993028]

[66]. Molina EJ, Palma J, Gupta D, et al. Reverse remodeling is associated with changes in extracellular matrix proteases and tissue inhibitors after mesenchymal stem cell (MSC) treatment of pressure overload hypertrophy. J Tissue Eng Regen Med 2009; 3(2): 85–91. [PubMed: 19065545]

[67]. Christoforou N, Gearhart JD. Stem cells and their potential in cell-based cardiac therapies. Prog Cardiovasc Dis 2007; 49(6): 396–413. [PubMed: 17498520]

[68]. Shintani S, Murohara T, Ikeda H, et al. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation 2001; 103(23): 2776–9. [PubMed: 11401930]

[69]. Aghi M, Cohen KS, Klein RJ, Scadden DT, Chiocca EA. Tumor stromal-derived factor-1 recruits vascular progenitors to mitotic neovasculature, where microenvironment influences their differentiated phenotypes. Cancer Res 2006; 66(18): 9054–64. [PubMed: 16982747]

[70]. Ceradini DJ, Gurtner GC. Homing to hypoxia: HIF-1 as a mediator of progenitor cell recruitment to injured tissue. Trends Cardiovasc Med 2005; 15(2): 57–63. [PubMed: 15885571]

[71]. Hu X, Dai S, Wu WJ, et al. Stromal cell derived factor-1 alpha confers protection against myocardial ischemia/reperfusion injury: role of the cardiac stromal cell derived factor-1 alpha CXCR4 axis. Circulation 2007; 116(6): 654–63. [PubMed: 17646584]

[72]. Lima e Silva R, Shen J, Hackett SF, et al. The SDF-1/CXCR4 ligand/receptor pair is an important contributor to several types of ocular neovascularization. Faseb J 2007; 21(12): 3219–30. [PubMed: 17522382]

[73]. Petit I, Jin D, Rafii S. The SDF-1-CXCR4 signaling pathway: a molecular hub modulating neo-angiogenesis. Trends Immunol 2007; 28(7): 299–307. [PubMed: 17560169]

[74]. Hoenig MR, Bianchi C, Sellke FW. Hypoxia inducible factor-1 alpha, endothelial progenitor cells, monocytes, cardiovascular risk, wound healing, cobalt and hydralazine: a unifying hypothesis. Curr Drug Targets 2008; 9(5): 422–35. [PubMed: 18473772]

[75]. Ju SY, Cho KA, Cho SJ, et al. Effect of hypoxic treatment on bone marrow cells that are able to migrate to the injured liver. Cell Biol Int 2009; 33(1): 31–5. [PubMed: 18992833]

[76]. Kubo M, Li TS, Kamota T, Ohshima M, Qin SL, Hamano K. Increased expression of CXCR4 and integrin alphaM in hypoxia-preconditioned cells contributes to improved cell retention and angiogenic potency. J Cell Physiol 2009; 220(2): 508–14. [PubMed: 19415696]

[77]. Li N, Lu X, Zhao X, et al. Endothelial nitric oxide synthase promotes bone marrow stromal cell migration to the ischemic myocardium via upregulation of stromal cell-derived factor-1alpha. Stem Cells 2009; 27(4): 961–70. [PubMed: 19353524]

[78]. Lin JS, Chen YS, Chiang HS, Ma MC. Hypoxic preconditioning protects rat hearts against ischaemia-reperfusion injury: role of erythropoietin on progenitor cell mobilization. J Physiol 2008; 586(Pt 23): 5757–69. [PubMed: 18845609]

[79]. Tang YL, Zhu W, Cheng M, et al. Hypoxic preconditioning enhances the benefit of cardiac progenitor cell therapy for treatment of myocardial infarction by inducing CXCR4 expression. Circ Res 2009; 104(10): 1209–16. [PubMed: 19407239]

[80]. Wang X, Li C, Chen Y, et al. Hypoxia enhances CXCR4 expression favoring microglia migration via HIF-1alpha activation. Biochem Biophys Res Commun 2008; 371(2): 283–8. [PubMed: 18435916]

[81]. Zagzag D, Esencay M, Mendez O, et al. Hypoxia-and vascular endothelial growth factor-induced stromal cell-derived factor-1alpha/CXCR4 expression in glioblastomas: one plausible explanation of Scherer's structures. Am J Pathol 2008; 173(2): 545–60. [PubMed: 18599607]

[82]. Shi M, Li J, Liao L, et al. Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice. Haematologica 2007; 92(7): 897–904. [PubMed: 17606439]

[83]. Kyriakou C, Rabin N, Pizzey A, Nathwani A, Yong K. Factors that influence short-term homing of human bone marrow-derived mesenchymal stem cells in a xenogeneic animal model. Haematologica 2008; 93(10): 1457–65. [PubMed: 18728032]

[84]. Venkatesan S, Rose JJ, Lodge R, Murphy PM, Foley JF. Distinct mechanisms of agonist-induced endocytosis for human chemokine receptors CCR5 and CXCR4. MolBiolCell 2003; 14(8): 3305–24.

[85]. Hur J, Yoon CH, Lee CS, et al. Akt is a key modulator of endothelial progenitor cell trafficking in ischemic muscle. Stem Cells 2007; 25(7): 1769–78. [PubMed: 17412896]

[86]. Schroeter MR, Leifheit M, Sudholt P, et al. Leptin enhances the recruitment of endothelial progenitor cells into neointimal lesions after vascular injury by promoting integrin-mediated adhesion. Circ Res 2008; 103(5): 536–44. [PubMed: 18658052]

[87]. Rezai N, Deisher TA, Heine HL, et al. Effects of granulocyte-colony stimulating factor on bone marrow-derived progenitor cells in murine cardiac transplantation. Cardiovasc Pathol 2010; 19(1): 36–47. [PubMed: 19144547]

[88]. Tatsumi K, Otani H, Sato D, et al. Granulocyte-colony stimulating factor increases donor mesenchymal stem cells in bone marrow and their mobilization into peripheral circulation but does not repair dystrophic heart after bone marrow transplantation. Circ J 2008; 72(8): 1351–8. [PubMed: 18654025]

[89]. Pallante BA, Duignan I, Okin D, et al. Bone marrow Oct¾+ cells differentiate into cardiac myocytes via age-dependent paracrine mechanisms. Circ Res 2007; 100(1): e1–11. [PubMed: 17122441]

[90]. Xie XJ, Wang JA, Cao J, Zhang X. Differentiation of bone marrow mesenchymal stem cells induced by myocardial medium under hypoxic conditions. Acta Pharmacol Sin 2006; 27(9): 1153–8. [PubMed: 16923335]

[91]. Zhang Z, Deb A, Zhang Z, et al. Secreted frizzled related protein 2 protects cells from apoptosis by blocking the effect of canonical Wnt3a. J Mol Cell Cardiol 2009; 46(3): 370–7. [PubMed: 19109969]

[92]. Schumann H, Holtz J, Zerkowski HR, Hatzfeld M. Expression of secreted frizzled related proteins 3 and 4 in human ventricular myocardium correlates with apoptosis related gene expression. Cardiovasc Res 2000; 45(3): 720–8. [PubMed: 10728394]

[93]. Shujia J, Haider HK, Idris NM, Lu G, Ashraf M. Stable therapeutic effects of mesenchymal stem cell-based multiple gene delivery for cardiac repair. Cardiovasc Res 2008; 77(3): 525–33. [PubMed: 18032392]

[94]. 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(11): 1139–45. [PubMed: 17967789]

[95]. Wei HJ, Chen CH, Lee WY, et al. Bioengineered cardiac patch constructed from multilayered mesenchymal stem cells for myocardial repair. Biomaterials 2008; 29(26): 3547–56. [PubMed: 18538386]

[96]. Shi RZ, Wang JC, Huang SH, Wang XJ, Li QP. Angiotensin II induces vascular endothelial growth factor synthesis in mesenchymal stem cells. Exp Cell Res 2009; 315(1): 10–5. [PubMed: 18973754]

[97]. Gnecchi M, He H, Noiseux N, et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. Faseb J 2006; 20(6): 661–9. [PubMed: 16581974]

[98]. Ishibe S, Joly D, Zhu X, Cantley LG. Phosphorylation-dependent paxillin-ERK association mediates hepatocyte growth factor-stimulated epithelial morphogenesis. Mol Cell 2003; 12(5): 1275–85. [PubMed: 14636584]

[99]. Wen Y, Zhang XJ, Ma YX, Xu XJ, Hong LF, Lu ZH. Erythropoietin attenuates hypertrophy of neonatal rat cardiac myocytes induced by angiotensin-II in vitro. Scand J Clin Lab Invest 2009; 69(4): 518–25. [PubMed: 19347742]

[100]. Lorenz K, Schmitt JP, Schmitteckert EM, Lohse MJ. A new type of ERK1/2 autophosphorylation causes cardiac hypertrophy. Nat Med 2009; 15(1): 75–83. [PubMed: 19060905]

[101]. Liu XH, Bai CG, Xu ZY, et al. Therapeutic potential of angiogenin modified mesenchymal stem cells: angiogenin improves mesenchymal stem cells survival under hypoxia and enhances vasculogenesis in myocardial infarction. Microvasc Res 2008; 76(1): 23–30. [PubMed: 18462761]

[102]. Li SC, Loudon WG. Stem Cell Therapy for Paediatric Malignant Brain Tumours: The Silver Bullet? ONcology News (UK), June-July 2008, ISSN 1751–4975, 2008; 3(1): 10–4. http://www.oncologynews.biz/pdf/jun_jul_08/ONJJ_stemcell.pdf (Accessed May 4, 2008).

[103]. Hagikura K, Fukuda N, Yokoyama SI, et al. Low invasive angiogenic therapy for myocardial infarction by retrograde transplantation of mononuclear cells expressing the VEGF gene. Int J Cardiol 2010; 142(1): 56–64. [PubMed: 19167769]

[104]. Guo J, Lin GS, Bao CY, Hu ZM, Hu MY. Anti-inflammation role for mesenchymal stem cells transplantation in myocardial infarction. Inflammation 2007; 30(3–4): 97–104. [PubMed: 17497204]

[105]. Fujio Y, Nguyen T, Wencker D, Kitsis RN, Walsh K. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. Circulation 2000; 101(6): 660–7. [PubMed: 10673259]

[106]. Kovacic S, Soltys CL, Barr AJ, Shiojima I, Walsh K, Dyck JR. Akt activity negatively regulates phosphorylation of AMP-activated protein kinase in the heart. J Biol Chem 2003; 278(41): 39422–7. [PubMed: 12890675]

[107]. Shiojima I, Yefremashvili M, Luo Z, et al. Akt signaling mediates postnatal heart growth in response to insulin and nutritional status. J Biol Chem 2002; 277(40): 37670–7. [PubMed: 12163490]

[108]. Taniyama Y, Walsh K. Elevated myocardial Akt signaling ameliorates doxorubicin-induced congestive heart failure and promotes heart growth. J Mol Cell Cardiol 2002; 34(10): 1241–7. [PubMed: 12392981]

[109]. Chimenti I, Smith RR, Li TS, et al. Relative roles of direct regeneration versus paracrine effects of human cardiosphere-derived cells transplanted into infarcted mice. Circ Res 2011; 106(5): 971–80.

[110]. Lee ST, White AJ, Matsushita S, et al. Intramyocardial injection of autologous cardiospheres or cardiosphere-derived cells preserves function and minimizes adverse ventricular remodeling in pigs with heart failure post-myocardial infarction. J Am Coll Cardiol 2011; 57(4): 455–65. [PubMed: 21251587]

[111]. Mazo M, Pelacho B, Prosper F. Stem cell therapy for chronic myocardial infarction. J Cardiovasc Transl Res 2011; 3(2): 79–88.

[112]. Noort WA, Feye D, Van Den Akker F, et al. Mesenchymal stromal cells to treat cardiovascular disease: strategies to improve survival and therapeutic results. Panminerva medica 2011; 52(1): 27–40.

[113]. Soejitno A, Wihandani DM, Kuswardhani RA. Clinical applications of stem cell therapy for regenerating the heart. Acta medica Indonesiana 2011; 42(4): 243–57.

[114]. Strauer BE, Yousef M, Schannwell CM. The acute and long-term effects of intracoronary Stem cell Transplantation in 191 patients with chronic heARt failure: the STAR-heart study. Eur J Heart Fail 2011; 12(7): 721–9.

[115]. Bironaite D, Baltriukiene D, Uralova N, et al. Role of MAP kinases in nitric oxide induced muscle-derived adult stem cell apoptosis. Cell Biol Int 2009; 33(7): 711–9. [PubMed: 19376253]

[116]. Jaquet K, Krause KT, Denschel J, et al. Reduction of myocardial scar size after implantation of mesenchymal stem cells in rats: what is the mechanism? Stem Cells Dev 2005; 14(3): 299–309. [PubMed: 15969625]

[117]. Li H, Yu B, Zhang Y, Pan Z, Xu W, Li H. Jagged1 protein enhances the differentiation of mesenchymal stem cells into cardiomyocytes. Biochem Biophys Res Commun 2006; 341(2): 320–5. [PubMed: 16413496]

[118]. Tang J, Xie Q, Pan G, Wang J, Wang M. Mesenchymal stem cells participate in angiogenesis and improve heart function in rat model of myocardial ischemia with reperfusion. Eur J Cardiothorac Surg 2006; 30(2): 353–61. [PubMed: 16829080]

[119]. Tokcaer-Keskin Z, Akar AR, Ayaloglu-Butun F, et al. Timing of induction of cardiomyocyte differentiation for in vitro cultured mesenchymal stem cells: a perspective for emergencies. Can J Physiol Pharmacol 2009; 87(2): 143–50. [PubMed: 19234578]

[120]. Wang T, Xu Z, Jiang W, Ma A. Cell-to-cell contact induces mesenchymal stem cell to differentiate into cardiomyocyte and smooth muscle cell. Int J Cardiol 2006; 109(1): 74–81. [PubMed: 16122823]

[121]. Xu W, Zhang X, Qian H, et al. Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype in vitro. Exp Biol Med (Maywood) 2004; 229(7): 623–31. [PubMed: 15229356]

[122]. Plotnikov EY, Khryapenkova TG, Vasileva AK, et al. Cell-to-cell cross-talk between mesenchymal stem cells and cardiomyocytes in co-culture. J Cell Mol Med 2008; 12(5A): 1622–31. [PubMed: 18088382]

[123]. Derval N, Barandon L, Dufourcq P, et al. Epicardial deposition of endothelial progenitor and mesenchymal stem cells in a coated muscle patch after myocardial infarction in a murine model. Eur J Cardiothorac Surg 2008; 34(2): 248–54. [PubMed: 18457957]

[124]. Arminan A, Gandia C, Bartual M, et al. Cardiac differentiation is driven by NKX2.5 and GATA4 nuclear translocation in tissue-specific mesenchymal stem cells. Stem Cells Dev 2009; 18(6): 907–18. [PubMed: 18983250]

[125]. Luiken JJ, Ouwens DM, Habets DD, et al. Permissive action of protein kinase C-zeta in insulin-induced CD36-and GLUT4 translocation in cardiac myocytes. J Endocrinol 2009; 201(2): 199–209. [PubMed: 19273501]

[126]. Lian F, Xue S, Gu P, Zhu HS. The long-term effect of autologous endothelial progenitor cells from peripheral blood implantation on infarcted myocardial contractile force. J Int Med Res 2008; 36(1): 40–6. [PubMed: 18230266]

[127]. Muscari C, Bonafe F, Carboni M, et al. Difluoromethylornithine stimulates early cardiac commitment of mesenchymal stem cells in a model of mixed culture with cardiomyocytes. J Cell Biochem 2008; 103(4): 1046–52. [PubMed: 18240140]

[128]. 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(2): 405–20. [PubMed: 17283208]

[129]. Koyanagi M, Brandes RP, Haendeler J, Zeiher AM, Dimmeler S. Cell-to-cell connection of endothelial progenitor cells with cardiac myocytes by nanotubes: a novel mechanism for cell fate changes? Circ Res 2005; 96(10): 1039–41. [PubMed: 15879310]

[130]. Gruh I, Beilner J, Blomer U, et al. No evidence of transdifferentiation of human endothelial progenitor cells into cardiomyocytes after coculture with neonatal rat cardiomyocytes. Circulation 2006; 113(10): 1326–34. [PubMed: 16520414]

[131]. Gerdes HH, Bukoreshtliev NV, Barroso JF. Tunneling nanotubes: a new route for the exchange of components between animal cells. FEBS Lett 2007; 581(11): 2194–201. [PubMed: 17433307]

[132]. Gerdes HH, Carvalho RN. Intercellular transfer mediated by tunneling nanotubes. Curr Opin Cell Biol 2008; 20(4): 470–5. [PubMed: 18456488]

[133]. Gurke S, Barroso JF, Gerdes HH. The art of cellular communication: tunneling nanotubes bridge the divide. Histochem Cell Biol 2008; 129(5): 539–50. [PubMed: 18386044]

[134]. Gurke S, Barroso JF, Hodneland E, Bukoreshtliev NV, Schlicker O, Gerdes HH. Tunneling nanotube (TNT)-like structures facilitate a constitutive, actomyosin-dependent exchange of endocytic organelles between normal rat kidney cells. Exp Cell Res 2008; 314(20): 3669–83. [PubMed: 18845141]

[135]. Gwak SJ, Bhang SH, Yang HS, et al. In vitro cardiomyogenic differentiation of adipose-derived stromal cells using transforming growth factor-beta1. Cell Biochem Funct 2009; 27(3): 148–54. [PubMed: 19319827]

[136]. Karra R, Wu SM. Multipotent stem cells in cardiac regenerative therapy. Regen Med 2008; 3(2): 189–98. [PubMed: 18307403]

[137]. Beguin PC, El-Helou V, Assimakopoulos J, et al. The phenotype and potential origin of nestin(+) cardiac myocyte-like cells following infarction. J Appl Physiol 2009; 107(4): 1241–8. [PubMed: 19679743]

[138]. Padin-Iruegas ME, Misao Y, Davis ME, et al. Cardiac progenitor cells and biotinylated insulin-like growth factor-1 nanofibers improve endogenous and exogenous myocardial regeneration after infarction. Circulation 2009; 120(10): 876–87. [PubMed: 19704095]

[139]. Stamm C, Choi YH, Nasseri B, Hetzer R. A heart full of stem cells: the spectrum of myocardial progenitor cells in the postnatal heart. Ther Adv Cardiovasc Dis 2009; 3(3): 215–29. [PubMed: 19443513]

[140]. Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. Science 2009; 324(5923): 98–102. [PubMed: 19342590]

[141]. Hayashi S, Inoue A. Cardiomyocytes re-enter the cell cycle and contribute to heart development after differentiation from cardiac progenitors expressing Isl1 in chick embryo. Dev Growth Differ 2007; 49(3): 229–39. [PubMed: 17394601]

[142]. Omatsu-Kanbe M, Matsuura H. A novel type of self-beating cardiomyocytes in adult mouse ventricles. Biochem Biophys Res Commun 2009; 381(3): 361–6. [PubMed: 19222989]

[143]. Yanagi K, Takano M, Narazaki G, et al. Hyperpolarization-activated cyclic nucleotide-gated channels and T-type calcium channels confer automaticity of embryonic stem cell-derived cardiomyocytes. Stem Cells 2007; 25(11): 2712–9. [PubMed: 17656646]

[144]. Yang L, Soonpaa MH, Adler ED, et al. Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. Nature 2008; 453(7194): 524–8. [PubMed: 18432194]

[145]. 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(6): 1137–50. [PubMed: 17123591]

[146]. Wu SM, Chien KR, Mummery C. Origins and fates of cardiovascular progenitor cells. Cell 2008; 132(4): 537–43. [PubMed: 18295570]

[147]. Christoffels VM, Mommersteeg MT, Trowe MO, et al. Formation of the venous pole of the heart from an Nkx2–5-negative precursor population requires Tbx18. Circ Res 2006; 98(12): 1555–63. [PubMed: 16709898]

[148]. Prall OW, Menon MK, Solloway MJ, et al. An Nkx2–5/Bmp2/Smad1 negative feedback loop controls heart progenitor specification and proliferation. Cell 2007; 128(5): 947–59. [PubMed: 17350578]

[149]. Aghila Rani KG, Jayakumar K, Srinivas G, Nair RR, Kartha CC. Isolation of ckit-positive cardiosphere-forming cells from human atrial biopsy. Asian Cardiovasc Thorac Ann 2008; 16(1): 50–6. [PubMed: 18245707]

[150]. Chamuleau SA, Vrijsen KR, Rokosh DG, Tang XL, Piek JJ, Bolli R. Cell therapy for ischaemic heart disease: focus on the role of resident cardiac stem cells. Neth Heart J 2009; 17(5): 199–207. [PubMed: 19484156]

[151]. Li Z, Lee A, Huang M, et al. Imaging survival and function of transplanted cardiac resident stem cells. J Am Coll Cardiol 2009; 53(14): 1229–40. [PubMed: 19341866]

[152]. Scobioala S, Klocke R, Kuhlmann M, et al. Up-regulation of nestin in the infarcted myocardium potentially indicates differentiation of resident cardiac stem cells into various lineages including cardiomyocytes. Faseb J 2008; 22(4): 1021–31. [PubMed: 17984177]

[153]. Tallini YN, Greene KS, Craven M, et al. c-kit expression identifies cardiovascular precursors in the neonatal heart. Proc Natl Acad Sci USA 2009; 106(6): 1808–13. [PubMed: 19193854]

[154]. Torella D, Ellison GM, Mendez-Ferrer S, Ibanez B, Nadal-Ginard B. Resident human cardiac stem cells: role in cardiac cellular homeostasis and potential for myocardial regeneration. Nat Clin Pract Cardiovasc Med 2006; 3 (Suppl 1): S8–13. [PubMed: 16501638]

[155]. Yu XY, Geng YJ, Li XH, et al. The effects of mesenchymal stem cells on c-kit up-regulation and cell-cycle re-entry of neonatal cardiomyocytes are mediated by activation of insulin-like growth factor 1 receptor. Mol Cell Biochem 2009; 332(1–2): 25–32. [PubMed: 19507001]

[156]. Cebotari S, Mertsching H, Kallenbach K, et al. Construction of autologous human heart valves based on an acellular allograft matrix. Circulation 2002; 106(12 Suppl 1): I63–I8. [PubMed: 12354711]

[157]. Eitan Y, Sarig U, Dahan N, Machluf M. Acellular cardiac extracellular matrix as a scaffold for tissue engineering: In-vitro cell support, remodeling and biocompatibility. Tissue Eng Part C Methods 2010; 16(4): 671–83. [PubMed: 19780649]

[158]. Elder BD, Eleswarapu SV, Athanasiou KA. Extraction techniques for the decellularization of tissue engineered articular cartilage constructs. Biomaterials 2009; 30(22): 3749–56. [PubMed: 19395023]

[159]. Iop L, Renier V, Naso F, et al. The influence of heart valve leaflet matrix characteristics on the interaction between human mesenchymal stem cells and decellularized scaffolds. Biomaterials 2009; 30(25): 4104–16. [PubMed: 19481252]

[160]. Knight RL, Wilcox HE, Korossis SA, Fisher J, Ingham E. The use of acellular matrices for the tissue engineering of cardiac valves. Proc Inst Mech Eng H 2008; 222(1): 129–43. [PubMed: 18335724]

[161]. Mirsadraee S, Wilcox HE, Watterson KG, et al. Biocompatibility of acellular human pericardium. J Surg Res 2007; 143(2): 407–14. [PubMed: 17574597]

[162]. Schmidt CE, Baier JM. Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering. Biomaterials 2000; 21(22): 2215–31. [PubMed: 11026628]

[163]. Tedder ME, Liao J, Weed B, et al. Stabilized collagen scaffolds for heart valve tissue engineering. Tissue Eng Part A 2009; 15(6): 1257–68. [PubMed: 18928400]

[164]. Tudorache I, Cebotari S, Sturz G, et al. Tissue engineering of heart valves: biomechanical and morphological properties of decellularized heart valves. J Heart Valve Dis 2007; 16(5): 567–73; discussion 74. [PubMed: 17944130]

[165]. Wainwright JM, Czajka CA, Patel UB, et al. Preparation of Cardiac Extracellular Matrix from an Intact Porcine Heart. Tissue Eng Part C Methods 2010; 16(3): 525–32. [PubMed: 19702513]

[166]. Yang M, Chen CZ, Wang XN, Zhu YB, Gu YJ. Favorable effects of the detergent and enzyme extraction method for preparing decellularized bovine pericardium scaffold for tissue engineered heart valves. J Biomed Mater Res B Appl Biomater 2009; 91(1): 354–61. [PubMed: 19507136]

[167]. Ye X, Zhao Q, Sun X, Li H. Enhancement of mesenchymal stem cell attachment to decellularized porcine aortic valve scaffold by in vitro coating with antibody against CD90: a preliminary study on antibody-modified tissue-engineered heart valve. Tissue Eng Part A 2009; 15(1): 1–11. [PubMed: 18759669]

[168]. Jo J, Nagaya N, Miyahara Y, et al. Transplantation of genetically engineered mesenchymal stem cells improves cardiac function in rats with myocardial infarction: benefit of a novel nonviral vector, cationized dextran. Tissue Eng 2007; 13(2): 313–22. [PubMed: 17518565]

[169]. Stock UA, Schenke-Layland K. Performance of decellularized xenogeneic tissue in heart valve replacement. Biomaterials 2006; 27(1): 1–2. [PubMed: 16026824]

[170]. Rieder E, Kasimir MT, Silberhumer G, et al. Decellularization protocols of porcine heart valves differ importantly in efficiency of cell removal and susceptibility of the matrix to recellularization with human vascular cells. J Thorac Cardiovasc Surg 2004; 127(2): 399–405. [PubMed: 14762347]

[171]. Schenke-Layland K, Vasilevski O, Opitz F, et al. Impact of decellularization of xenogeneic tissue on extracellular matrix integrity for tissue engineering of heart valves. J Struct Biol 2003; 143(3): 201–8. [PubMed: 14572475]

[172]. Schenke-Layland K, Opitz F, Gross M, et al. Complete dynamic repopulation of decellularized heart valves by application of defined physical signals-an in vitro study. Cardiovasc Res 2003; 60(3): 497–509. [PubMed: 14659795]

[173]. Ott HC, Matthiesen TS, Brechtken J, et al. The adult human heart as a source for stem cells: repair strategies with embryonic-like progenitor cells. Nat Clin Pract Cardiovasc Med 2007; 4 Suppl 1: S27–39. [PubMed: 17230213]

[174]. Ott HC, Matthiesen TS, Goh SK, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med 2008; 14(2): 213–21. [PubMed: 18193059]

[175]. Forte G, Carotenuto F, Pagliari F, et al. Criticality of the biological and physical stimuli array inducing resident cardiac stem cell determination. Stem Cells 2008; 26(8): 2093–103. [PubMed: 18499898]

[176]. Niebruegge S, Bauwens CL, Peerani R, et al. Generation of human embryonic stem cell-derived mesoderm and cardiac cells using size-specified aggregates in an oxygen-controlled bioreactor. Biotechnol Bioeng 2009; 102(2): 493–507. [PubMed: 18767184]

[177]. Li SC, Loudon WG. A novel and generalizable organotypic slice platform to evaluate stem cell potential for targeting pediatric brain tumors. Cancer Cell Int 2008; 8: 9. [PubMed: 18498656]

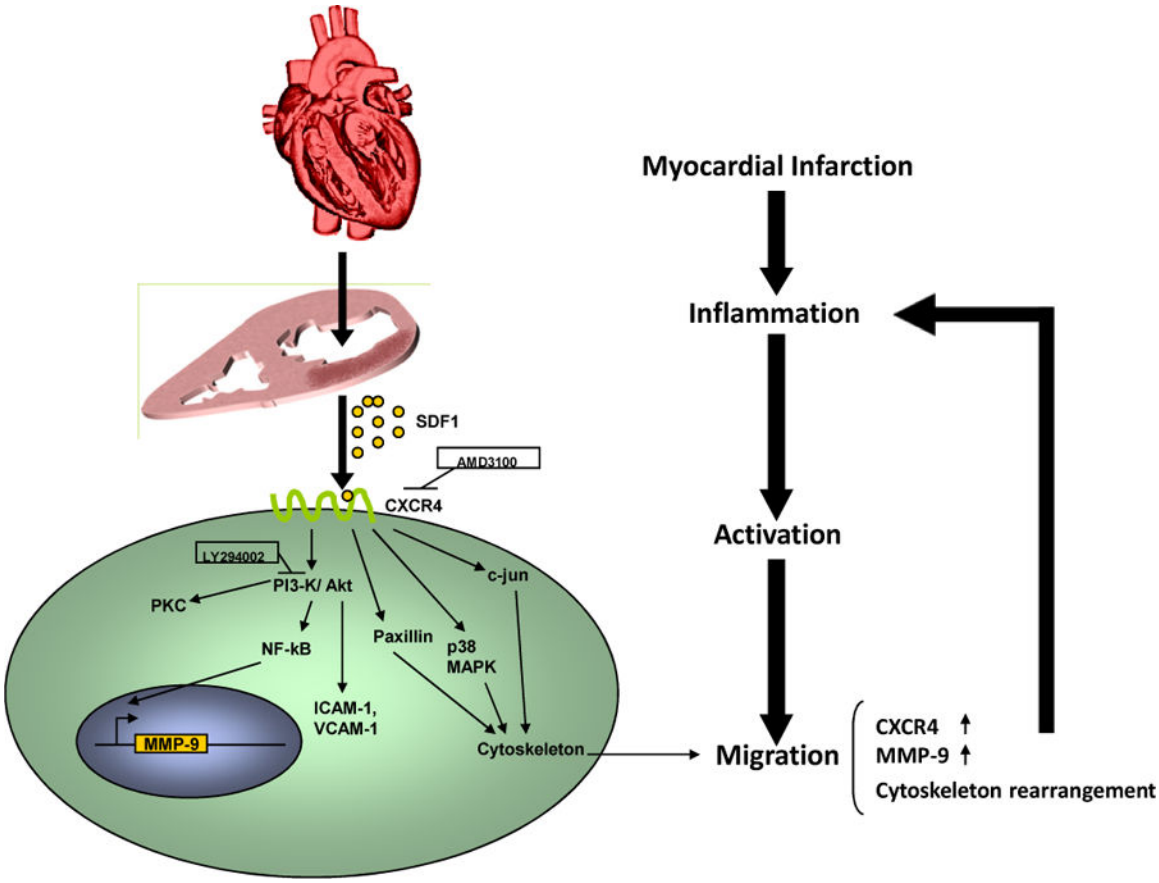

Fig. (1).

The PI3K/Akt signal pathway is crucial to SDF1/CXCR-mediated homing of endothelial progenitor cells (EPCs) after myocardiac infarction. Ischemia-associated cell death triggered by myocardiac infarction initiates an immune response by producing various cytokines, such as SDF-1. SDF-1 activates its corresponding receptor in circulating EPCs *via* activation of CXCR4. This binding in turn mediates the PI-3K/AKT signaling pathway that induces the progenitor cell migration toward myocardial infarcts to initiate the tissue repair by increasing CXCR4 and MMP-9 as well as cytoskeleton rearrangement. Either AMD3100, an inhibitor for CXCR4, or LY294002, an inhibitor for PI3K, can downregulate this signaling event.

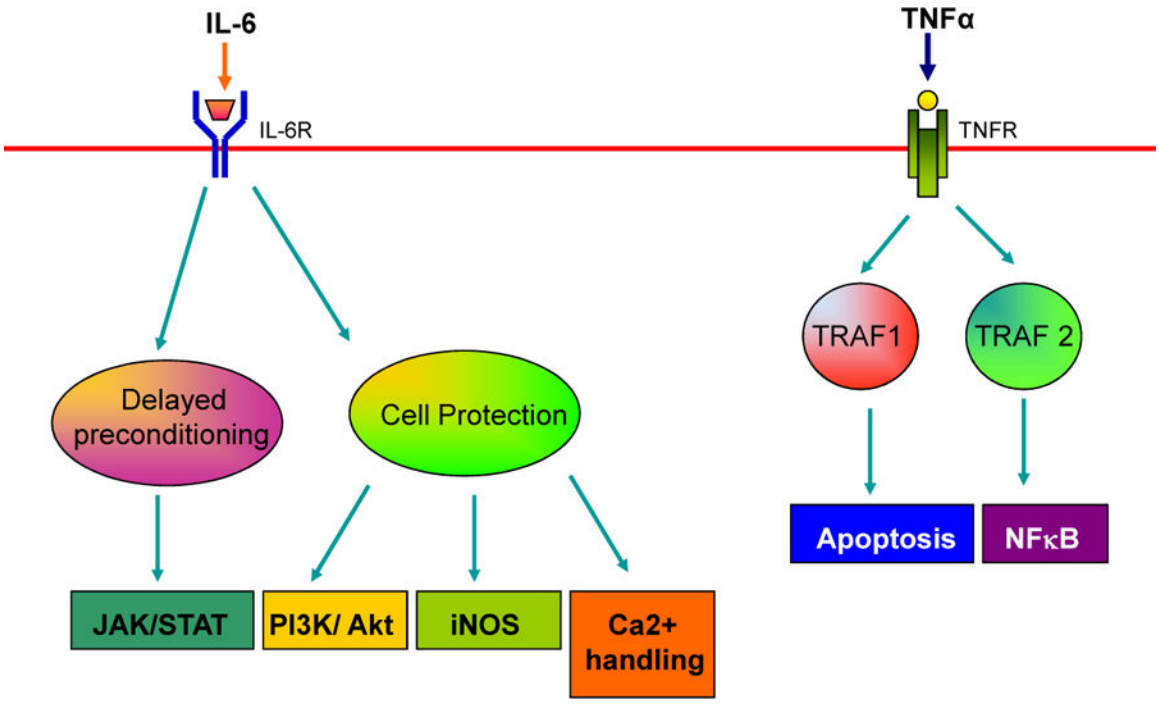

Fig. (2).

Ischemia-associated cell death triggers an immune response that produces various cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFα). Cytokine-mediated signaling pathways modulate precursor cellular activity. IL-6 triggers preconditioning for precursor cell migration and cell protection while TNFα promotes apoptosis and may facilitate the survival and engraftment of precursor cells.

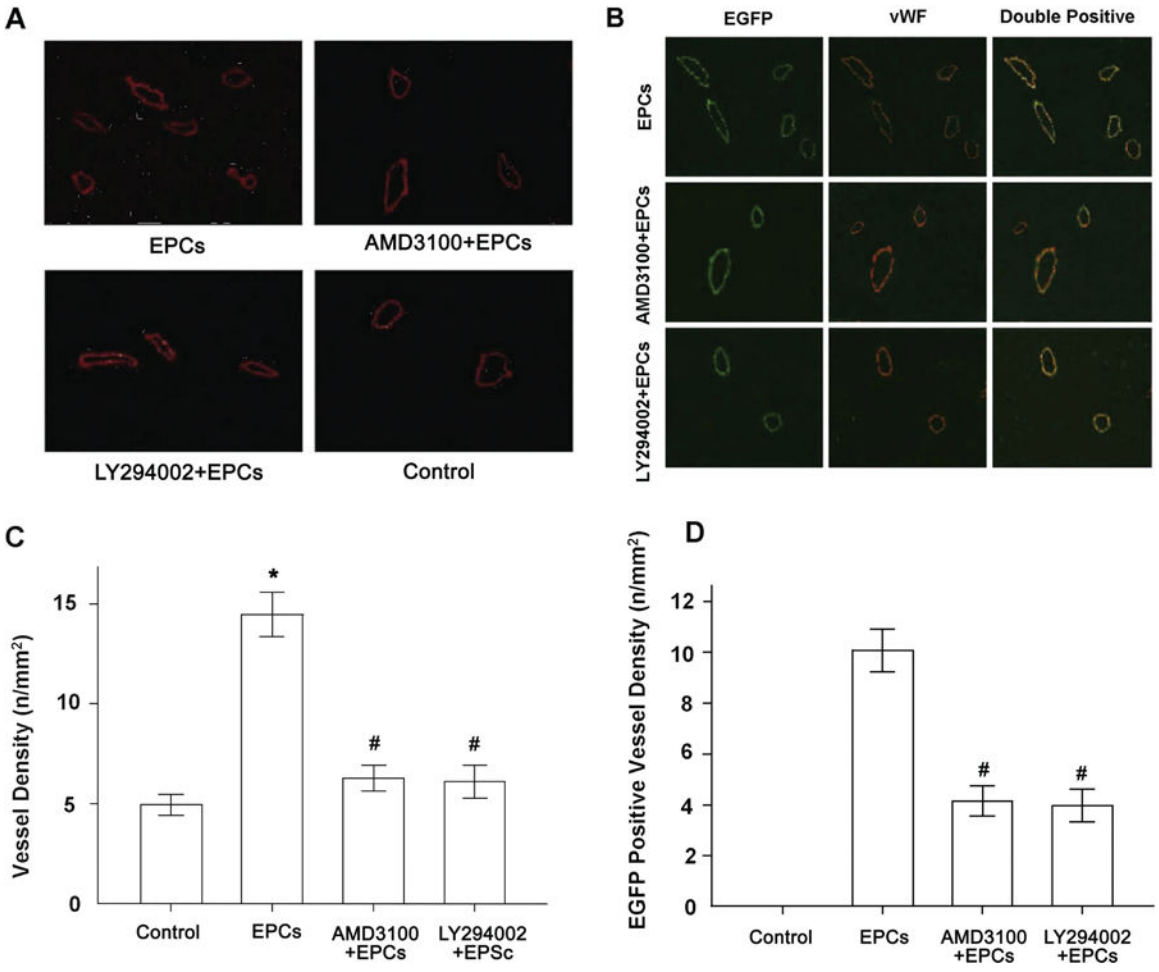

Fig. (3).

In the rat myocardiac infarction model, neovascularization activity is induced. Endothelial precursor cells (EPCs) were implanted *via* tail vein injection 24 hours after coronary left anterior coronary artery ligation in rats. Vessel density is determined by staining with vWF 14 days after EPC injection (original magnification, 200×).

A. Immunofluorescence staining of vWF (red) shows the vessel density 14 days after EPC injection.

B. 14 days after EPC injection, EGFP (green) shows that EPCs home to the peri-infarct region with formation of lumen-like structures. vWF staining confirms that the lumen-like structures are new vessels.

C. Quantitative analysis of vessel density. Briefly, EPCs pretreated with AMD3100 (10 μg/ml) or LY294002 (20 μM) for 30 minutes. 5×10^6 EPCs in 1ml EBM-2 or 1 ml EBM-2 were injected *via* tail vein 24 hours after left anterior coronary artery ligation in rats. Control vs EPCs vs AMD3100+EPCs vs LY294002+EPCs: 4.93±0.64 vs 14.47±1.37 vs 6.27±0.82 vs 6.10±1.02 n/mm^2. *$p<0.05$ vs control, #$p<0.05$ vs EPCs group. There was no statistical difference between the AMD3100+EPCs and LY294002+EPCs group.

D. Quantitavive analysis of EGFP-positive vessel density. There were no EGFP-positive vessels in the control group. EPCs vs AMD3100+EPCs vs LY294002+EPCs: 10.07±1.03 vs

4.13 ±0.73 vs 3.97±0.78 n/mm^2. #p<0.05 vs EPCs group. There was no statistical difference between the AMD3100+EPCs and LY294002+EPCs group.

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Table 1.

Selected Studies of Transplanted Stem Cells for Cardiac Repair

Cell Source/ Type	Donor/Host Animal	# of Cells Injected	Delivery Method	Cell Survival Time	% Survival	Phenotype	Regenerative Advantage Conferred Over Vehicle	Authors & Year
EPC	Pig	2.5×10^6	intracoronary infusion	4 weeks	20%	ND	sp-FGF1-modified EPCs induced neovascularization	Chen *et al.*, 2009
MSC	Rat	6×10^6	injected at six injection sites	6 weeks	n=10, n= 9, n= 6	Endothelial,	Bcl-2 gene resulted in high-level VEGF expression in response to the hypoxic condition, improved LV remodeling and function	Li *et al.*, 2007
MSC	Rat	5×10^6 in 0.1 ml	6 sites laterally and anterior of ischemic tissue	>6 weeks	ND	ND	Angiogenin enhanced MSCs promoted scar repopulation, angiogenesis, and prevented LV remodeling	Liu *et al.*, 2008
MSC	Rat	3×10^6	Intramyocardial injection	3 months	-	Cardiomyocytes, smooth muscle	MSC co-overexpression of Ang-1 and Akt incorporate into scar tissue and increase neovascularization	Shujia *et al.*, 2008
MSC	Rat	2.5×10^6	Tail vein injection	3 days	200 cells/ high-power field	Endothelial	CXCR4-MSCs showed increased homing ability into ischemic tissue and increased end diastolic pressure	Cheng *et al.*, 2008
MSC	Rat	2×10^6	Tail Vein injection	4days to 5 weeks	130 cells/ mm^2 60 cells/ mm^2	None observed	SDF1-MSCs protect existing cardiomyocytes from apoptosis and improve electrical conduction in scar tisssue	Zhang (MING) *et al.*, 2008
MSC	Rat	1×10^6	Aortic root	28 days	ND	ND	left and right ventricular pressure overload	Molina *et al.*, 2008

Table 2.

Clinical Trials Using Stem Cells for Treatment of Injured Cardiac Tissues to Improve Heart Function in Patients (Refer to http://clinicaltrials.gov/ct2/results?term=msc+heart)

Clinical Trials (gov ID)	Phase	Study Type	Target Heart Disease	Cell Type	Sponsor	Administration Route
NCT00587990	I/ II	Interventional	Left Ventricular Dysfunction	MSC	National Heart, Lung, and Blood Institute	Intramyocardial Injection
NCT00114452	I	Interventional	Myocardial Infarction	MSC	Osiris Therapeutics	Provacel™ (PUMP1)
NCT00768066	I/ II	Interventional	Heart Failure/ Left Ventricular Dysfunction	MSC	University of Miami	Transendocardial Injection
NCT00308633	-	Observational	Coronary Artery Disease	EPC	National Heart, Lung, and Blood Institute	-
NCT00053456	-	Observational	Coronary Arteriosclerosis	EPC	National Heart, Lung, and Blood Institute	-
NCT00644410	I/ II	Interventional	Congestive Heart Failure	MSC	Rigshospitalet, Denmark	Intramyocardial Injection