This Review is part of a thematic series on Cellular Therapy, which includes the following articles:
The Stem Cell Movement
Aging and Disease as Modifiers of Efficacy of Cell Therapy

Genetic Enhancement of Stem Cell Engraftment, Survival, and Efficacy

Paracrine Signaling in Cell Transplantation
Assessment and Optimization of Cell Engraftment After Transplantation
Immune Biology of Stem Cells
Cardiogenic Differentiation and Transdifferentiation of Stem Cells
Stem Cell Homing to Sites of Injury
Regulatory Considerations in Cell Transplantation

Eduardo Marbán, Editor

Genetic Enhancement of Stem Cell Engraftment, Survival, and Efficacy
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Abstract—Cell-based therapies for the prevention and treatment of cardiac dysfunction offer the potential to significantly modulate cardiac function and improve outcomes in patients with cardiovascular disease. To date several clinical studies have suggested the potential efficacy of several different stem cell types; however, the benefits seen in clinical trials have been inconsistent and modest. In parallel, preclinical studies have identified key events in the process of cell-based myocardial repair, including stem cell homing, engraftment, survival, paracrine factor release, and differentiation that need to be optimized to maximize cardiac repair and function. The inconsistent and modest benefits seen in clinical trials combined with the preclinical identification of mediators responsible for key events in cell-based cardiac repair offers the potential for cell-based therapy to advance to cell-based gene therapy in an attempt to optimize these key events in the hope of maximizing clinical benefit. Below we discuss potential key events in cardiac repair and the mediators of these events that could be of potential interest for genetic enhancement of stem cell–based cardiac repair. (Circ Res. 2008;102:1471-1482.)

Key Words: gene transfer ■ myocardial repair ■ stem cells ■ heart failure ■ acute myocardial infarction

Cell based therapies for the prevention or treatment of cardiac dysfunction have received significant interest given the aging population and the increasing prevalence of patients with chronic heart failure. Whereas early data suggested that the delivery of adult stem cells led to the regeneration of myocardial tissue including endothelium, new vasculature, and new contractile and functional cardiac myocytes correctly oriented on a fibrous skeleton,1,2 more recent data would suggest that the process is not quite as simple as previously thought.3-5 Although the notion that endothelial cells are renewed by a population of bone marrow–derived progenitor cells has gained traction,6 the idea that cardiac myocytes can be similarly regenerated continues to be vigorously debated. Although a population of resident cardiac stem cells has been demonstrated,7-10 unless harnessed, its reparative capacity remains muted.
An indication of how little consensus there is in this fledgling field is suggested by the sheer number of cell types that are being investigated as the "magic bullet" to provide reparative capacity to the heart. A recent review reminds us that the cell types currently under clinical investigation include: Bone marrow mononuclear cells, circulating progenitor cells, CD133+ cells, CD34+ cells, skeletal myoblasts, mesenchymal stem cells, endothelial progenitor cells, and bone marrow–derived cells.11 The lack of appropriate and specialized markers, cell-surface marker characterization, coupled with the near complete lack of standardized nomenclature, contributes to the overwhelming confusion in this field. One man’s bone marrow–derived cell may very well be another’s mesenchymal stem cell! If the varieties of cell types being investigated in basic science research laboratories such as embryonic stem cells, fetal cardiac myocytes, cells generated from somatic cell nuclear transfer, neonatal cardiac myocytes were involved in this discussion, it would be very difficult to sort through any potentially realistic therapeutic target.

Furthermore, there is mounting data to suggest that the benefits of adult cell therapy for cardiac repair are less related to direct cellular participation in cardiac regeneration and more attributable to paracrine effects that lead to enhanced ability of native cardiac myocytes to resist death in the setting of ischemic insults and improved remodeling.12–15 Proponents of this theory have argued forcefully that the magnitude of cardiac repair seen in cell transplant experiments cannot be explained by cell transplantation alone. The volume of myocardium lost, and repaired, cannot be explained solely by numbers of cells being transplanted into the damaged area of the heart. This argument is particularly forceful when large numbers (sometimes upwards of 90%) of transplanted cells are lost during the injection process, and after implantation into a hostile substrate with little to no extracellular matrix, supporting cells or tissues, oxygen or nutritive substrates, but with a massive excess of free-radicals, neutrophils, and scavenger cells.

However, the results from clinical trials to date suggest that adult cell therapy for the prevention and treatment of cardiac dysfunction is safe and potentially efficacious15–18 and that a small and consistently measurable extent of improvement is seen after cell transplantation alone. Although the mechanisms behind such improvements are poorly understood, one means by which better results could be accomplished might be to deliver massive excesses of cells into the target area of the injured heart. This experiment, however, to the best of our knowledge has not been done; and the limitation in this regard clearly is obtaining large amounts of homogenous and well characterized cells. It has been estimated that more than 100 billion adult cardiac myocytes would be required to reconstruct the human left ventricular free wall after a massive myocardial infarction. For biologists, these numbers are daunting. One means to circumvent this limitation is by potentially using cells as local “drug delivery” units. Small numbers of specifically and uniquely designed cells may be able to exploit paracrine pathways that have been worked out so as to maximize the biological and clinical effects after cell delivery.12,14,19–24 In doing so, resident populations of stem cells that are recruited to the injured area and participate in limited degrees of repair may be recruited in far greater numbers and may actually perform clinically meaningful and relevant degrees of repair.

The therapeutic gene targets are almost too limitless to fathom, but certain broad categories can be sketched out. Cell survival, homing, migration, engraftment, efficiency, deposition of matrix, and production of factors that recruit endogenous progenitor type cells.

What remains to be determined is what cell type would be used to this end. Which is most amenable to gene therapy? As the Table shows, several cell types are of interest. Importantly, to date, only mesenchymal stem cells (MSCs) and multipotent adult progenitor cells have been shown to have efficacy in the allogeneic setting without the need for immunosuppressive agents.25,26 However, because cell-based gene therapy has the potential to harness the effects of gene therapy combined with cell-based regenerative medicine, through the genetic modification of self-renewing and replicating cells that can be found in the adult animal, a detailed understanding of the cell types involved is important. The features listed in the Table relate to their ability to be used in autologous or allogeneic strategies; their ability to participate in angiogenesis or vasculogenesis, their ability to home to injured myocardium, their ability to survive long-term after engraftment in newly injured myocardium; whether they have been shown to have any clinical efficacy to date, and whether investigators have begun genetically engineering these cells in preclinical trials.

**Endothelial Progenitor Cells for Vascular Reendothelialization**

Endothelial progenitor cells (EPCs) arise from bone marrow–derived hemangioblasts and can be mobilized into peripheral blood in response to ischemia or cytokines. Thus, EPCs...
provide a scalable source of autologous endothelial cells for therapeutic purposes. Our group has demonstrated that local administration of EPCs results in rapid reendothelialization of the denuded arterial bed with 85% coverage at day 7 and 70% to 80% at day 14. Kaushal et al have shown that EPCs can be stably seeded onto decellularized porcine iliac artery grafts, which then gain the ability to produce NO, enabling relaxation and contraction of the neovessel.2 If such a strategy can restore endothelial function vessels treated this way it could theoretically demonstrate reduced graft thrombosis and stenosis. Genetic modification of EPCs can yield endothelial cells with enhanced functions such as antithrombotic, profibrinolytic, and antiinflammatory activities. Furthermore, such grafts could theoretically function as “organoids” in which modified endothelial cells produce gene products with paracrine or endocrine actions.

Endothelial Progenitor Cells and Angioblasts for Angiogenesis

Although human EPCs are mobilized from the bone marrow during ischemic episodes, endogenous reserves may not always provide a critical mass capable of rescuing tissue from ischemic injury. However, augmenting circulating or local levels of cells has proven to be a successful therapeutic strategy. Systemic intravenous injection of human angioblasts into ischemic nude athymic rat hearts resulted in an increase in capillary density, which in turn, protected against cardiomyocyte apoptosis, decreased local collagen deposition, and improved cardiac function.28 In separate experimental studies, EPCs engineered to overexpress VEGF demonstrated improved proliferative and adhesive capabilities both in vitro and in vivo. Ischemic hind-limbs treated with these VEGF-EPCs demonstrated improved blood flow and less limb loss when compared to limbs treated with Lac-Z-transduced EPC.29 A strategy including genetic modification may hold additional promise for therapeutic angiogenesis, or for antiangiogenesis (eg, in antitumorigenic therapy).

Myocardial Regeneration Using Stem Cells

The damaged heart responds to the loss of functional tissue by undergoing remodeling, which involves replacement of infarcted tissue by fibrous scar and compensatory hypertrophy of surviving myocytes. Cardiomyocytes are unable to more than double in size before they succumb to eventual exhaustion, and although the mitotic index of cardiomyocytes in the border zone increases after infarction their replicative potential is limited and of little therapeutic value. Persistent increased wall stress results in pathological alteration of ventricular geometry and perpetuates progressive loss of cardiomyocytes, eventually leading to cardiac failure. It has been hypothesized that repopulating the damaged zone with contractile cells, coupled with appropriate matrix modulation, may normalize the hemodynamic load on the surviving cardiomyocytes, thereby avoiding the deleterious consequences of ventricular remodeling.

Although myocardial skeletal myoblast transplantation has been reported to improve both systolic and diastolic myocardial function, the cells are incapable of electro-mechanical coupling with native cardiomyocytes, and proliferate in uncontrolled fashion. These asynchronous islands of intramyocardial skeletal muscle can result in lethal arrhythmias in mice31–33 and can distort intraventricular geometry.34 Despite these potential limitations, a safety and feasibility study of skeletal myoblast transplantation for human heart failure has been undertaken in an uncontrolled, open-label fashion. Furthermore the randomized placebo controlled MAGIC trial was recently published showing a decrease in left ventricular end-systolic volume in those patients who received skeletal myoblast injections along with coronary artery bypass grafting compared to those that received revascularization along.35 Importantly, overall there was no difference in episodes of ventricular tachycardia over time.

Although cell types such as fetal cardiomyocytes and cardiomyocytes derived from murine or human embryonic stem cells are capable of electromechanical coupling, their clinical use has unfortunately been hampered by technical, ethical, moral, social, and legal hurdles.

Over the last 3 years, the existence of cardiac myocyte precursor cells in the bone marrow of adult animals has received a lot of attention. The potential of harnessing this population for an autologous therapeutic strategy involving cardiac regeneration has great appeal for obvious reasons. Transplantation of bone marrow–derived cells into the heart, with or without pretreatment with 5-azacytidine, has been reported to augment ventricular function.36 The precise identity and lineage of the cell type capable of myogenesis remains unclear. Jackson et al have demonstrated that the hematopoietic progenitor SP population is capable of participating in cardiac regeneration.37 Orlic et al have demonstrated that c-kit+ cells can be isolated from the bone marrow and participate in angiogenesis, vasculogenesis, and myogenesis when injected into the ischemic murine heart.1 Furthermore, this group has demonstrated that these cells are mobilized from the bone marrow after systemic administration of granulocyte colony stimulating factor and stem cell factor and “home” to the myocardium, where they induce myocardial repair after acute infarction, and reduce mortality.2 The bone marrow origin of this cell, and its ability to migrate into the heart, has been validated by the demonstration of Y-chromosome labeled cardiomyocytes and resident c-kit+ cells in hearts transplanted from female donors to male recipients.38 Mangi et al have characterized a highly purified population of MSCs harvested from the bone marrow of adult animals, which is easily expandable and scalable. These cells are amenable to ex vivo genetic manipulation and induces recovery of cardiac function after myocardial infarction by differentiating into cardiomyocytes in vivo.19 Thus, genetic engineering of MSCs may be an important strategy to yield myocytes with enhanced functions for therapeutic cell transplantation.

Several unknown issues need to be investigated before we believe that human cardiac stem cell transplantation can be safely started. These questions include: What percent of transplanted stem cells survive transplantation, remain viable, and successfully engraft? What is the proliferative and regenerative capacity of the transplanted cells? What are the local myocardial signals and mediators of homing, trafficking, proliferation, and differentiation of these cells? What is
the optimal timing of transplantation—that is, is it better to transplant during the acute ischemic event or several weeks thereafter? What are the relative contributions of CD34+ and CD34- cells to angiogenesis and myogenesis? Do angiogenesis and myogenesis complement one another? What are the most appropriate criteria to evaluate long-term clinical benefit of myocardial stem cell transplantation? Do regenerated cardiomyocytes induce primarily systolic or diastolic improvement, or both? Is this approach clinically scalable, reproducible, and safe?

Figure 1 is one proposed conceptualization on how cardiovascular cell therapy may develop over time in several different cell types, and attempts to place where cell-based gene therapy may fit over time. Based on this proposal, the field is currently in Phase I where unmodified adult stem cells are being delivered to patients in the peri-infarct period or with ischemic cardiomyopathy. Important, there are undoubtedly multiple relevant pathways involved in the efficacy of any cell type. Furthermore, the relevant pathways for a given cell type can be significantly different between the peri-infarct period and ischemic cardiomyopathy. In Figure 2, we have categorized the targets that can potentially be manipulated via cell-based gene therapy in an attempt to optimize the results of stem cell therapy. The processes of interest include the homing of stem cells to the organ of interest; the migration of the stem cells through the injured organ; the engraftment of the stem cells at the site of injury; and then the differentiation of the stem cell to an end-organ cell (eg, cardiac myocyte) or release of paracrine factors that result in end-organ cell survival or improved tissue remodeling. Many of these processes are the topic of other articles in this series; below we focus our discussion specifically on how cells have been or could be genetically engineered to optimize each process.

Finally, Phase 3 as conceptualized in Figure 1 will involve the use of totipotent stem cells including embryonic stem cells or adult derived embryonic like stem cells. Using these cells it is likely we will be able to achieve a significant and reproducible level of myocardial tissue regeneration including blood vessels and cardiac myocytes. It is of course a presumption that as we achieve greater levels of regeneration we will simultaneously achieve greater levels of efficacy. That said, the knowledge and clinical strategies potentially developed in Phase 2 will still be highly relevant, as we will likely always strive to optimize the functional aspects of the native tissue along the way to introducing regenerated tissue.

Stem Cell Homing

Several chemokines and growth factors have been shown to be responsible for stem cell homing to the myocardium including: Stromal-cell derived factor-1 (SDF-1α), monocyte chemotactant protein-3 (MCP-3), hepatocyte growth factor (HGF) fibroblast growth factor-2 (FGF-2) and insulin growth factor-1 (IGF-1). These different stem cell homing factors recruit different populations of stem cells. SDF-1 recruits CXCR4 expressing stem cells including hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), cardiac stem cells (CSCs) and CXCR4 expressing MSCs. MCP-3 homes MSCs, GRO-1 homes bone marrow–derived EPCs, and FGF-2, HGF, and IGF-1 activate CSCs. Furthermore, VEGF overexpression can recruit VEGFR2-positive cells, angiogenesis through local endothelial cell proliferation. The transient overexpression of VEGF-165 in skeletal myoblasts in a cell-based gene therapy strategy has been shown to increase vascular density and cardiac function.

In the setting of acute myocardial infarction, many of these stem cell homing factors are expressed leading to varying extents of stem cell homing to the newly injured tissue obviating the need to engineer cells to express these factors in the period immediately after a myocardial infarction. However, many of these stem cell homing factors are expressed for a short period of time after myocardial infarction. For example, SDF-1 expression is expressed by the injured period immediately after a myocardial infarction. How-ever, many of these stem cell homing factors are expressed for a short period of time after myocardial infarction. For example, SDF-1 expression is expressed by the injured myocardium less than 1 week after myocardial infarction and FGF-2, HGF, and IGF-1 activate CSCs.45–48 Furthermore, VEGF overexpression can recruit VEGFR2-positive cells, angiogenesis through local endothelial cell proliferation. The transient overexpression of VEGF-165 in skeletal myoblasts in a cell-based gene therapy strategy has been shown to increase vascular density and cardiac function.

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fer, leading to prolongation, or re-establishment of SDF-1 expression at times late after myocardial infarction leads to stem cell homing, an increase in vascular density, activation and homing of cardiac stem cells, and improvement in cardiac function. Furthermore, engineering of MSCs or HSCs to overexpress SDF-1 receptor CXCR4 similarly leads to greater homing of engineered MSCs and improved left ventricular function compared to control MSCs or HSCs when the cells were delivered within 24 hours of myocardial infarction.55–57

Thus, engineering cells to induce the expression of stem cell homing factors in myocardial tissue can lead to reestablishment or prolongation of stem cell homing of bone marrow–derived and cardiac stem cells to the injured myocardium. Conversely, overexpressing the receptor of relevant stem cell homing factors in exogenously delivered stem cells can increase the homing of stem cells to the myocardium.

Stem Cell Migration and Engraftment
Several studies have focused on strategies to optimize stem cell migration through injured myocardial tissue. Specific areas of focus are the role of adhesion molecules and integrins in the mobilization and engraftment of stem cells, proteases in modulating stem cell migration through injured myocardial tissue, and modulation of the connective tissue microenvironment to improve stem cell engraftment.

Proteases of Interest
Several proteases have been identified to have significant effects on stem cell mobilization or stem cell migration and engraftment in cardiac tissue. Many of these proteases are potential targets for gene based modulation of stem cells before transplantation.

Inhibitors of protease activation such as PAI-1 have been shown to have significant effects on leukocyte infiltration and left ventricular remodeling. Uprogulation of PAI-1 expression leading to decreased tissue plasminogen activity results in decreased leukocyte infiltration, tissue degradation, and decreased left ventricular dilation. Alternatively, downregulation of PAI-1 levels and increased tissue plasmin activity through the delivery of a sequence-specific catalytic deoxyribozyme targeting PAI-1 at the time of acute myocardial infarction has been shown to increase the engraftment of exogenously delivered CD34+ cells in the infarct zone. The increase in stem cell engraftment was attributable to enhanced vitronectin-dependent transendothelial migration of the human bone marrow–derived CD34+ cells from the blood stream into the infarct zone. This increase in stem cell engraftment after PAI-1 inhibition after acute myocardial infarction has recently been shown to be associated with a decrease in cardiac myocyte apoptosis and an increase in cardiac function.

Endothelial nitric oxide synthase (eNOS) expression has been shown to be linked to multiple responses that enhance the effects of cell therapy after acute myocardial infarction. With respect to stem cell migration and engraftment, eNOS expression has been shown to be critical for the mobilization of stem cells. eNOS expression leads to an upregulation of MMP-9, leading to an increase in stem cell mobilization at the time of acute myocardial infarction. Interestingly, eNOS-mediated MMP-9 upregulation has been shown to be estradiol dependent, suggesting a potential explanation for gender differences after acute myocardial infarction. Similarly, HMG-CoA reductase inhibitors, which lead to an increase in eNOS expression, have been shown to increase stem cell mobilization, suggesting a potential pleuripotent mechanism for the benefits of statin therapy observed in patients with acute myocardial infarction.

Mediators of Migration
As discussed above, the overexpression of the receptors of stem cell homing factors like CXCR4 can improve the migratory and engraftment of stem cells after infusion in the peri-infarct period. Other strategies that achieve similar effects have been studied. Uprogulation of eNOS expression has also been shown to improve the migratory capacity of bone marrow–derived stem cells. Stem cells from patients with coronary artery disease have been shown to have decreased migratory capacity compared to bone marrow–derived stem cells from healthy controls. Treatment of stem cells with an eNOS transcription enhancer resulted in restoration of SDF-1–mediated stem cell migration. Pretreatment of bone marrow–derived stem cells from patients with coronary artery disease and ischemic cardiomyopathy with the transcription enhancer significantly increased neovascularization after infusion of cells into a hind-limb ischemia model. Although the efficacy of these cells was significantly enhanced, they were still significantly less functional than cells from healthy controls. Recently the effect of eNOS cDNA delivery to the myocardium has been shown to improve local endothelial cell proliferation and perfusion in a chronic ischemia porcine model. These results suggest that transient overexpression of cDNA in bone marrow–derived stem cells before delivery to the myocardium could significantly enhance the effects of stem cell–based treatment of newly injured myocardium.

Integrins of Interest
Several studies have focused on key cell surface receptors involved in stem cell recruitment and migration. Each of these cell surface receptors is a potential target for genetic enhancement before stem cell delivery. They are also targets for studies to determine whether the presence of chronic inflammatory diseases such as coronary artery disease and chronic heart failure results in the decrease in stem cell surface expression of these receptors.

Genetic manipulation of stem cell integrin expression could take several forms. Transient integrin expression in delivered cells could significantly improve stem cell engraftment increasing the efficiency of stem cell therapy. With such an approach it is possible that integrin expression for such an approach could be transient, allowing for the delivery of paracrine factors to the injured tissue, or for other cellular processes to induce long term engraftment. Conversely, it is possible that long-term integrin expression is necessary for long-term stem cell engraftment and functional benefit. What is unclear is the extent to which specific integrin overexpres-
sion could alter stem cell differentiation toward a cardiac phenotype. These questions need to be addressed in future studies that focus on the efficiency of stem cell delivery and engraftment in myocardial tissue.

Key receptors and integrins that regulate stem cell migration have been shown to be dependent on stem cell type. As discussed above, EPCs have been shown to home to newly injured myocardium in response to signaling via the SDF-1–CXCR4 axis. Similarly it appears that CD18 (β2 integrin) expression by the EPCs and its interaction with endothelial cell surface ICAM-1 is necessary. Blockade of CD18–ICAM-1 binding through the administration of a CD18 neutralizing antibody nearly completely inhibited EPC engraftment after acute myocardial infarction.

There are several potential targets for genetic enhancement of EPC recruitment and engraftment. Integrin linked kinase (ILK) regulates EPC ICAM-1 expression. It has recently been demonstrated that under hypoxic conditions, ILK is stabilized by HSP-90. The resulting increase in ILK activity leads to an increase in ICAM-1 expression through NFκB- and HIF-1α-mediated signaling. The overexpression of ILK alone in normoxic cells is sufficient to induce ICAM-1 expression suggesting that it is a sufficiently upstream target to modulate integrin mediated adhesion and migration.

Migration of mesenchymal stem cells is not necessarily in response to the SDF-1–CXCR4 axis. We have previously demonstrated the importance of the MCP-3–CCR1/2 axis to induce mesenchymal stem cell homing. Interestingly, mesenchymal stem cell migration is mediated by integrin β1, not integrin β2 or alpha4, as is seen with hematopoietic stem cell–derived populations such as endothelial progenitor cells.

Mediators of Extracellular Matrix

Several studies have focused on optimization of the extracellular matrix for stem cell engraftment in an attempt to improve the efficacy of cell therapy. Many of these studies have implemented a tissue engineering approach attempting to identify matrices that optimize myocardial grafts. Some of these studies have identified factors that either directly intercalate into the extracellular matrix or induce remodeling of the extracellular matrix leading to increases in stem cell engraftment and cardiac function. Of particular interest are 3 factors, tenascin-C, relaxin, and periostin.

Tenascin-C (TN-C) is an extracellular matrix molecule that is expressed during wound healing in various tissues including myocardium after acute myocardial infarction. It is believed to be associated with profibrotic effects and is downregulated in response to aldosterone, suggesting that its downregulation may be associated with improved outcomes in patients with chronic heart failure. Although its downregulation may be associated with improved outcomes at times, remote from acute myocardial infarction, in the peri-infarct period upregulation of tenasin-C may be crucial to normal healing. Tenascin-C has been shown to be present in the infarct zone in areas of myofibroblast engraftment. Tenascin-C has been shown to accelerate fibroblast cell migration and α-SMA expression. After acute myocardial infarction, in tenascin-C–null mice there is a delay in myofibroblast recruitment to the infarct zone that is normalized by 3 days after acute myocardial infarction. Whether this delay in myocardial healing is significant is still unknown and awaits further study; however, data suggest that increased circulating levels of tenascin-C predict patients with increased pathological remodeling.

Relaxin is a hormone and a member of the relaxin superfamily, which also includes insulin-like peptides. Targets of relaxin includes the blood vessels and the heart. Relaxin is a potent vasodilator through liberation of nitric oxide. Relaxin has been administered intravenously at the time of reperfusion in a porcine ischemia-reperfusion model. In this study the administration of relaxin led to decreases in myonecrosis, cardiac myocyte apoptosis, and leukocyte infiltration into the injured myocardium. These results have led to porcine studies in which control or human relaxin overexpressing C2C12 myoblasts were transplanted into a model of chronic ischemia. The chronic expression of relaxin led to the increased local expression of MMP-2 and VEGF. Animals that received the relaxin-overexpressing cells demonstrated a greater increase in vascular density and cardiac function compared to untreated and control C2C12 treated animals.

Periostin is a secreted extracellular matrix protein that has been shown to have significant effects on left ventricular remodeling, stem cell engraftment, and differentiation in multiple pathological states in the heart. Periostin was first identified in tumors and is involved in cell survival and angiogenesis within the tumor. Normally expressed by cardiac fibroblasts after injury, periostin in the extracellular matrix of the heart at least in part regulates cardiac myocyte hypertrophy after myocardial infarction and pressure overload. Periostin null mice have been shown to have increased myocardial rupture rates; however, those animals that survive have less myocardial fibrosis and improved cardiac function. Similarly, pressure overload in periostin-null animals leads to less fibrosis and cardiac myocyte hypertrophy compared to wild-type controls. When periostin is injected into the infarct zone after acute myocardial infarction there is evidence of increased cardiac myocyte proliferation, decreased infarct size, increased angiogenesis, and improved cardiac function. Similar improvements in cardiac function were observed when sustained periostin delivery was achieved using impregnated gelfoam placed on the epicardial surface at a time remote from acute myocardial infarction.

Each of these matrix proteins or modulators of matrix deposition offer potentially unique molecular targets that could be introduced through genetic enhancement of stem cells before infusion or injection. Like many of the other potential genetic enhancements of interest, timing and length of time of gene expression is likely critical. Furthermore, as the molecular mechanisms that regulate the expression of these proteins are further defined, the number of strategies for induction of gene expression will increase. For example, periostin expression can be induced by either TGF-β or BMP-2; therefore, periostin expression could be upregulated by either direct introduction of cDNA encoding periostin, or
Seventy percent decrease in cardiac myocytes undergoing apoptosis 4 days after acute myocardial infarction, 200% increase in ejection fraction 5 weeks later.12,13,87 These observations support the paracrine hypothesis of myocardial repair, and suggest not only the potential for genetic enhancement of stem cell therapies, but also suggest that myocardial gene transfer of appropriate gene(s) could offer the same degree of benefit observed with cell therapy without the need for cells.

Differential array analyses comparing control and phosphorylated Akt overexpressing mesenchymal stem cells have identified some potential gene of interest that at least in part may account for the enhanced paracrine effects observed in genetically enhanced mesenchymal stem cells. Some of the factors identified, each of which could be a candidate for genetic enhancement of stem cells or direct myocardial gene transfer, include VEGF, FGF-2, HGF, IGF-1, and thymosin β4.13 Of note, HGF, IGF-1, and FGF-2 have been shown to induce cardiac stem cell activation and migration,46,47 and thymosin b4 has been shown to form a complex with PINCH and integrin-linked kinase (ILK), resulting in activation of Akt within cardiac myocytes in the infarct border zone.88 It is unknown whether there are synergistic effects or cross talk among these multiple factors or whether delivery of one of these factors would yield similar results. Clearly further defining the relevant pathways responsible for benefits seen with cell therapy is crucial. It has been suggested that secreted frizzled related protein 2 (Sfrp 2) is the key stem cell paracrine factor that mediates myocardial survival and repair after ischemic injury. When Sfrp2 is suppressed in Akt-MSCs, the ability of these cells to protect injured myocardium is lost. Similarly, Sfrp2 solely has the ability to protect cardiac myocytes from ischemic injury, and accomplishes this by upregulating nuclear and cellular βcatenin level, mimicking the canonical Wnt signaling antiapoptotic pathways.87 Furthermore, the degree to which Bcl-2–modified mesenchymal stem cells have similar changes in their secretome remains undefined.

SDF-1 has been shown by several groups to lead to improved cardiac function when delivered in the peri-infarct period or at times remote from myocardial infarction.5,12,32 It has recently been demonstrated that cardiac myocytes express CXCR4 after ischemic preconditioning or within hours of a myocardial infarction. Thus, as activation of the SDF-1–CXCR4 axis has been shown to increase hematopoietic and mesenchymal stem cell12 survival, it can similarly improves cardiac myocyte survival.12 Perhaps importantly, in addition to improving cardiac myocyte survival, SDF-1 also results in the homing of blood borne and cardiac derived stem cells leading to neovascularization and alterations in electric conduction at the infarct border zone.54 We have recently demonstrated that genetic enhancement of mesenchymal stem cells to induce overexpression of SDF-1 leads to ~60% decrease in cardiac myocytes undergoing apoptosis 4 days after acute myocardial infarction, ~150% increase in the number of surviving cardiac myocytes, and ~200% increase in ejection fraction 5 weeks later.12

Thus, genetic engineering of stem cells has led to the identification of several factors that ultimately can be used to genetically enhance stem cells in the future, or lead to the development of novel protein therapies that will either need...
to be delivered locally to the heart or allow for the development of systemic therapies.

**Cell–Cell Communication**

Sudden cardiac death is a significant cause of mortality in patients with chronic heart failure. Thus, to broadly improve patient outcomes after acute myocardial infarction cell therapy needs to address both the mechanical and electric consequences of cardiac dysfunction. Sudden cardiac death is usually a result of reentrant arrhythmias. Reentrant circuits require an area of scar, unidirectional block, and slow conduction.91,92 The risk of developing reentrant ventricular tachycardia is increased with volume of scar. Thus, if cells are able to functionally decrease the electric size of the scar, even in the absence of regenerating functional tissue, there is a chance to decrease the risk of sudden cardiac death. We have recently developed distinct electric effects of different cell types. In one study we demonstrated that despite an increase in cardiac function with skeletal myoblasts or engineered skeletal myoblasts, we observed a significant increase in arrhythmogenic potential in the tissue.32 These data would suggest that the electric and mechanical effects of cell therapy are independent. More recent studies have suggested that the degree of arrhythmogenic potential is correlated with the degree of connexin protein expression.33 MSCs which express connexins 40, 43, and 45 resulted in a significant decrease in arrhythmogenic potential; whereas skeletal myoblasts, which increased arrhythmogenic potential, do not express any connexin proteins in vivo. The potential for cell-based gene therapy to modulate arrhythmogenic potential was recently demonstrated using skeletal myoblasts engineered to express connexin 43. Transplantation of these engineered skeletal myoblasts led to significantly less ventricular tachycardia compared to control skeletal myoblasts.93 These findings suggest that in the future, transplantation of engineered cells could replace or augment tissue ablation for the treatment of recurrent ventricular tachycardia.

**Directed Stem Cell Differentiation to Cardiac Myocytes**

The effects of adult stem cell therapies to date appear to be achieved in the absence of significant levels of myocardial regeneration.3,4 The mechanisms understood at this time relate to decreasing cardiac myocyte death, optimization of left ventricular remodeling, and increases in vascular density. While these pathways appear robust and have the real potential for having clinical benefit in patients with acute myocardial infarction16 and chronic heart failure,17 achieving the consistent regeneration of cardiac myocytes is still of great interest.

Potentially strategies of genetic enhancement for directing stem cell are beginning to emerge. Significant work has been performed to further our understanding of regulatory pathways involved in embryonic stem cell differentiation to cardiac myocytes.94–96 These studies have suggested potential pathways that could be activated in adult stem cells in an attempt to have them take on a cardiac phenotype.94,96,97

Perhaps the most studied strategy to date with adult stem cells is the effect of 5-azacytidine, a DNA demethylation reagent, on cardiac protein expression in mesenchymal stem cells.98,99 Several studies have demonstrated an increase in cardiac protein expression after treatment of mesenchymal stem cells with 5-azacytidine, and some have observed spontaneously beating cells.98 Importantly, studies have consistently demonstrated improvement in cardiac function after the transplantation of 5-azacytidine–treated mesenchymal stem cells compared to control mesenchymal stem cells.99–101 As we begin to define the pathways activated after 5-azacytidine treatment, we will be able to use genetic enhancement to activate those pathways in an attempt to further optimize cardiac differentiation and functional effects.101

Another approach that is being developed to direct cardiac differentiation of adult stem cells is the delivery of chimeric proteins encoding cell penetrating peptides and cardiac specific transcription factors.24,102 Cell penetrating peptides (CPP) cause nonsecreted proteins to be secreted and to be internalized by surrounding cells. We have demonstrated that the transplantation into the myocardium of cells genetically enhanced to express a CPP-GFP protein results in GFP expression in native cardiac myocytes.102 To determine whether we could deliver functional transcription factors to the myocardium, we developed a CPP-GATA4 construct and transplanted cardiac fibroblasts that were stably transfected with the CPP-GATA4 construct 1 month after myocardial infarction in the Lewis rat. The infarct border zone of animals that received CPP-GATA4 demonstrated increased cardiac myosin and Bcl-2 expression.24 The modulation of GATA4 responsive gene expression led to hypertrophy of the cardiac myocytes at the infarct border zone and global improvement in cardiac function.24 These findings suggest that combining genetic enhancement of stem cells to deliver cell penetrating peptide–transcription factor chimeric proteins along with either stem cell homing agents or additional stem cells could lead to an increase in cardiac protein expression in the stem cells, cardiac myocyte regeneration, and further improvement in cardiac function.

**Strategies for Gene Transfer to Stem Cells**

There are several multiple strategies that can be used to genetically modify stem cell populations. There are several features decision points that need to be addressed before choosing the appropriate strategy, including the length of expression desired, the efficiency of transfection needed, and the proliferative state of the population to be transfected. For a more detailed discussion we refer the reader to a comprehensive review.103 The following discussion will presume ex vivo transfection of stem cells populations of interest before infusion of transplantation.

To achieve long-term gene expression there are several options including adeno-associated virus, retrovirus, and lentivirus.

**Adeno-Associated Virus (AAV)**

AAV vectors do not express any viral gene products, rendering them significantly less immunogenic. This vector results in efficient and long-term expression of transgene with a minimal inflammatory response.104 AAV vectors can infect
replicating and nonreplicating cells and are believed to be nonpathogenic to humans. Despite these advantages, limitations of AAV vectors include the limited size of the transgene that they will accept, they can be difficult to produce in large quantities, and they appear to possess the potential for insertional mutagenesis.49

Retrovirus
Retroviruses have several features including their ability to stably and precisely integrate into the host genome providing long-term transgene expression. These vectors can be manipulated ex vivo to eliminate infectious gene particles to minimize the risk of systemic infection and patient-to-patient transmission. However, a number of limitations exist including (1) high titer stocks are difficult to maintain, (2) retroviruses can only transfect proliferating cells, and (3) the fact that random integration of retroviral vectors poses a risk of insertional mutagenesis or transformation of the host cell. Clinical trials using ex vivo retroviral transfaction of hematopoietic stem cells have been undertaken in patients with severe combined immunodeficiency disease. Although this strategy has been shown to restore immune function in patients,106,107 unfortunately t-cell leukemia was observed in the 2 youngest of the treated cohort.108 Lentiviral vectors are a form of retrovirus that can infect both proliferating and nonproliferating cells.

Short-term gene expression can be induced through plasmid transfection or adenoviral vectors.

Adenovirus
Replication-defective adenoviral vectors allow for high efficiency transfection of multiple cell types through cell entry via the coxsackie virus receptor. Adenoviral vectors are rendered replication incompetent by deleting the early (E1A and E1B) genes responsible for viral gene expression from the genome and are stably integrated into the host cells in an extrachromosomal form. This decreases the risk of integration into the host cell genome and mutagenesis. These vectors have also demonstrated the ability to transfect both replicating and nonreplicating cells. In addition, adenoviral vectors can be produced at very high titers allowing efficient gene transfer with small volumes of virus. Transfection of skeletal myoblasts ex vivo leads to gene expression for approximately 18 days with a peak of expression at 10 to 12 days.32 One disadvantage of adenoviral strategy for gene transfer is that adenovirus can be immunogenic; however, studies have demonstrated that ex vivo transfection can limit the immunogenicity of the adenoviral vector.49

Plasmid
Multiple studies have demonstrated the feasibility of gene transfer to cells by exposing cells in culture to plasmid DNA along with a transfection agent. While this is a reliable strategy for gene transfer it is not very efficient with transfection rates varying from <5% to ~30% depending on the cells being transfected. If the goal is to deliver a secreted factor like VEGF or SDF-1 a plasmid based approach may be sufficient; however, if the gene of interest encodes an intracellular protein and needs to be expressed by the target cell to have an effect, as may be the case with integrins, then plasmid transfection may be too inefficient.

There are several strategies for genetically modifying stem cells ex vivo prior to cell transplantation. As with the decision of which gene to expression, the choice of which strategy to implement depends on the biology that is trying to be modified, the clinical situation in which the modified cell product will be delivered, and the size of the transgene and regulatory elements needed.

Summary
Tissue healing in mammals after acute ischemic events is biased toward scar formation and not regeneration of functional tissue. The heart may be at a greater disadvantage than other organ systems like the brain or liver because cardiac myocytes are to a significant extent not prone to proliferation, and, at least naturally, there appears to be minimal regeneration from endogenous stem cells. Cell-based therapies have demonstrated that there is significant potential to improve myocardial healing after an acute myocardial infarction. Studies focused on understanding the mechanisms of stem cell–based myocardial repair have led to the identification of naturally occurring mediators of myocardial healing that if exploited may be able to enhance stem cell–based repair with or without the delivery of exogenous stem cells. One strategy for modulating the expression of these mediators is genetic enhancement of stem cells before introducing the stem cells in the peri-infarct period or at time remote from myocardial injury. As discussed above, there are multiple potential pathways along each step of the process of stem cell–based myocardial healing (Figure 4), including stem cell delivery, engraftment, differentiation, and function, that genetic enhancement could be used to optimize cardiac function and patient outcomes.

Sources of Funding
This work was supported by grants from the NHLBI HL74400 and HL84142, the State of Ohio, and the Skirball Foundation.
Disclosures

M.S. Penn is a coinventor on a pending patent filed by the Cleveland Clinic Foundation that relates to the use of SDF-1 in acute myocardial infarction.

References


11. Laflamme MA, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Christerson HK, Murray CE. Regenerating the heart.


55. Lee SF, Youn SW, Choi HJ, Li L, Kim TY, Youk HS, Chung JW, Hur J, Yoon CH, Park KW, Oh BH, Park YB, Kim HS. Integrin-linked...
kinase, a hypoxia-responsive molecule, controls postnatal vasculo-
genesis by recruitment of endothelial progenitor cells to ischemic tissue. 

73. Abbott JD, Huang Y, Liu D, Hickey R, Krause DS, Giordano FJ. Stromal 
cell-derived factor-1alpha 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.

74. Imanaka-Yoshida K, Hiroe M, Nishikawa T, Ishiyama S, Shimojo T, 
Ohta Y, Sakakura T, Yoshida T. Tenascin-C modulates adhesion of 
mast cells by aldosterone-mediated inflammation: a model study. 

75. Kudo Y, Siriwardena BS, Hatano H, Ogawa I, Takata T. Periostin: novel 

76. Noiseux N, Gnecchi M, Lopez-Ilasaca M, Zhang L, Solomon SD, Dzau VJ, 
Pratt RE. Mesenchymal stem cells overexpressing Akt 
dramatically repair infarcted myocardium and improve cardiac function 
despite infrequent cellular fusion or differentiation. Mol Ther. 2006;14: 
840–850.

77. Litvin J, Blagg A, Mu A, Matiwalla S, Montgomery M, Berretta R, 
Penna AM, Masini E, Nistri S, Bani ST, Bigazzi M, Bani D. Human 
recombinant relaxin reduces heart injury and improves ventricular per-
formance in a swine model of acute myocardial infarction. Ann N Y 

78. Katsuragi N, Morishita R, Nakamura N, Ochiai T, Taniyama Y, 
Horie M, Imanaka-Yoshida K. Eplerenone attenuates myocardial 
fibrosis in the angiotensin II-induced hypertensive mouse: 
electrophysiologic and anatomic correlation. Circulation. 2008;77:
589–606.

79. Quan W, Rudy Y. Unidirectional block and reentry of cardiac excitation: 

80. Masters S, Tirosh I, Cedeldorf R, Firestein S, Scholer HR. BMP-2 
encodes a domain that promotes the cardiomyogenic potential of embryonic 

A, Brunskill EW, Dorn GW, Conway SJ, Arokwow BJ, Robbins J, 
Molkentin JD. Genetic manipulation of periostin expression reveals a 
role in cardiac hypertrophy and ventricular remodeling. Circ Res. 2007; 

D, Kinoshita N, Yazaki Y, Hiroe M. Serum tenascin-C might be a novel 
factor responsible for ventricular dilatation. Circulation. 2004;110: 
1806–1813.

83. Nishioh K, Suzuki M, Onishi K, Takakura N, Inada H, Yoshida T, 
Hiroe M, Imanaka-Yoshida K. Eplerenone attenuates myocardial 
infarction but is not sufficient to induce homing in the absence of injury. 

84. Li W, Ma N, Ong LL, Nesselmann C, Klopsch C, Ladilov Y, Furlani D, 
Piecaczek C, Moebius JM, Lutzow K, Lendlein A, Stamm C, Li RK, 
Steinhoff G. Bel-2 engineered MSCs inhibited apoptosis and improved 

85. Kabani N, Guevara-Davis M, Zhang L, Solomon SD, Doh A, Dzau VJ, 
Pratt RE. Mesenchymal stem cells overexpressing Akt 
dramatically repair infarcted myocardium and improve cardiac function 
despite infrequent cellular fusion or differentiation. Mol Ther. 2006;14: 
840–850.

86. Li W, Ma N, Ong LL, Nesselmann C, Klopsch C, Ladilov Y, Furlani D, 
Piecaczek C, Moebius JM, Lutzow K, Lendlein A, Stamm C, Li RK, 
Steinhoff G. Bel-2 engineered MSCs inhibited apoptosis and improved 

87. Li W, Ma N, Ong LL, Nesselmann C, Klopsch C, Ladilov Y, Furlani D, 
Piecaczek C, Moebius JM, Lutzow K, Lendlein A, Stamm C, Li RK, 
Steinhoff G. Bel-2 engineered MSCs inhibited apoptosis and improved 
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Circ Res. 2008;102:1471-1482
doi: 10.1161/CIRCRESAHA.108.175174

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/102/12/1471

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