Empowering Adult Stem Cells for Myocardial Regeneration

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Abstract: Treatment strategies for heart failure remain a high priority for ongoing research due to the profound unmet need in clinical disease coupled with lack of significant translational progress. The underlying issue is the same whether the cause is acute damage, chronic stress from disease, or aging: progressive loss of functional cardiomyocytes and diminished hemodynamic output. To stave off cardiomyocyte losses, a number of strategic approaches have been embraced in recent years involving both molecular and cellular approaches to augment myocardial structure and performance. Resultant excitement surrounding regenerative medicine in the heart has been tempered by realizations that reparative processes in the heart are insufficient to restore damaged myocardium to normal functional capacity and that cellular cardiomyoplasty is hampered by poor survival, proliferation, engraftment, and differentiation of the donated population. To overcome these limitations, a combination of molecular and cellular approaches must be adopted involving use of genetic engineering to enhance resistance to cell death and increase regenerative capacity. This review highlights biological properties of approached to potentiate stem cell–mediated regeneration to promote enhanced myocardial regeneration, persistence of donated cells, and long-lasting tissue repair. Optimizing cell delivery and harnessing the power of survival signaling cascades for ex vivo genetic modification of stem cells before reintroduction into the patient will be critical to enhance the efficacy of cellular cardiomyoplasty. Once this goal is achieved, then cell-based therapy has great promise for treatment of heart failure to combat the loss of cardiac structure and function associated with acute damage, chronic disease, or aging. (Circ Res. 2011; 109:1415-1428.)

Key Words: regeneration ■ stem cell ■ infarction ■ myocardium
Substantial resources have been expended over the last decade in pursuit of interventional strategies to treat the unmet need of heart failure patients to restore myocardial structure and function. In the wake of thousands of research reports and hundreds of clinical studies we remain perplexed, which is reassuring in the context of the Gibran quote that begins this review. Although there remains a lot to learn, knowledge is coalescing into understanding that, in turn, refines the search for answers into ever more fruitful investigations. However, it has become abundantly clear from both clinical and basic research settings that full restoration of myocardial structure and function in the wake of pathological injury remains outside our reach at present but may be achievable with a combination of ongoing research, creativity, perseverance, and maybe a little luck. This review endeavors to summarize the run up to current understanding, where the road is blocked or splits apart, and how utilization of enhanced stem cells may provide the means to surpass current results and further the efficacious implementation of regenerative cell therapy for heart failure.

Part 1: In the Beginning There Were a Couple of Ideas

Ideas are like rabbits. You get a couple and learn how to handle them, and pretty soon you have a dozen.

—John Steinbeck

Today in a new age of enlightenment, students and trainees regard their mentors with bemused incredulousness when told that, until recently, the prevailing dogma held the myocardium as a fully postmitotic tissue incapable of regeneration. At the turn of this century, cell therapy approaches were essentially limited to adoptive transfer of various noncardiac cell types into the pathologically injured heart in the hopes of stimulating myocardial and vasculature14 had been isolated and, as a consequence of experimental studies and published reports now numbering in the thousands, the reputation of the heart as an organ incapable of cell regeneration has been transformed.15,16 No longer slumbering in postmitotic quiescence, the heart is a dynamic organ capable of repair, cellular replacement over aging, and a fertile milieu for the panoply of stem cells sourced from adults, embryos, and induced fibroblasts. With subtypes of each cell category seemingly multiplying like proverbial rabbits, the field has morphed from a lack of suitable regenerative cell populations to an overabundance of possibilities. A brief examination of the embryonic/inducible pluripotent camp versus adult cells is in order to understand the empowerment issues involved.

With a goal of recreating tissue in mind, the use of cells that give rise to all the tissue types in our bodies in early development seems a logical and promising choice. Indeed, embryonic stem cells (ESC) derived from human blastocysts have been around since the end of the last century.17 These pluripotent cells exhibit normal karyotypes and high telomerase activity and express cell surface markers that characterize ESCs before lineage commitment. Human embryonic stem cells (hESCs) can, conceptually, give rise to cells in any somatic cell line. Differentiation of hESCs can be regulated by different culture conditions and growth factors.18,19 Animal studies using ESCs have demonstrated restoration of cardiac function, but teratoma formation and immunologic rejection restricts therapeutic utility of this cell type, in addition to ethical considerations.20,21 Tumorigenic potential of ESCs persists in various differentiated stages regardless of cell population leading to teratoma formation,22 which clearly illustrates safety concerns associated with purportedly “differentiated” hESC-derived material intended for clinical mediation repair remained the holy grail of cardiac regenerative medicine. Coincidentally, while skeletal myoblast transfer studies stalled in 2001, a new era was concurrently dawning with the advent of bone marrow adoptive cell transfer for repair of the infarcted heart.6,7 Regardless of the maelstrom of debate that ensued about the findings of these seminal studies,8,9 these publications represented a turning point in the perspective of how myocardial repair could be effected. The following decade witnessed numerous clinical trials with bone marrow and bone marrow–derived cells to assess the clinical application of stem cells, as summarized in excellent reviews and meta-analyses.10–13 In brief, cardiac clinical trials from the past decade have mainly been based on different cell subsets of autologous bone marrow. The general conclusion is that bone marrow stem cell therapy is safe and associated with a moderate (1.93–5.40%) increase in ejection fraction. This improvement appears to be temporary,11 presumably due to limitation of remodeling or relief of angina through paracrine effects, rendering this approach possibly efficacious in biologically old patients but a suboptimal choice for the majority of the middle patient population. Long-term functional improvement requires application of stem cells possessing true cardiomyogenic and vascular differentiation potential and contributing to new cell and vessel formation in the myocardium. This rationale underpinned the announcement that resident cardiac progenitor cells (CPCS) derived from human samples capable of generating myocardium and vasculature14 had been isolated and, as a consequence of experimental studies and published reports now numbering in the thousands, the reputation of the heart as an organ incapable of cell regeneration has been transformed.15,16 No longer slumbering in postmitotic quiescence, the heart is a dynamic organ capable of repair, cellular replacement over aging, and a fertile milieu for the panoply of stem cells sourced from adults, embryos, and induced fibroblasts. With subtypes of each cell category seemingly multiplying like proverbial rabbits, the field has morphed from a lack of suitable regenerative cell populations to an overabundance of possibilities. A brief examination of the embryonic/inducible pluripotent camp versus adult cells is in order to understand the empowerment issues involved.

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application, with chromosomal instability reported in later passages of these cells in culture.23 Human induced pluripotent cells (hiPSCs) are similar to embryonic cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell specific genes, telomerase activity,24 and cardiac potential.25 Along with these attributes, hiPSCs also show similarities with hESCs regarding teratoma formation, tumorigenicity, and somatic coding mutations.26 In addition, incomplete reprogramming or accumulation of genetic abnormalities during the iPSCs derivation process may render even autologous iPSCs lines immunogenic.27 iPSCs coculture studies with various cell types have revealed that until now, no cell type has been able to generate the cell type of interest with higher than 95% purity. Heterogeneity in the iPSC population raises risk of cellular transdifferentiation and susceptibility to teratogenesis. However, the dilemma of heterogeneity is not limited to predifferentiation, as maturation stages are also not uniform in iPSC culture, including cardiomyocyte phenotypes.27,28 Variable differentiation is of major concern in the heart, where synchrony and structure is of fundamental importance. In summary, at the time of this review, clinical cardiac application of ESC and hiPSCs populations must traverse a deep chasm that can only be bridged by harnessing overenthusiastic proliferative potential, gaining control over cell fate determination signals, and coping with issues of allogeneic rejection for the ESC. As such, these cell types have yet to make an appearance in a clinical trial for treatment of heart failure.

Safety concerns over the utilization of ESC or hiPSCs contrasts with the lack of adverse events associated with administration of adult stem cells derived from bone marrow or cardiac tissue explants. Although ontogeny of adult cardiac stem cells remains unresolved, collective findings from multiple laboratories validate the cardiogenic potential of these cells.29–32 The presumption for presence of tissue resident adult stem cells is their participation in normal cellular renewal due to consequences of aging over the lifetime of an organism. Therein lies the crux of the problem, because the resident adult cell population never evolved for rapid creation of new tissue in the wake of injury. The positive aspect of an adult stem cell’s limited proliferative potential is the fact that not a single incidence of oncogenic transformation has been documented, and this distinction from their embryonic brethren has enabled clinical trials with adult stem cells to move forward. In the SCiPIO trial (cardiac Stem Cell Infusion in Patients with Ischemic Cardiomyopathy), cardiac stem cells are isolated from patients undergoing a coronary artery bypass grafting (CABG) procedure for autologous reintroduction after expansion in culture when they are percutaneously infused into the scar tissue 4 months after CABG (http://clinicaltrials.gov/ct2/show/study/NCT00474461). Although the SCiPIO trial is mainly based on determining the feasibility and safety of harvesting adult cardiac progenitors for autologous reintroduction, there is also optimism toward obtaining functional hemodynamic improvement.

The good news is that clinical utilization of adult stem cells is a reality today, and the results appear promising as well as safe as shown by a SWOT (Strengths, Weaknesses, Opportunities, and Threats) analysis of stem cell types (Figure 1).
The caveat is that whereas embryonic or induced pluripotent cells possess an inherently youthful phenotype, heart failure patients who provide tissue for autologous stem cells isolation are usually above the age of 60 years and have coronary occlusions, possibly multiple events, and previous cardiac procedures. Indeed, aging may be, in part, a “stem cell disease” characterized by the ravages of time on the resident adult cell population that renders them increasingly stressed in the progressively dysfunctional tissue environment of aging myocardium. Stem cells would be well suited for regeneration if they clung to the exuberance of youth while also maintaining self-control that comes with maturity.

Part 2: Getting Older, Not Necessarily Better

By the time we’ve made it, we’ve had it.

—Malcom Forbes

In adult mammalian tissue, stem cells participate in normal tissue homoeostasis through repair and regeneration on damage. Stem cell niches are profoundly affected by signals and growth factors from the local and systemic environment. Thus, a younger niche is exposed to a different local milieu than an older or injured niche. Because normal regeneration is a function of local stem cell niches, the accretion of age-related changes such as DNA damage, impaired catabolism, altered epigenetics, and environmental stress prompt decline in stem cell function. In the process of DNA replication, alterations such as single- and double-strand DNA breaks, chromosomal translocations, telomere shortening, and single base mutations can occur and lead to replicative cellular senescence. In addition to replicative senescence, adult stem cells in the heart are susceptible to chronological aging, reflected by aggregation of damaged proteins, lipids, and other macromolecules due to a decrease in cellular autophagy. Inefficient catabolism leads to accumulation of dysfunctional organelles and cellular substructures over time, which in turn reduces quality and efficiency of cellular and molecular biological processes required to maintain homoeostasis and survival. As an organ matures, the well-orchestrated regulation of sequential expression timing and intensity for genes such as Wnt, Notch, and Hedgehog in the stem cell pool can be epigenetically disrupted leading to changes cell progeny. Disturbance of gene expression cascades into production of misprogrammed daughter cell progeny that fail to maintain tissue structure and function. The accumulation of aberrant cells can be significant with advancing age, as predictive calculations reveal the entire myocyte compartment is replaced 15 times in women and 11 times in men from 20 to 100 years of age, meaning an average of 13 replications in 80 years. As an indication of repetitive rounds of replication, shortening of telomeres in the adult cardiac stem cell pool was paralleled by appearance of myocytes with severe telomere attrition, suggesting that older CPCs are the likely source for phenotypically old myocyte progeny. Last but not least in this cavalcade of detrimental insults are the exogenous stresses that stem cells endure in a pathologically compromised heart. For example, cardiac stem cells from a CABG patient probably have not only suffered from replicative and chronological aging but have also been forced to persevere in a genotoxic environment of reactive oxygen species and chemical substances, promoting a process called stress-induced premature senescence. Stress-induced premature senescence, in turn, leads to DNA damage and mitochondrial DNA destruction, which ultimately influences stem cell replicative capacity and progeny. Premature senescence also occurs through the renin-angiotensin-aldosterone system and chronic elevation of angiotensin II levels. Because the majority of the target patient population for stem cell therapy suffers from sympathetic hyperactivity, such patients also carry a stem cell pool compromised by adverse repercussions of the renin-angiotensin-aldosterone system. The emerging paradigm of cellular senescence also portrays senescent cells as active participants in communicating their decrepitude by profoundly affecting their microenvironment in a paracrine fashion through an altered secretome that inhibits proliferation and modulates immune responses. These processes initiate a vicious circle of negative events on stem cell function and progeny, ultimately compromising the regenerative potential of the tissue as a whole.

Taken collectively, evidence indicates that adult stem cells are unlikely to be equivalent in their regenerative potential. Moreover, the very target population of aged and infirmed patients destined to be at the forefront of interventional therapy also possess the most compromised stem cell population in terms of functional capacity and regenerative potential. Like so many biological problems, the solution is conceptually simple but fraught with technical challenges. Simply put, we would want to metaphorically “turn back the clock” on aged adult stem cells and empower them with the phenotypic characteristics of youthful vigor while not obviating their programming for context-dependent recognition of the environment and appropriate integration into the local environment in a salubrious fashion.

Part 3: May-December Wedding Between Science and Stem Cells

You’ve got to go out on a limb sometimes because that’s where the fruit is.

—Will Rogers

As researchers pursue the ultimate goal of therapeutic implementation for regenerative medicine, the journey slowly yields hard-won fruits of knowledge gathered through innovation and creativity. Transformational ideas alter longstanding paradigms and redefine approaches to creating and delivering stem cells, but major issues concerning the therapeutic application of stem cells remain unresolved. Success of adoptively transferred adult stem cells remains modest primarily as a consequence of 3 factors: poor survival, marginal proliferation, and limited functional engraftment/commitment within the host tissue. Adoptively transferred stem cells must be primed against apoptotic, necrotic, and hypoxic conditions prevalent within the damaged tissue. Furthermore, the aforementioned deterioration of proliferative capacity in old age adversely affects the stem cell regenerative capacity. Finally, if cells persist and even proliferate but are functionally incapable of appropriate lineage commitment and functional
integration, then the end result is a cell predisposed to oncogenic transformation. Therefore, combating a constellation of negative factors affecting stem cell–mediated regeneration must be balanced against the need for restraint and appropriate participation in direct or indirect tissue repair. Threading this figurative “eye of the needle” is the purview of stem cell empowerment as detailed in the remainder of the review, wherein current concepts, research efforts, and problems associated with stem cell modification to enhance function are enumerated (Figure 2).

**Survival**

Poor survival and marginal retention of adoptively transferred cells into the pathologically challenged heart is widely accepted as a significant barrier to enhancing efficacy of regenerative therapy, and there is no controversy over the assertion that live cells do a better job of mediating biologically relevant effects than dead ones. Yet, researchers readily acknowledge massive losses of donated stem cells and failure to engraft in the damaged organ takes place within the first few days after delivery.5435 If most of the effects we observe are mediated by cells that disappear within a week, then imagine the possibilities for enhanced repair if the donated population persisted for weeks, months, or even became incorporated permanently into the heart tissue. Clearly, this is one of the front lines in the battle to enhance efficacy of adoptive transfer cell therapy. Stem cell survival is influenced by a number of factors such as ischemic conditions, inflammatory response,56 and quality of donor cells,57 and research has focused on enhancement of stem cell survival within host environment to augment repair.

“The preconditioning” in the context of stem cells refers to treatment with growth factors, hypoxic shock, or anti-aging compounds for augmentation of stem cell potency. Preconditioning promotes cyto-protection that enhances resistance stem cell survival against oxidative stress in vitro and in vivo58–61 as well as promotes migration and recruitment to ischemic myocardium.60 Cytokines and chemokine preconditioning strategies augment stem cell recruitment to injured tissue after intracardiac delivery of erythropoietin,62 hepatocyte growth factor (HGF), or vascular endothelial growth factor (VEGF).63 Other factors used to enhance stem cell function included BMP-2, IGF-1, fibroblast growth factor (FGF)-2,64 HGF, Hsp70, and atorvastatin.65–69 Similarly, mesenchymal stem cells under hypoxic conditions exhibit increased proliferation and differentiation70 associated with prosurvival and proangiogenic signaling. The mechanistic signal transduction basis for these preconditioning effects promoting cell survival involves activation of PI3K/AKT and ERK1/2 signal transduction and activation of STAT356 as well as scenarios involving ERK1/2 expression.71 Preconditioning can be initiated by multiple different cytokines that differentially influence downstream targets; therefore, multiple signaling pathways participate in mediating stem cell survival. The advantage of preconditioning is that the treatments are often simple, take advantage of cellular endogenous responses, and do not depend on genetic manipulation that is time-consuming and introduces foreign DNA into the
treatment regimen. The duration of the protective response is a significant limitation of the preconditioning approach, as cell surface receptors are downregulated, desensitized, or internalized in response to stimulation. Therefore, protection afforded by ex vivo preconditioning treatment before delivery probably will improve donated cell survival, but only by hours to days.

Alternative to preconditioning, genetic modification of stem cells to express prosurvival factors also enhances endurance of stem cells in the hostile environment of a pathologically damaged heart. Moreover, genetic manipulation allows for cells to serve as a source of growth factors that initiate intracrine, autocrine, and paracrine effects, which augment activity of the donated population, endogenous cells, and their local environment. Candidate molecules used for genetic modification of cells include canonical mediators of cell survival in the context of cardiomyocytes or oncogenically transformed cells (see Tables 1 through 3) and are

| Table 1. Preconditioning by Factors to Empower Stem Cells to Augment Myocardial Repair |
|-----------------------------------------------|-----------------|-----------------|-----------------------------------|-----------------|
| Factor Used                                  | Delivery Method  | Model           | Outcome                                         | References       |
| Hepatocyte growth factor                     | Intravenous injection | Mice           | Improved cardiac function, promotes migration, proliferation, and angiogenesis | 155, 156         |
| Vascular endothelial growth factor           | Preconditioning of MSCs | Mice           | Improved cardiac function, reduced fibrosis    | 157, 158         |
| IGF-1                                        | Intramyocardial injection, MSC preconditioning | Mice           | Improved cardiac function, promotes engraftment and differentiation | 66, 114, 159     |
| TGF-1α                                       | MSC preconditioning | Rat            | Improved cardiac function                      | 160              |
| Atorvastatin                                 | Oral             | Pigs            | Improved cardiac function, promotes cell survival | 68              |
| Erythropoietin                               | Intracardiac injection | Rat            | Improved cardiac function, promotes migration | 62              |

| Table 2. Genetic Modification by Integrated or Episomal DNA to Empower Stem Cells |
|-----------------------------------------------|-----------------|-----------------|-----------------------------------|-----------------|
| Factor Used                                  | Delivery Method  | Model           | Outcome                                         | References       |
| AKT                                          | Retrovirus      | Rat             | Improved cardiac function and prevent remodeling | 79, 81           |
| Hepatocyte growth factor                     | Liposome-mediated cell transfection | Mice, rat       | Improved cardiac function, promotes migration, proliferation and angiogenesis | 155, 156, 161   |
| Vascular endothelial growth factor           | Adenovirus      | Rats            | Improved cardiac function, reduced fibrosis    | 157, 158         |
| IGF-1                                        | Adenovirus      | Rats            | Improved cardiac function, promotes engraftment, differentiation, and cardiac function | 162             |
| Pim-1                                        | Lentivirus      | Mice            | Improved cardiac function, promotes engraftment, cell survival, and differentiation | 32              |
| Ang-1                                        | Adenovirus      | Rats            | Improved cardiac function and angiogenesis     | 162             |
| GSK3-ß                                       | Adenovirus      | Mice            | Improved cardiac function, promotes survival and angiogenesis | 103             |
| GATA4                                        | Retrovirus      | Rat             | Improved cardiac function, increased angiogenesis and cell survival | 98              |
| CXCR4                                        | Adenovirus      | Mice            | Improved cardiac function, reduced fibrosis and angiogenesis | 163             |
| Survivin                                     | Lentivirus      | Rat             | Improved cardiac function, increased capillary density, reduced infarct | 75              |
| Heme oxygenase-1                             | Adenovirus      | Rats            | Improved cardiac function, promotes angiogenesis | 164             |
| Bcl-2                                        | Transduction    | Rats            | Improved cardiac function, cell survival       | 72, 73           |
| HSP-20                                       | Adenoviral      | Rats            | Improved cardiac function, promotes cell survival | 165             |
| Nuclear targeted Akt                         | Lentivirus transduction | Mice           | Improved cell proliferation, inhibits cardiac commitment | 85              |
briefly delineated in the next few paragraphs.

Apoptosis is a serious threat faced by transplanted cells into a hostile environment, so modifying stem cells to circumvent apoptotic signaling increases cell survival. The Bcl-2 protein family regulates caspase activation and mitochondrial integrity through dual actions of antiapoptotic and proapoptotic members. Bcl-2 engineering of mesenchymal stem cells (MSCs) increases survival after acute myocardial infarction. Bcl-2–modified mesenchymal stem cells ameliorated left ventricular remodeling and improved left ventricular function. Exogenous delivery of Bcl-2 in MSCs activates a survival pathway that is sufficient to suppress hypoxia-induced apoptosis and adeno viral Bcl-2 transgene expression—attenuated early donor cell death in cardiomyoblast transplantation. Heme oxygenase-1 is an antiapoptotic stress-inducible enzyme with antioxidant cytoprotective activity under ischemic conditions. Overexpression of Heme oxygenase-1 in mesenchymal stem cells promotes angiogenesis and reduces fibrotic area after transplantation in ischemic myocardium. Transplantation of survivin-engineered mesenchymal stem cells also enhanced cellular survival after transplantation. Similarly, other survival molecules including SDF-1, Angiotsin-1, and CXCR4 significantly improve survival of transplanted cells.

This approach has proven successful with MSCs expressing myristolated AKT that augments heart function resulting in significant infarct size reduction and inhibition of ventricular remodeling 72 hours after transplantation despite the fact that donated cells did not significantly contribute to formation of new myocardium. Paracrine effects of these AKT-expressing modified cells were postulated to play an important role in protection, with identification of genes including VEGF, FGF-2, HGF, IGF, and notably thymosin β4 that complexes with PINCH and integrin-linked kinase, resulting in the activation of AKT within cardiomyocytes of the border zone. Secreted frizzled related protein 2 (Sfrp 2) was also identified as a key paracrine factor mediating myocardial survival and repair after ischemic injury since protection of injured myocardium by AKT-modified mesenchymal stem cells was lost after suppression of Sfrp 2.

**Proliferation**

Another important factor for consideration to improve the efficacy of cellular therapy is to augment the rate of proliferation of transplanted adult stem cells, which leads to persistence and expansion of the donated cell population and increases the number of cells available for engraftment.

Combined with enhanced survival, increasing proliferation can serve as a powerful combinatorial approach to expand the impact of donated stem cells, as shown in studies using cardiac progenitor cells modified to express Pim-1 kinase. Similarly, overexpression of nucleostemin in cultured cells cardiac stem cells increased proliferation accompanied by preservation of telomere length. However, an important caveat is that enhancing proliferation at the expense of lineage commitment and functional engraftment may not provide significant long-term benefits, as was the case when cardiac progenitor cells were modified to express nuclear-targeted Akt, resulting in expansion and persistence of the donated cells. This study points out the importance of balancing the trifecta of desirable cell properties judiciously, as the optimal outcome can only be achieved when appropriate cell phenotypic properties accompany enhanced survival, proliferation, and commitment to cardiogenic fate.

**Commitment**

Ideally, donated stem cells will ultimately participate directly in repair of damaged tissue by becoming new myocardium through synthesis of de novo myocytes, vessels, and endothelium. Regulatory pathways involved in embryonic stem cell differentiation to cardiomyocytes provide insight into how such cell fate decisions might be controlled and influenced. A commonly used pharmacological strategy to promote differentiation is exposure to the DNA demethylation reagent 5-azacytidine as performed on mesenchymal stem cells, bone marrow–derived stem cells, or cardiac progenitor cells. Long-term stimulation of cardiac stem cells with TGF-β1 also favors acquisition of a cardiomyocyte phenotype. Such approaches are unlikely to have significant clinical implications because of regulatory concerns about the effects of such treatments on stem cells, but examining molecular processes induced by such treatments facilitates unraveling the pathways involved in optimizing cardiac differentiation of transplanted cells. An interesting alternative approach is the delivery of cardiac transcription factors as chimeric proteins fused to cell-penetrating peptides to promote differentiation into cardiac phenotypes. Paracrine factors secreted by adoptively transferred mesenchymal stem cells may play an important role in orchestrating recruitment and lineage commitment of endogenous responses by promoting vasculogenesis and inhibiting apoptosis through VEGF, FGF, HGF, Angiotsin-1, GATA4, or angiopoietin. However, at present, the most intriguing multifaceted player in the signaling cascade of cardiac stem cell

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### Table 3. Pharmacological Treatment With Chemicals to Empower Stem Cells In Vitro

<table>
<thead>
<tr>
<th>Factor Used</th>
<th>Delivery Method</th>
<th>Model</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Azacytidine</td>
<td>In culture media</td>
<td>In vitro cells (MSCs, bone marrow–derived cells)</td>
<td>Formation of myotubes and cardiomyocyte-like structure</td>
<td>89–91</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>In culture media</td>
<td>In vitro cells (CPCs)</td>
<td>Beating myocytes</td>
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<tr>
<td>eNOS</td>
<td>Genetic deletion of Nos-3</td>
<td>Nos-3/−/− mice</td>
<td>Inhibit mobilization of stem cells</td>
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<tr>
<td>MCP-3-CCR1/2</td>
<td>In culture media and through cardiac fibroblast expressing MCP-3-CCR1/2</td>
<td>MSCs, rats</td>
<td>Mobilization of MSCs</td>
<td>113</td>
</tr>
</tbody>
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myogenic determination is Notch, which regulates commitment\textsuperscript{100} as well as survival.\textsuperscript{101} Notch is also a key regulator in smooth muscle differentiation as noted in epicardium-derived cells.\textsuperscript{102} Therefore, manipulating stem cells with Notch seems a likely avenue for enhancing stem cell commitment and persistence. Similarly, GSK3-\(\beta\) induces cardiomyocyte differentiation, with myocardial injection of bone marrow mesenchymal stem cells overexpressing GSK3-\(\beta\) associated with cardiomyocyte differentiation and angiogenesis.\textsuperscript{103}

Rejuvenation

One additional consideration alluded to earlier in this review is the problem of stem cell exhaustion caused by aging. Autologous cell therapy on an aging target population probably will be hampered by the biological limitations of endogenous stem cells and the advent of senescence in the myocardial cell pool. Ideally, empowering the explanted stem cell population requires attention to antagonizing senescence and “turning back the clock.” Although relatively little has been accomplished in the myocardial context, there are signaling pathways that seem connected to reversing the passage of time. For example, experimental activation of Notch restored “youthful” myogenic responses to satellite muscle cells isolated from 70-year-old humans, rendering them similar to cells from 20-year-old humans.\textsuperscript{104} Declining proliferation in hepatic progenitor cells has been ascribed to formation of a complex involving cEBP-\(\alpha\) and the chromatin remodeling factor brahma (Brm) that inhibits the regenerative capacity of aged liver.\textsuperscript{105} The mTOR pathway has been studied in the context of hematopoietic stem cells, where rapamycin increased life span and restored self-renewal and hematopoiesis in aged mice, implicating mTOR signaling in aging and showing the potential of mTOR inhibitors to restoring hematopoiesis in the elderly.\textsuperscript{106} Manipulation of the telomere-telomerase axis was suggested in 1998, when 2 different human cell lines, retinal pigment epithelial cells and foreskin fibroblasts, were transfected with vectors encoding for human telomerase catalytic subunit. Overexpression of telomerase resulted in elongated telomeres, invigorated cell division, and reduced expression of senescence markers.\textsuperscript{107} Increased telomerase activity correlates with telomere elongation in stem cell–derived activated T cells.\textsuperscript{108}

Genetic Modulation for Guidance and Trafficking

Stem cell homing through injured myocardium represents another relevant key facet for furthering stem cell–based regeneration for both donated as well as endogenous cell populations. Multiple molecular players are involved in the journey from a niche or injection site to the battleground of border zone or infarct region. Adhesion molecules such as integrins\textsuperscript{109} as well as proteases work in concert to facilitate migration of stem cells through damaged tissue. Several integrins have been identified on stem cells and found to be involved in the recruitment, mobilization, and homing of stem cells to the site of injury.\textsuperscript{110,111} Directional motility for mesenchymal stem cells was enhanced by engineered expression of the SDF-1/CXCR4 axis.\textsuperscript{112–114} MSCs have also been involved in recruiting endogenous stem cells improving myocardial repair.\textsuperscript{115,116} Similarly, cellular recruitment is enhanced for endothelial progenitor cells in infarcted myo-cardium by CD18/ICAM\textsuperscript{117} or the MCP-3-CCR1/2 axis.\textsuperscript{113} Selected proteases and their inhibitors have been touted as candidates to influence stem cell trafficking such as PAI-1, a protease inhibitor that blunts trafficking of mobilized CD34 + bone marrow cells and influences ventricular remodeling.\textsuperscript{112} Endothelial nitric oxide synthetase also enhanced stem cell homing after acute myocardial infarction\textsuperscript{118} through increases in MMP-9 and SDF-1.\textsuperscript{119} Thus, engineering of stem cells to induce expression of stem cell mobilization and homing factors can augment recruitment and retention of prodigal stem cells in their effort to find the right place to exert their reparative effects.

Collectively, information in this section of the review shows that modification of adult stem cells can adopt many forms and vary in method of implementation but always shares the singular goal of enhancing regeneration. Optimization of stem cell modification will depend on an approach or combination of approaches that maximizes all aspects of the regenerative process encompassing survival, proliferation, trafficking, lineage commitment, and functional engraftment. Published results using various strategies such as Pim-1 kinase support the premise that engineering of stem cells is a viable option to enhance the reparative process. Pim-1 is unique among the molecules used thus far as a combinatory mediator of enhanced survival, proliferation, lineage commitment, and functional engraftment. Some might argue that using such powerful molecular interventions with adult stem cells is going out on a limb and taking a risk, but cellular reprogramming by genetic engineering yielded inducible pluripotency that is unquestionably one of the greatest advances of stem cell biology. Just like inducible pluripotent cells, the challenge is not in seeing the destination for where adult stem cell engineering must go but rather how to get there as quickly and safely as possible.

Part 4: Clinical Implementation and the Challenge of Genetically Engineered Stem Cell Empowerment

If you find a path with no obstacles, it probably doesn’t lead anywhere.

—Anthony Michael Hall

The primary hurdle in empowering stem cells for clinical application does not rest primarily with lack of knowledge on molecular mechanisms and pathways but rather how best to deliver the engineered solution to the stem cell population in an acceptable and feasible solution. Traditional gene delivery relies on recombinant protein expression through viral vectors that possess the inherently desirable characteristics of easy cell delivery of the engineered construct, use of replication deficient vectors and cell-type–specific vectors to limit spread and target delivery, and the persistent expression of introduced genetic material by incorporation into the genome or maintained epidosal presence in nondividing cells. Adenoviral, adeno-associated, lentiviral, and retroviral vectors are widely used for gene delivery in the experimental setting and to a limited extent in clinical trials.\textsuperscript{121–125} Each type of delivery vector has a different set of strengths and weaknesses in the context of empowering stem cells. Lenti-
viruses have the ability to infect nondividing cells, whereas retroviruses express in a proliferative cell population. The primary advantage of lentiviral and retroviral-based engineering is the persistent incorporation of the viral genome (and with it the gene of interest) into the host genome so that the genetic modification can be selected for and propagated in daughter cell progeny. Although incorporation of the transgene into the host cell genome makes these vectors an excellent choice for engineering cells, risk of insertional mutagenesis and difficulty in regulating expression of the introduced gene limits utilization of these vectors in the clinical setting. Ongoing research is focused on addressing these issues,126–129 in an effort to make the lentiviral and retroviral vectors more palatable to regulatory agencies.

Adenoviruses deliver their genomes to the nucleus of both dividing and nondividing cells, are relatively cheap to produce in high titers, and have a broad tropism to target cells especially within the cardiovascular system, which makes them widely used in myocardial gene therapy.130 However, virus-specific cellular immune responses eventually lead to destruction of the adenoviral genetically modified cells131 that can provoke adenoviral-induced myocarditis.132 As such, the temporal expression of adenoviral-encoded proteins is relatively short lived (10–14 days). As of May 2001, 532 adenoviral gene therapy protocols had been approved for evaluation in clinical trials conducted predominantly in oncological patients; however, only 5 of these trials had been evaluated in phase III testing. Multiple side effects including fever, chills, shivering, myalgias, and even death were reported in these clinical trials.133 As long as the inherent problem of high immunogenicity of these vectors remains unsolved, their production and application will remain restricted essentially to experimental and academic purposes.

The contemporary virus of choice is the adeno-associated virus (AAV) that is able to infect nondividing human cells, stably integrate into a specific locus on chromosome 19,134 shows serotype-specific tissue tropism to direct expression,135 and thus far shows no reported pathological consequences. AAVs have been used in numerous clinical trials,136–139 including treatment of heart failure by increasing cardiac myocyte contractility in 2007.140 Balancing these positive attributes, AAV vectors are hampered by restricted carrying capacity for DNA, are more challenging to produce in high titer than other viral vector types, and their viral backbones render them susceptible to eventual epigenetic modification.

Development of minicircles for gene delivery shows promise as a viable option for DNA delivery in engineering of stem cells. Minicircles are episomal DNA vectors produced as circular expression cassettes devoid of any bacterial plasmid DNA backbone. Their smaller molecular size enables more efficient transfections and offers sustained expression over a period of weeks as compared with standard plasmid vectors that only work for a few days. By virtue of the production methodology in minicircle creation, the expression plasmid no longer contains the bacterial origin of replication or the antibiotic resistance markers. Thus, delivering only the minicircles to cells lengthens the expression of the transgene over traditional transient transfections of plasmids. For dividing cells, expression of the minicircles lasts up to 14 days. For nondividing cells, expression drops slightly after the first week but then can continue expressing the transgenes for months. The lack of a bacterial backbone, the small size of the vector, potential expression duration of months, lack of genomic integration, and low cost of production make this delivery technique superior to viral delivery methods for ex vivo gene delivery involved in autologous stem cell modification. Moreover, the ability to produce minicircle vectors in bacterial expression systems devoid of animal by-products such as serum together with the ability to perform high-quality, good manufacturing practice to control for batch-to-batch quality makes them attractive from a regulatory perspective. However, efficiency of transfection for stem cells may be variable, and the persistence of transfected DNA in the context of an adoptively transferred adult stem cell population remains unknown at present.

Intramyocardial injection of viral vectors has led to transgene expression in a variety of organs other than the hearts such as the thymus, lung, and liver.141–144 Delivering specificity to myocardial gene therapy involves incorporation of targeting mechanisms to restrict transgene expression. Viral vector tropism has been altered through insertion of peptide ligands,145 hypervirulent envelope glycoproteins,146 or antibodies to specific cell targets.147 Modification of viral envelope can disrupt natural fusion with the host cell, leading to reduced viral titers and transduction efficiencies.145–147 Cell-type–specific promoters, which avoid expression in nontarget cells, improve efficacy and safety of gene transfer with myocardial specific promoters; myosin light chain, α-myosin heavy chain, and cardiac Troponin T.148–151 Although specificity of tissue-specific promoters remains a major concern for in vivo use of vectors, genetically stable stem cell lines are engineered ex vivo and then subsequently introduced. As such, tissue specificity of promoters might be an additional gain by restricting prosurvival or proliferative signals to myocardial or vascular progeny of the adoptively transferred cell population, thereby diminishing fears of oncogenic transformation for undifferentiated cells.

Epilogue

By prevailing over all obstacles and distractions, one may unfailingly arrive at his chosen goal or destination.

—Christopher Columbus

Success in the future of stem cell therapy for currently incurable conditions rests primarily in maintaining the unshakable faith espoused by Columbus. Advances in the field of regenerative medicine are coming fast and furious, both in the figurative and literal sense of those words. Controversies and disagreements are to be expected in any endeavor as complex and perplexing as stem cell research, especially in view of the high stakes placed on funding the “magic bullet” that will hit the target of clinically relevant intervention. This review has focused predominantly on adult stem cells simply because from a clinical perspective that population is far more advanced than ESC or iPS cell types, not because adult-derived cells are the “best” type of cell. With decades of successful bone marrow reconstitution procedures now commonplace in hospitals, it is clear that adult stem cell therapy
works, but can it work in a structurally complex tissue such as the heart, where proliferative activity is so limited? That question lies at the root of a massive international effort to understand the signals and cues necessary to coax stem cells into functionally relevant cardiac engraftment now entering the second decade of study.

The journey to a New World of regenerative medicine has been phenomenally productive, as evidenced by a quick scan of the more than 7500 references available today in a PubMed search for the key words “cardiac, stem cell, heart,” with almost 1800 of those references being review articles to summarize our current understanding—all this for a field of research that was essentially nonexistent a little over a decade ago. In view of this overwhelming body of literature, it seems pointless to debate whether cardiac regeneration occurs, as that question has now been asked and affirmatively answered many times.1424 Circulation Research 2011;109:2658–2665.

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Disclosures

None.

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