ANTIOXIDANTS & REDOX SIGNALING
Volume 13, Number 12, 2010

DOI: 10.1089/ars.2010.3388

FORUM REVIEW ARTICLE

De Novo Myocardial Regeneration: Advances and Pitfalls

Khawaja Husnain Haider, Stephanie Buccini, Rafeeq P.H. Ahmed, and Muhammad Ashraf

Abstract

The capability of adult tissue-derived stem cells for cardiogenesis has been extensively studied in experimental animals and clinical studies for treatment of postischemic cardiomyopathy. The less-than-anticipated improvement in the heart function in most clinical studies with skeletal myoblasts and bone marrow cells has warranted a search for alternative sources of stem cells. Despite their multilineage differentiation potential, ethical issues, teratogenicity, and tissue rejection are main obstacles in developing clinically feasible methods for embryonic stem cell transplantation into patients. A decade-long research on embryonic stem cells has paved the way for discovery of alternative approaches for generating pluripotent stem cells. Genetic manipulation of somatic cells for pluripotency genes reprograms the cells to pluripotent status. Efforts are currently focused to make reprogramming protocols safer for clinical applications of the reprogrammed cells. We summarize the advancements and complicating features of stem cell therapy and discuss the decade-and-a-half-long efforts made by stem cell researchers for moving the field from bench to the bedside as an adjunct therapy or as an alternative to the contemporary therapeutic modalities for routine clinical application. The review also provides a special focus on the advancements made in the field of somatic cell reprogramming. *Antioxid. Redox Signal.* 13, 1867–1877.

Introduction

ISCHEMIC HEART DISEASE is the leading cause of death and morbidity worldwide (2). The massive loss of functioning cardiomyocytes subsequent to infarction episode greatly reduces the normal cardiac function. Additionally, the ischemic region is infiltrated by inflammatory cells and remains filled with inflammatory cytokines that can damage the surrounding myocardium. Irreversible fibrous scar tissue fills in the injured area in the heart as a part of the intrinsic repair mechanism (108). Although the scar formation maintains structural integrity, it lacks the properties of healthy cardiomyocytes and therefore remains electromechanically disconnected from the surrounding myocardium (11, 44). Contemporary treatment options for ischemic heart disease only provide symptomatic relief and none are curative in terms of addressing the root cause of the problem (96). In this regard, last decade has seen the emergence of stem cell-based therapeutic approach that holds the promise of *de novo* myocardial regeneration and replaces the damaged myocardium with new functionally competent myocytes and improves regional blood flow. Despite immense progress made in this regard, the ideal stem cell type, with best physiological behavior and differentiation characteristics together with ease of availability and safety, remains largely unknown. Moreover, the current protocols for isolation, propagation, processing, and transplantation have not yet been fully optimized to exploit fully the therapeutic potential of stem cells. We summarize the data published from various research groups and the current progress on the use of various types of stem and progenitor cells for myocardial repair.

Adult Stem Cells in Cardiovascular Therapy

Stem cells derived from different adult tissues have been extensively assessed for their regenerative potential in both small as well as large experimental animal models of myocardial ischemia (22, 26, 33, 37, 38, 58, 62, 91). These studies provided sufficient evidence for the safety, feasibility, and effectiveness of cell therapy approach that, in most instances, showed attenuated infarct size and improvement in the indices of the left ventricular contractile function. Although the actual mechanism of the functional outcome remains contentious and is considered as multifactorial (23, 27, 30, 41, 52, 116), it was generally reported that both the cell types after transplantation differentiated to adopt myogenic phenotype, and improved angiogenic response and restoration of regional blood flow in the infarcted myocardium (6, 59, 78, 93, 115, 116). The first-in-man cellular cardiomyoplasty was performed with skeletal muscle-derived myoblasts (72). The encouraging results of this study paved the way for subsequent clinical studies that mostly involved either skeletal

Department of Pathology, University of Cincinnati, Cincinnati, Ohio.

myoblast or bone marrow-derived cell transplantation either as an adjunct therapy to the routinely used revascularization procedures or as a sole therapy (32, 95, 97, 102, 106, 107). These studies have been carried out in various clinical centers worldwide and provided evidence of safety and feasibility of cell therapy approach. Encompassing their advantages, both skeletal myoblsts and bone marrow stem cells have near-ideal characteristics as donor cells for the heart cell therapy. However, there are issues that need to be addressed before their routine clinical use. The arrythmogenic nature of skeletal myoblasts due to lack of electromechanical integration with the host myocytes postengraftment in the heart remains a cause for concern (28, 31). Moreover, both skeletal myoblasts and bone marrow stem cells are heterogeneous in nature, and therefore it remains difficult to ascertain the actual sublineages of the regenerating cells. Similarly, more recent studies have questioned the myogenic potential of bone marrow-derived stem cells and reported this as limited (1, 4, 77, 85) although this potential has been reaffirmed by others (42, 47, 55, 93). Similar to skeletal myoblasts, a controversy about safety of bone marrow cells has raised concerns about their clinical use (17). Studies have shown that due to their multilineage potential, taking cues from the cytokine-rich microenvironment of the infarcted heart wherein fibrosis is rampant as part of the intrinsic repair process, bone marrow stem cells may adopt unwanted phenotypes (127). It is therefore important to identify sublineages of both skeletal myoblasts and the bone marrow cells with therapeutic potential and define their biological properties before these cells can be used in patients. Alternatively, skeletal myoblast engraftment may be combined with bone marrow stem cell transplantation for mutual beneficial effect of the two cell types (71). Studies are already underway to subfractionate and purify more primitive populations from the two cell types and showing multilineage differentiation potential, including cardiac lineage (114).

Cardiac Stem/Progenitor Cells

The existence of resident cardiac/progenitor cell population in the heart dates back to the pioneering work by Anversa and colleagues, who isolated c-kit$^+$/Lin$^-$ cells from rat hearts that showed self-renewal, clonogenicity, and multipotentiality by differentiating into myogenic cell, smooth muscle cell, or endothelial cell lineages (7). These data were later supported by other research groups (14, 67, 73, 86, 88, 113). Several studies have independently described cardiac stem/progenitor cell isolation from various species, including murine, rat, feline, pig, dog, and human, that display stem/progenitor cell characteristics (5, 18, 60, 73, 88, 117). These cells have been identified on the basis of surface markers such as stem cell antigen-1$^+$, c-kit$^+$, or side population or on transcription factors Mesoderm posterior-1, T box-18, Nkx2.5, Islet-1 (Isl1) and their ability to form cardiospheres. More recently, a diverse set of human fetal Isl1$^+$ cardiovascular progenitors has been identified that are capable of self-renewal and expansion before differentiation to form cardiac, smooth muscle, and endothelial cell lineages (12). The origin of these cells, however, is unknown, but it is considered that the different putative adult cardiac stem and progenitor cells represent different developmental and/or physiological stages of a unique resident adult cardiac stem cell (24). The results from experimental animal studies have established cardiac stem/progenitor cells as candidates for cardiac regeneration and support an approach in which the heart's own stem cells could be collected, expanded, and stored for subsequent therapeutic repair (21, 101, 118). Molecular studies imply Notch1 receptor as the mediator of cardiac fate commitment of cardiac stem/progenitor cells *via* Nkx2.5 upregulation (10). Despite encouraging results, there are some issues to solve for future success. The definition of cardiac stem/progenitor cells is still ambiguous. One of the main reasons of this uncertainty is the difference in the isolation and evaluation methods in the individual studies. Moreover, to translate the knowledge about cardiac stem/progenitor cells for clinical applications, the difference between various subpopulations of these cells among various species should be determined.

Embryonic Stem Cells

Embryonic stem (ES) cells are the most primitive and versatile stem cells from the inner cell mass in the blastocyst with enormous developmental potential (98). ES cells show excellent stem cell characteristics of clonality, self-renewal, and pluripotency to differentiate into all the three primary germ layers, ectoderm, mesoderm, and endoderm (39, 99). In comparison with the adult stem cells (found in the adult tissues) that are multipotent, the most distinguishing feature of ES cells is their pluripotent nature, which enables these cells to generate all cell types in the body. Extensive studies that involved analyses of numerous human and mouse ES cell lines have shown generic similarities and differences between the two types of ES cells at both the transcriptional and functional level. Gene expression profiling of mouse and human ES cells by immunocytochemistry, reverse transcription polymerase chain reaction, and membrane-based focused cDNA array analysis has shown that these cells, despite many similarities, show species specificity for the expression of vimentin, beta-III tubulin, alpha-fetoprotein, eomesodermin, Hela E box, aryl hydrocarbon receptor nuclear translocator, and Forkhead box D3, as well as in expression of the leukemia inhibitory factor receptor complex LIFR/IL6ST (gp130) besides cycle regulation, control of apoptosis, and cytokine expression (35).

The presence of various populations of cardiac progenitor cells expressing Brachyury/Flk1, Isl1/Flk1/Nkx2-5, cKit/Nkx2-5, or Nkx2-5 have been reported in ES cells that can differentiate to adopt cardiac, smooth muscle, and endothelial phenotypes (20, 48, 75). The intramyocardially delivered ES cells successfully engraft in the recipient infarcted heart, differentiate to adopt cardiac phenotype that develop electrophysiological coupling with the host myocytes, and positively influence the cardiac structure with significant contractile function recovery of the heart (16, 50, 57, 80). Human ES cell-derived cardiomyocytes formed human myocardium after transplantation into the noninfarcted hearts of immunosuppressed pigs and developed as pacemakers, which indicated their ability to electrically integrate with the host myocytes (51). Besides, it is believed that improvement in myocardial contractility is related with the release of cardioprotective and cardiac differentiation factors by the transplanted ES cells (74, 100). ES cell-derived cardiomyocytes exhibit characteristics of early chamber myocardium and show significantly higher proliferative potential *in vitro* as compared with the adult heart-derived cardiomyocytes (29, 57, 68).

Despite the obvious advantage of cardiogenic potential, the use of ES cells is not without limitations (Table 1). The main obstacles associated with the therapeutic applications of ES cells are the immunological rejection of ES cell-derived myocytes, their tendency to form teratomas when injected *in vivo*, besides the ethical issues involved in their availability (84). Various strategies have been successfully adopted to address these issues. Genetic selection of differentiated ES cells or their partial differentiation into cardiomyocytes or endothelial cells *in vitro* before engraftment has given encouraging results in experimental animal models (13, 14, 34, 76). Further insight from the heart development studies and discovery of the signaling pathways involved in ES cell differentiation will facilitate isolation of the pure population of cardiomyocytes, thereby limiting the risk of teratoma formation. Although the mechanism of spontaneous beating of ES cell-derived cardiomyocytes needs to be explored in-depth, earlier studies have shown that expression of cardiac specific gene markers in the spontaneously differentiating cardiomyocytes occurred in a sequential manner in line with the cardiac development (94). Besides the gene expression profile, the electrophysiological characteristics of early ES cell-derived cardiomyocytes, which typically resemble with primary myocardium, differ from the terminally differentiated ES cell-derived cardiomyocytes that are more comparable with the postnatal cardiomyocytes (9). Although ES cells provide us with a valuable tool for cardiac regeneration and much advancement has been made in overcoming the technical limitations and hindrances in the use of ES cells, the ethical and moral issues regarding their availability still warrant the need to search for an alternative source of stem cells.

Induced Pluripotent Stem Cells

One way to avoid the problems associated with the use of ES cells is to reprogram somatic cells for induction of pluripotency (Fig. 1). Somatic cell reprogramming is not an entirely a new idea. Earlier work established that somatic cells can be reprogrammed by methods such as nuclear transfer or by fusion with ES cells (66, 109). These studies indicated that some unidentified factors in ES cells were responsible for the maintenance of their undifferentiated self-renewal and pluripotent status. Takahashi and Yamanaka were the first to postulate that these factors also played an important role in inducing pluripotency in somatic cells (112, 124). After selecting some 24 candidate transcription factor genes, mouse embryonic fibroblasts and adult tail tip fibroblasts were isolated with a drug resistance marker inserted into the *Fbx15* gene, a gene that is expressed downstream of Octamer 4 (Oct4) in mouse ES cells. Using Maloney-based retroviral vectors, they reported that ectopic expression of a combination of Oct4, *Sry* box containing gene 2 (Sox2), Kruppel like factor-4 (Klf4), and c-myelocytomatosis (c-Myc) was adequate to reprogram mouse fibroblasts to a pluripotent state that resembled mouse ES cells, though at a low reprogramming efficiency. These cells were later termed as induced pluripotent cells (iPS cells). The iPS cells thus generated through induction of a set of pluripotency defining transcription factors were morphologically similar to mouse ES cells and expressed ES cell marker genes. Interestingly, Nanog, a transcription factor that shares many target genes with Oct4 and Sox2 in ES cells, was dispensable for somatic cell reprogramming (125).

Table 1. Comparison of Induced Pluripotent Cells and Embryonic Stem Cells as Donor Cells

iPS cells	*Es cells*
Pluripotent	Pluripotent
—	Ethical issues
Availability from autologous source	—
—	Immunogenic considerations
Patient/disease specific	—
Cardiac differentiation	Cardiac differentiation

iPS, induced pluripotent stem cells; ES, embryonic stem cells.

Although mouse iPS cells obtained using Fbx15 selection expressed some ES cell marker genes, they were not identical to ES cells (112). The Fbx15-selected iPS cells did not display the exact gene expression and DNA methylation patterns as ES cells, and these cells did not contribute to the formation of normal adult chimeric mice (87). To circumvent this issue, reactivation of either Nanog or Oct4 was used as a more stringent selection marker. This allowed the isolation and expansion of fully reprogrammed cells (63, 87, 120). In another new development, iPS cells have been generated from adult mouse hepatocytes and gastric epithelial cells using Fbx15 selection and these cells contributed to adult chimeras (3).

Generation of Human iPS Cells

The generation of iPS cells from mouse fibroblasts paved the way for developing a system that could be extrapolated to produce iPS cells from human somatic cells, thus allowing patient-specific iPS cell generation (90). More specifically, human iPS cells may provide an alternative source of stem cells for therapeutic use in patients. Although the methods for generation and maintenance of human iPS cells and mouse iPS cells are similar, the latter requires some modifications to improve efficiency and safety of the protocols for human use of the cells. For example, it is imperative to maintain xeno-free culture conditions for generation, maintenance, and propagation of human iPS cells (110). Additionally, human somatic cells can be more difficult to reprogram, especially when only a limited number of cells can be obtained from a patient. One major issue with the iPS cell reprogramming protocol was that genetic alteration of target cells with drug resistance gene driven by ES cell markers was required to select the reprogrammed cells. Recent studies have shown that iPS cells could be generated from mouse embryonic fibroblasts that were genetically unaltered for drug resistance marker inserted into either the Oct4 or Nanog loci (45, 69). The cells were generated and expanded solely based on their colony characteristics and closely resembled ES cells in morphology and at molecular level. The efficiency of mouse iPS protocols to obtain human iPS cells from human fibroblasts has already been reported (90, 111). Pluripotency induction in human fibroblasts has also been achieved with the addition of Nanog to the original four factors and without using drug selection. Alternatively, human fibroblasts have been re-programmed by using a different set of Oct4, Sox2, Nanog, and LIN28 transcription factors and expressed human ES cell markers, displayed normal karyotypes, and differentiated into the three germ layers (128). Given that reactivation of the c-Myc virus contributed to

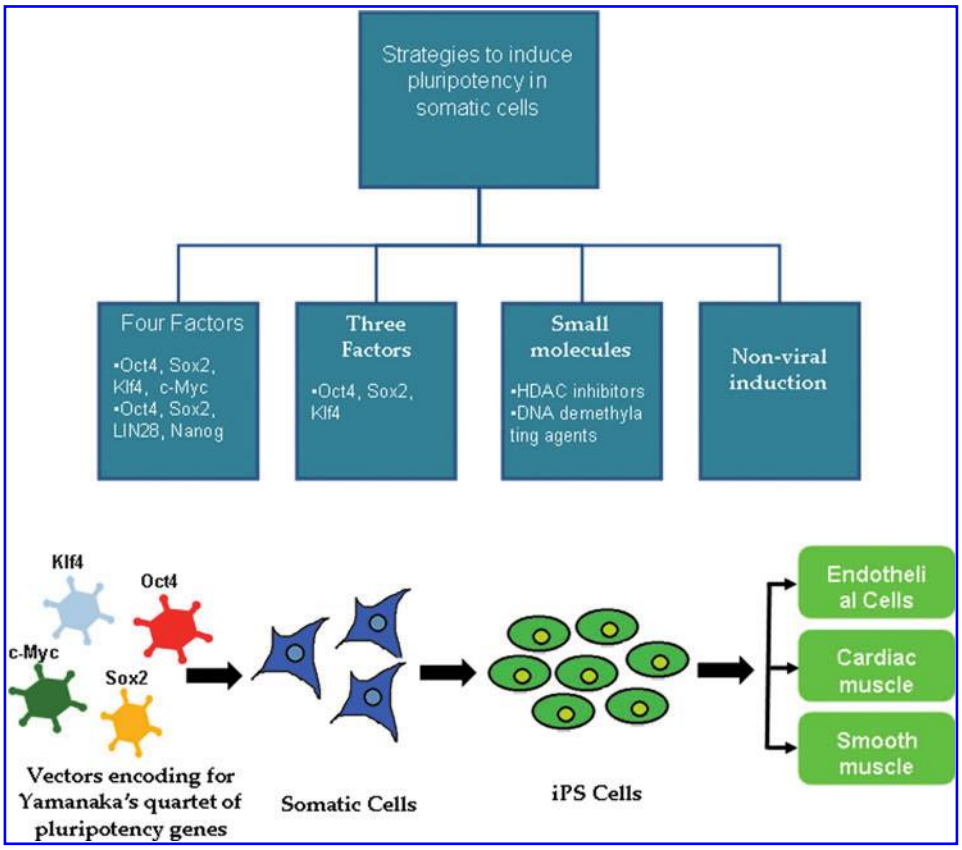

FIG. 1. Methods of iPS cell generation. (A) Flow diagram showing general classification of the methods and **(B)** a simplified overview to generate iPS cells by induction of pluripotency genes. C-Myc, myelocytomatosis; HDAC, histone deacetylase; Klf4, Kruppel like factor-4; iPS, induced pluripotent cells; Sox2, *Sry* box containing gene 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

increased tumorgenicity in the iPS cell-derived chimeras (87), efforts are underway to exclude c-Myc from the select quartet of pluripotency factors to curtail tumorgenicity of iPS cells. Some research groups in this regard have already shown that c-Myc was dispensable for somatic cell reprogramming, and therefore iPS cells may be generated using only three factors (79, 120). The three-factor iPS cells were equivalent to the previously described four-factor iPS cells, though with less incidence of tumor formation than chimeras derived from four-factor iPS cells. Nevertheless, the reprogramming process was significantly slowed and much less efficient as compared to using four factors (79). A recent study has shown that iPS cells reprogrammed by the exclusion of c-Myc from Yamanaka quartet develops innate cardiogenic potential with an as yet undefined mechanism (64).

Advances in Pluripotency Induction Protocols

Although the risk of tumorigenicity is considerably reduced by removal of c-Myc from the group of reprogramming factors, the safety concerns for human use of iPS cells due to multiple transductions with retroviral and lentiviral vectors for expression of putative oncogenes need to be addressed (79, 104). Incidentally, fibroblasts and human keratinocytes have been reprogrammed with a drug-inducible polycistronic lentivirus containing all four reprogramming factors (15, 103). The use of autonomous self-cleaving 2A peptides approach in a single polycistronic vector instead of internal ribosomal entry sites helped to overcome the problem of nonstoichiometric and low level expression of multiple genes under the control of one promoter. Not only did the polycistronic lentiviral vector approach enhance the efficiency of the reprogramming procedure, but it was also significant in terms of alleviating the problem of insertional mutagenesis due to multiple pro-viral integrations. Although, the use of adenoviral vectors with low frequency of genomic integration may be a better choice in this regard (105), protocols have also been designed to eliminate the use of viral vector delivery systems altogether and use transient trasfection methods employing nonviral vectors. Repeated transfections of mouse embryonic fibroblasts with two separate plasmids, one encoding for Oct4, Sox2, and Klf4, and the other for c-Myc, successfully reprogrammed the cells into iPS cells (87). The nonviral approach has been further refined to develop a single multigene expression vector containing the four factors and was successfully used to reprogram mouse and human fibroblasts (46). Although the reprogramming efficiency of nonviral vector protocol is still low, the biggest advantage of nonviral vector approach is the complete elimination of exogenous reprogramming factors in the iPS cells. Further, nonviral vector approach reduces the safety concern for iPS cell generation and their clinical application. Efforts are underway to develop nonviral vector-based protocols to enhance their reprogramming efficiency.

Another nonviral alternative is the use of small molecule compounds that cause global reversion of the somatic epigenome into an ES-like state (63). Small molecules such as inhibitors of histone deacetylase and DNA demethylation agents can improve reprogramming efficiency of somatic cells. This approach was therefore combined with the three factor transduction approach to improve the efficiency of reprogramming. Mouse fibroblasts that were transduced with

the three reprogramming factors (excluding c-Myc) and treated with valproic acid showed increased reprogramming efficiency compared to the control fibroblasts that were reprogrammed with four factors without valproic acid treatment (40). Another approach gaining popularity is the search for cells having endogenous high level expression of one or more pluripotency markers that may allow simplifying the reprogramming protocol. Mouse neural stem cells with higher endogenous Sox2 and Klf4 levels were reprogrammed by exogenous expression of either Oct4 alone or together with c-Myc (53, 54). Although adult stem cells may have a higher reprogramming efficiency due to the less differentiated state of these cells compared to somatic cells, it would be difficult to obtain neural stem cells from patients. To circumvent the availability issues, the authors have opted for bone marrow-derived stem cells and skeletal muscle-derived myoblasts and successfully reprogrammed the cells into iPS cells using Yamanaka quartet of factors. Using bone marrow-derived stem cells from Oct4-green fluorescence protein^{+} transgenic mouse, we have successfully generated iPS cells lines that were identified on the basis of their ES cell-like morphology and expression of green fluorescence protein postsuccessful reprogramming (Figs. 2 and 3). Both skeletal myoblasts as well as bone marrow-derived stem cells are readily available from patients in sufficient number and without ethical issues. This will ensure iPS cells from an autologous source in the clinical setting. The lower level differentiation status of both bone marrow stem cells and skeletal myoblasts as compared to the terminally differentiated fibroblasts makes these cells better candidates for reprogramming with fewer factors.

Cardiogenesis with iPS Cells

There is considerable interest in the use of iPS cells and iPS cell-derived cardiac progenitors and cardiomyocytes as an alternative cell source for myocardial repair. Various research groups have already shown that similar to ES cells, iPS cells differentiate into functional cardiomyocytes that may

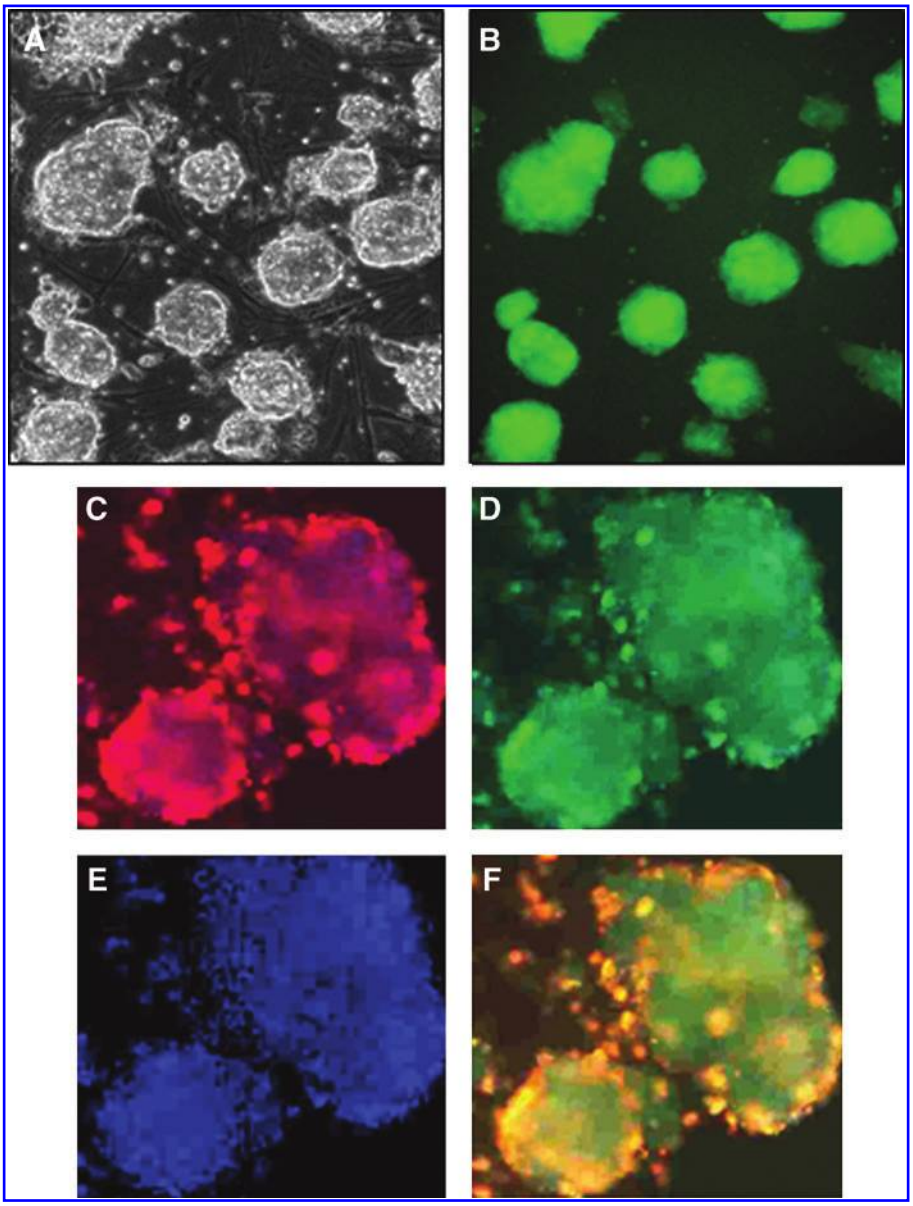

FIG. 2. iPS cells derived from bone marrow cells. (A) Phase-contrast and **(B)** GFP fluorescent photomicrographs of iPS cells derived from mouse bone marrow cells showing an embryonic stem cell-like morphology. Bone marrow was isolated from Oct4-GFP mice and reprogrammed using transduction with viral vectors encoding for Oct4, Sox2, Klf4, and c-Myc. Only the colonies expressing endogenous Oct4 (GFP-positive) were expanded to obtain a pure population of iPS cells. **(C–F)** Fluorescent immunostaining of bone marrow cell-derived iPS cells for expression of Sox2 (red; **C**). Endogenous Oct4 expression (GFP green; **D**) continued during *in vitro* expansion of iPS cells. The nuclei were observed by DAPI staining (blue; **E**). Figure 3F represents the merged image of **A–C** (original magnifications = 200×). GFP, green fluorescence protein. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

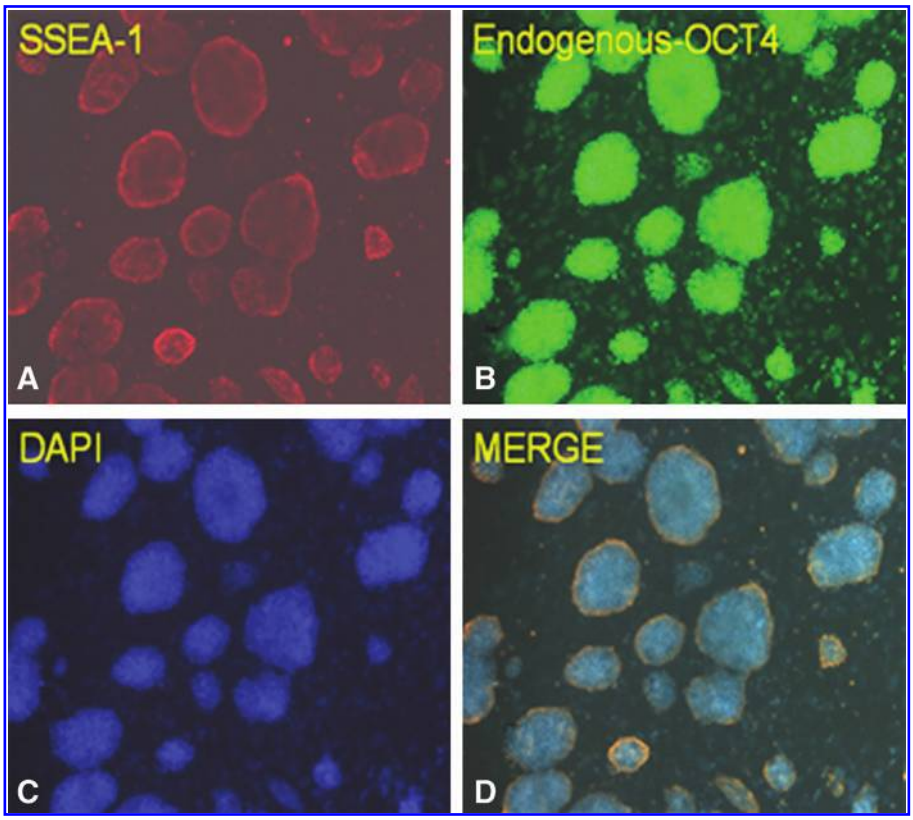

FIG. 3. Characterization of bone marrow iPS cells for pluripotency. Fluorescent immunostaining of bone marrow cell-derived iPS cells for expression of pluripotency stage-specific embryonic antigen-1 (SSEA-1; red, **A**). Endogenous Oct4 expression (GFP green; **B**) continued during *in vitro* expansion of iPS cells. **(C)** The nuclei were observed by 4′,6-diamidino-2-phenylindole staining. **(D)** shows the merged image (original magnifications = 400×). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

integrate and electromechanically couple with cardiomyocytes (8, 81). *In vitro* studies showed that iPS cells formed functional cardiomyocytes with established hormonal regulation pathways and functionally expressed cardiac ion channels besides adopting ventricular phenotype (56). Electrophysiology studies indicated that iPS cells have a capacity like ES cells for differentiation into nodal-, atrial-, and ventricular-like phenotypes based on action potential characteristics. Both iPS and ES cell-derived cardiomyocytes exhibited responsiveness to beta-adrenergic stimulation as was apparent from an increased spontaneous rate and decreased action potential duration (129).

Most of the studies published in this regard have focused on the *in vitro* differentiation of iPS cells, with only a few studies focusing on the *in vivo* behavior and myocardial reparability of the transplanted iPS cells. Nelson *et al.* have recently shown that intramyocardially delivered iPS cells engrafted and survived in the immunocompetent host myocardium without disrupting the cyto-architecture (81). These iPS cells were developed from murine fibroblasts after viral induction with Yamanaka quartet of pluripotency genes and they also showed inherent cardiogenic potential. The allogenic iPS cells thus engrafted in the immunocompetent murine model of acute myocardial infarction rescued the postischemic myocardial structure and contractile function in comparison with parental fibroblast injection. Immunoshistochemistry provided evidence of their myogenic and endothelial differentiation in the heart. However, the study results did not show their coupling with the host myocytes. The same group of researchers has also reported that iPS cells, with proficient cardiogenic potential, may also be generated by three factor induction without c-Myc (65).

Future Directions

One of the fundamental challenges facing stem cell therapy in cardiovascular therapeutics is selection of the right type of cells with ideal characteristics in terms of biology, differentiation potential, and ease of availability without ethical issues. To date, only skeletal myoblasts and bone marrow-derived stem cells have made it to clinical use. The recent changes in National Institutes of Health policy regarding the use of ES cells and the groundbreaking discovery that somatic cells can be reprogrammed to generate pluripotent stem cells have significantly enhanced the interest of researchers in the use of pluripotent stem cells in regenerative medicine. The biggest advantage of iPS cells would be the availability of patient-specific/disease-specific cells from an autologous source (43, 49, 89, 121). Moreover, due to realization on part of the research community that stem cell therapy alone may be insufficient to address the multi-aspects of the disease process in the infarcted myocardium, efforts are now underway to adopt a combinatorial treatment approach (37, 61, 82, 126). Gene therapy, which was previously considered rival emerging therapeutic intervention to stem cell therapy, is now being successfully combined to get the best of the two approaches (36, 83, 119).

iPS cell technology is still in its infancy. For its progress and adoption for clinical use, there are some fundamental issues that need to be resolved. The issues of viral vectors and genetic manipulation involved during generation of iPS cells are required to be replaced with safer methods of induction because the future lies in generating iPS cells without genetic manipulation to make it safer for human use. The most recent discovery of micro RNAs (miRs; a select group of small,

noncoding RNAs) that regulate cell functionality is being combined with stem cell therapy to enhance their post-engraftment characteristics such as survival, differentiation, and paracrine behavior (19, 45, 70, 122). Given the pivotal role of miRs in determining the primitive nature of stem cells, reprogramming protocols based on miR manipulation are therefore being developed (70, 123). These strategies would curtail teratogenicity of the iPS cells thus generated, which can further be alleviated by partial differentiation of iPS cells before engraftment. Likewise, the regenerative potential of iPS cells can best be exploited with more information on the properties of the cell type. Hence, in-depth studies are required to characterize the physiological behavior and electromechanical properties of iPS cells and iPS cell-derived cardiomyocytes. Similar to ES cells (25), one of the important features of iPS cells is that in their undifferentiated status, iPS cells have a very small number of mitochondria and mitochondrial DNA copy number. These mitochondrial changes occur during the reprogramming process wherein the somatic cell mitochondria revert to ES cell like immature morphology and distribution (92). However, following their commitment to adopt cardiac phenotype, which has quite distinct metabolic requirements as compared to the undifferentiated iPS cells, the cells would need appropriate mitochondrial changes to match the functional requirements of the new phenotype. Therefore, future research may be required to focus that differentiation protocols for iPS cells to adopt cardiac phenotype should ensure analogous maturation of mitochondria to sustain functionally effective status postengraftment in the heart.

Acknowledgments

This work was supported by National Institutes of Health (NIH) Grants # R37-HL074272, HL-080686, and HL-087246 (to M.A) and HL-087288 and HL-089535 (to Kh.H.H).

References

1. Agbulut O, Mazo M, Bressolle C, Gutierrez M, Azarnoush K, Sabbah L, Niederlander N, Abizanda G, Andreu EJ, Pelacho B, Gavira JJ, Perez-Ilzarbe M, Peyrard S, Bruneval P, Samuel JL, Soriano-Navarro M, Garcia-Verdugo JM, Hagege AA, Prosper F, and Menasche P. Can bone marrow-derived multipotent adult progenitor cells regenerate infarcted myocardium? *Cardiovasc Res* 72: 175–183, 2006.
2. AHA. Heart Disease and Stroke Statistics, 2009 Update. *Circulation* 119: e21–e181, 2009.
3. Aoi T, Yae K, Nakagawa M, Ichisaka T, Okita K, Takahashi K, Chiba T, and Yamanaka S. Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science* 321: 699–702, 2008.
4. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, and Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature* 428: 668–673, 2004.
5. Bearzi C, Rota M, Hosoda T, Tillmanns J, Nascimbene A, De Angelis A, Yasuzawa-Amano S, Trofimova I, Siggins RW, Lecapitaine N, Cascapera S, Beltrami AP, D'Alessandro DA, Zias E, Quaini F, Urbanek K, Michler RE, Bolli R, Kajstura J, Leri A, and Anversa P. Human cardiac stem cells. *Proc Natl Acad Sci USA* 104: 14068–14073, 2007.
6. Behfar A and Terzic A. Optimizing adult mesenchymal stem cells for heart repair. *J Mol Cell Cardiol* 42: 283–284, 2007.
7. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, and Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114: 763–776, 2003.
8. Blin G, Nury D, Stefanovic S, Neri T, Guillevic O, Brinon B, Bellamy V, Rucker-Martin C, Barbry P, Bel A, Bruneval P, Cowan C, Pouly J, Mitalipov S, Gouadon E, Binder P, Hagege A, Desnos M, Renaud JF, Menasche P, and Puceat M. A purified population of multipotent cardiovascular progenitors derived from primate pluripotent stem cells engrafts in postmyocardial infarcted nonhuman primates. *J Clin Invest* 120: 1125–1139, 2010.
9. Boheler KR, Czyz J, Tweedie D, Yang HT, Anisimov SV, and Wobus AM. Differentiation of pluripotent embryonic stem cells into cardiomyocytes. *Circ Res* 91: 189–201, 2002.
10. Boni A, Urbanek K, Nascimbene A, Hosoda T, Zheng H, Delucchi F, Amano K, Gonzalez A, Vitale S, Ojaimi C, Rizzi R, Bolli R, Yutzey KE, Rota M, Kajstura J, Anversa P, and Leri A. Notch1 regulates the fate of cardiac progenitor cells. *Proc Natl Acad Sci USA* 105: 15529–15534, 2008.
11. Boudoulas KD and Hatzopoulos AK. Cardiac repair and regeneration: the Rubik's cube of cell therapy for heart disease. *Dis Model Mech* 2: 344–358, 2009.
12. Bu L, Jiang X, Martin-Puig S, Caron L, Zhu S, Shao Y, Roberts DJ, Huang PL, Domian IJ, and Chien KR. Human ISL1 heart progenitors generate diverse multipotent cardiovascular cell lineages. *Nature* 460: 113–117, 2009.
13. Bugorsky R, Perriard JC, and Vassalli G. Genetic selection system allowing monitoring of myofibrillogenesis in living cardiomyocytes derived from mouse embryonic stem cells. *Eur J Histochem* 52: 1–10, 2008.
14. Cai L, Ye Z, Zhou BY, Mali P, Zhou C, and Cheng L. Promoting human embryonic stem cell renewal or differentiation by modulating Wnt signal and culture conditions. *Cell Res* 17: 62–72, 2007.
15. Carey BW, Markoulaki S, Hanna J, Saha K, Gao Q, Mitalipova M, and Jaenisch R. Reprogramming of murine and human somatic cells using a single polycistronic vector. *Proc Natl Acad Sci USA* 106: 157–162, 2009.
16. Caspi O, Huber I, Kehat I, Habib M, Arbel G, Gepstein A, Yankelson L, Aronson D, Beyar R, and Gepstein L. Transplantation of human embryonic stem cell derived cardiomyocytes improves myocardial performance in infarcted rat hearts. *J Am Coll Cardiol* 50: 1884–1893, 2007.
17. Chang MG, Tung L, Sekar RB, Chang CY, Cysyk J, Dong P, Marban E, and Abraham MR. Proarrhythmic potential of mesenchymal stem cell transplantation revealed in an *in vitro* coculture model. *Circulation* 113: 1832–1841, 2006.
18. Chen X, Wilson RM, Kubo H, Berretta RM, Harris DM, Zhang X, Jaleel N, MacDonnell SM, Bearzi C, Tillmanns J, Trofimova I, Hosoda T, Mosna F, Cribbs L, Leri A, Kajstura J, Anversa P, and Houser SR. Adolescent feline heart contains a population of small, proliferative ventricular myocytes with immature physiological properties. *Circ Res* 100: 536–544, 2007.
19. Chivukula RR and Mendell JT. Abate and switch: miR-145 in stem cell differentiation. *Cell* 137: 606–608, 2009.
20. Christoforou N, Miller RA, Hill CM, Jie CC, McCallion AS, and Gearhart JD. Mouse ES cell derived cardiac precursor cells are multipotent and facilitate identification of novel cardiac genes. *J Clin Invest* 118: 894–903, 2008.
21. Dawn B, Stein AB, Urbanek K, Rota M, Whang B, Rastaldo R, Torella D, Tang XL, Rezazadeh A, Kajstura J, Leri A,

Hunt G, Varma J, Prabhu SD, Anversa P, and Bolli R. Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proc Natl Acad Sci USA* 102: 3766–3771, 2005.
22. Dib N, Campbell A, Jacoby DB, Zawadzka A, Ratliff J, Miedzybrocki BM, Gahremanpour A, Diethrich EB, and Opie SR. Safety and feasibility of percutaneous autologous skeletal myoblast transplantation in the coil-infarcted swine myocardium. *J Pharmacol Toxicol Methods* 54: 71–77, 2006.
23. Ebelt H, Jungblut M, Zhang Y, Kubin T, Kostin S, Technau A, Oustanina S, Niebrugge S, Lehmann J, Werdan K, and Braun T. Cellular cardiomyoplasty: improvement of left ventricular function correlates with the release of cardioactive cytokines. *Stem Cells* 25: 236–244, 2007.
24. Ellison GM, Galuppo V, Vicinanza C, Aquila I, Waring CD, Leone A, Indolfi C, and Torella D. Cardiac stem and progenitor cell identification: different markers for the same cell? *Front Biosci (Schol Ed)*2: 641–652, 2010.
25. Facucho-Oliveira JM and St John JC. The relationship between pluripotency and mitochondrial DNA proliferation during early embryo development and embryonic stem cell differentiation. *Stem Cell Rev* 5: 140–158, 2009.
26. Farahmand P, Lai TY, Weisel RD, Fazel S, Yau T, Menasche P, and Li RK. Skeletal myoblasts preserve remote matrix architecture and global function when implanted early or late after coronary ligation into infarcted or remote myocardium. *Circulation* 118: S130–S137, 2008.
27. Fazel S, Cimini M, Chen L, Li S, Angoulvant D, Fedak P, Verma S, Weisel RD, Keating A, and Li RK. Cardioprotective c-kit + cells are from the bone marrow and regulate the myocardial balance of angiogenic cytokines. *J Clin Invest* 116: 1865–1877, 2006.
28. Fernandes S, Amirault JC, Lande G, Nguyen JM, Forest V, Bignolais O, Lamirault G, Heudes D, Orsonneau JL, Heymann MF, Charpentier F, and Lemarchand P. Autologous myoblast transplantation after myocardial infarction increases the inducibility of ventricular arrhythmias. *Cardiovasc Res* 69: 348–358, 2006.
29. Fijnvandraat AC, van Ginneken AC, Schumacher CA, Boheler KR, Lekanne Deprez RH, Christoffels VM, and Moorman AF. Cardiomyocytes purified from differentiated embryonic stem cells exhibit characteristics of early chamber myocardium. *J Mol Cell Cardiol* 35: 1461–1472, 2003.
30. Formigli L, Perna AM, Meacci E, Cinci L, Margheri M, Nistri S, Tani A, Silvertown J, Orlandini G, Porciani C, Zecchi-Orlandini S, Medin J, and Bani D. Paracrine effects of transplanted myoblasts and relaxin on post-infarction heart remodelling. *J Cell Mol Med* 11: 1087–1100, 2007.
31. Fouts K, Fernandes B, Mal N, Liu J, and Laurita KR. Electrophysiological consequence of skeletal myoblast transplantation in normal and infarcted canine myocardium. *Heart Rhythm* 3: 452–461, 2006.
32. Gavira JJ, Herreros J, Perez A, Garcia-Velloso MJ, Barba J, Martin-Herrero F, Canizo C, Martin-Arnau A, Marti-Climent JM, Hernandez M, Lopez-Holgado N, Gonzalez-Santos JM, Martin-Luengo C, Alegria E, and Prosper F. Autologous skeletal myoblast transplantation in patients with nonacute myocardial infarction: 1-year follow-up. *J Thorac Cardiovasc Surg* 131: 799–804, 2006.
33. Ghostine S, Carrion C, Souza LC, Richard P, Bruneval P, Vilquin JT, Pouzet B, Schwartz K, Menasche P, and Hagege AA. Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction. *Circulation* 106: I131–I136, 2002.
34. Gimond C, Marchetti S, and Pages G. Differentiation of mouse embryonic stem cells into endothelial cells: genetic selection and potential use *in vivo*. *Methods Mol Biol* 330: 303–329, 2006.
35. Ginis I, Luo Y, Miura T, Thies S, Brandenberger R, Gerecht-Nir S, Amit M, Hoke A, Carpenter MK, Itskovitz-Eldor J, and Rao MS. Differences between human and mouse embryonic stem cells. *Dev Biol* 269: 360–380, 2004.
36. Gnecchi M, He H, Melo LG, Noiseaux N, Morello F, de Boer RA, Zhang L, Pratt RE, Dzau VJ, and Ingwall JS. Early beneficial effects of bone marrow-derived mesenchymal stem cells overexpressing Akt on cardiac metabolism after myocardial infarction. *Stem Cells* 27: 971–979, 2009.
37. Haider H, Jiang S, Idris NM, and Ashraf M. IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1alpha/CXCR4 signaling to promote myocardial repair. *Circ Res* 103: 1300–1308, 2008.
38. Haider H, Ye L, Jiang S, Ge R, Law PK, Chua T, Wong P, and Sim EK. Angiomyogenesis for cardiac repair using human myoblasts as carriers of human vascular endothelial growth factor. *J Mol Med* 82: 539–549, 2004.
39. Hodgson DM, Behfar A, Zingman LV, Kane GC, Perez-Terzic C, Alekseev AE, Puceat M, and Terzic A. Stable benefit of embryonic stem cell therapy in myocardial infarction. *Am J Physiol Heart Circ Physiol* 287: H471–H479, 2004.
40. Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, Muhlestein W, and Melton DA. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol* 26: 1269–1275, 2008.
41. Inoue S, Popp FC, Koehl GE, Piso P, Schlitt HJ, Geissler EK, and Dahlke MH. Immunomodulatory effects of mesenchymal stem cells in a rat organ transplant model. *Transplantation* 81: 1589–1595, 2006.
42. Jiang S, Haider H, Idris NM, Salim A, and Ashraf M. Supportive interaction between cell survival signaling and angiocompetent factors enhances donor cell survival and promotes angiomyogenesis for cardiac repair. *Circ Res* 99: 776–784, 2006.
43. Jin ZB, Okamoto S, Mandai M, and Takahashi M. Induced pluripotent stem cells for retinal degenerative diseases: a new perspective on the challenges. *J Genet* 88: 417–424, 2009.
44. Joggerst SJ and Hatzopoulos AK. Stem cell therapy for cardiac repair: benefits and barriers. *Expert Rev Mol Med* 11: e20, 2009.
45. Judson RL, Babiarz JE, Venere M, and Blelloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. *Nat Biotechnol* 27: 459–461, 2009.
46. Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, and Woltjen K. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature* 458: 771–775, 2009.
47. Kajstura J, Rota M, Whang B, Cascapera S, Hosoda T, Bearzi C, Nurzynska D, Kasahara H, Zias E, Bonafe M, Nadal-Ginard B, Torella D, Nascimbene A, Quaini F, Urbanek K, Leri A, and Anversa P. Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell fusion. *Circ Res* 96: 127–137, 2005.
48. Kattman SJ, Huber TL, and Keller GM. Multipotent flk-1 + cardiovascular progenitor cells give rise to the cardiomyocyte, endothelial, and vascular smooth muscle lineages. *Dev Cell* 11: 723–732, 2006.

49. Kazuki Y, Hiratsuka M, Takiguchi M, Osaki M, Kajitani N, Hoshiya H, Hiramatsu K, Yoshino T, Kazuki K, Ishihara C, Takehara S, Higaki K, Nakagawa M, Takahashi K, Yamanaka S, and Oshimura M. Complete genetic correction of ips cells from Duchenne muscular dystrophy. *Mol Ther* 18: 386–393, 2010.
50. Kehat I and Gepstein L. Electrophysiological coupling of transplanted cardiomyocytes. *Circ Res* 101: 433–435, 2007.
51. Kehat I, Khimovich L, Caspi O, Gepstein A, Shofti R, Arbel G, Huber I, Satin J, Itskovitz-Eldor J, and Gepstein L. Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol* 22: 1282–1289, 2004.
52. Khan M, Kutala VK, Vikram DS, Wisel S, Chacko SM, Kuppusamy ML, Mohan IK, Zweier JL, Kwiatkowski P, and Kuppusamy P. Skeletal myoblasts transplanted in the ischemic myocardium enhance *in situ* oxygenation and recovery of contractile function. *Am J Physiol Heart Circ Physiol* 293: H2129–H2139, 2007.
53. Kim JB, Greber B, Arauzo-Bravo MJ, Meyer J, Park KI, Zaehres H, and Scholer HR. Direct reprogramming of human neural stem cells by OCT4. *Nature* 461: 649–643, 2009.
54. Kim JB, Zaehres H, Wu G, Gentile L, Ko K, Sebastiano V, Arauzo-Bravo MJ, Ruau D, Han DW, Zenke M, and Scholer HR. Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature* 454: 646–650, 2008.
55. Kudo M, Wang Y, Wani MA, Xu M, Ayub A, and Ashraf M. Implantation of bone marrow stem cells reduces the infarction and fibrosis in ischemic mouse heart. *J Mol Cell Cardiol* 35: 1113–1119, 2003.
56. Kuzmenkin A, Liang H, Xu G, Pfannkuche K, Eichhorn H, Fatima A, Luo H, Saric T, Wernig M, Jaenisch R, and Hescheler J. Functional characterization of cardiomyocytes derived from murine induced pluripotent stem cells *in vitro*. *FASEB J* 23: 4168–4180, 2009.
57. Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O'Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, and Murry CE. Cardiomyocytes derived from human embryonic stem cells in prosurvival factors enhance function of infarcted rat hearts. *Nat Biotechnol* 25: 1015–1024, 2007.
58. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, and Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci USA* 100: 7808–7811, 2003.
59. Li X, Yu X, Lin Q, Deng C, Shan Z, Yang M, and Lin S. Bone marrow mesenchymal stem cells differentiate into functional cardiac phenotypes by cardiac microenvironment. *J Mol Cell Cardiol* 42: 295–303, 2007.
60. Linke A, Muller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, Castaldo C, Cascapera S, Bohm M, Quaini F, Urbanek K, Leri A, Hintze TH, Kajstura J, and Anversa P. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. *Proc Natl Acad Sci USA* 102: 8966–8971, 2005.
61. Lu G, Haider HK, Jiang S, and Ashraf M. Sca-1 + stem cell survival and engraftment in the infarcted heart: dual role for preconditioning-induced connexin-43. *Circulation* 119: 2587–2596, 2009.
62. Ma N, Ladilov Y, Kaminski A, Piechaczek C, Choi YH, Li W, Steinhoff G, and Stamm C. Umbilical cord blood cell transplantation for myocardial regeneration. *Transplant Proc* 38: 771–773, 2006.
63. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R, Plath K, and Hochedlinger K. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* 1: 55–70, 2007.
64. Martinez-Fernandez A, Nelson TJ, Ikeda Y, and Terzic A. c-MYC independent nuclear reprogramming favors cardiogenic potential of induced pluripotent stem cells. *J Cardiovasc Transl Res* 3: 13–23, 2010.
65. Martinez-Fernandez A, Nelson TJ, Yamada S, Reyes S, Alekseev AE, Perez-Terzic C, Ikeda Y, and Terzic A. iPS programmed without c-MYC yield proficient cardiogenesis for functional heart chimerism. *Circ Res* 105: 648–656, 2009.
66. Matsumura H and Tada T. Cell fusion-mediated nuclear reprogramming of somatic cells. *Reprod Biomed Online* 16: 51–56, 2008.
67. Matsuura K, Nagai T, Nishigaki N, Oyama T, Nishi J, Wada H, Sano M, Toko H, Akazawa H, Sato T, Nakaya H, Kasanuki H, and Komuro I. Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *J Biol Chem* 279: 11384–11391, 2004.
68. McDevitt TC, Laflamme MA, and Murry CE. Proliferation of cardiomyocytes derived from human embryonic stem cells is mediated via the IGF/PI 3-kinase/Akt signaling pathway. *J Mol Cell Cardiol* 39: 865–873, 2005.
69. Meissner A, Wernig M, and Jaenisch R. Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nat Biotechnol* 25: 1177–1181, 2007.
70. Melton C, Judson RL, and Blelloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. *Nature* 463: 621–626, 2010.
71. Memon IA, Sawa Y, Miyagawa S, Taketani S, and Matsuda H. Combined autologous cellular cardiomyoplasty with skeletal myoblasts and bone marrow cells in canine hearts for ischemic cardiomyopathy. *J Thorac Cardiovasc Surg* 130: 646–653, 2005.
72. Menasche P, Hagege AA, Scorsin M, Pouzet B, Desnos M, Duboc D, Schwartz K, Vilquin JT, and Marolleau JP. Myoblast transplantation for heart failure. *Lancet* 357: 279–280, 2001.
73. Messina E, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso F, Salio M, Battaglia M, Latronico MV, Coletta M, Vivarelli E, Frati L, Cossu G, and Giacomello A. Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res* 95: 911–921, 2004.
74. Min JY, Yang Y, Sullivan MF, Ke Q, Converso KL, Chen Y, Morgan JP, and Xiao YF. Long-term improvement of cardiac function in rats after infarction by transplantation of embryonic stem cells. *J Thorac Cardiovasc Surg* 125: 361–369, 2003.
75. Moretti A, Caron L, Nakano A, Lam JT, Bernshausen A, Chen Y, Qyang Y, Bu L, Sasaki M, Martin-Puig S, Sun Y, Evans SM, Laugwitz KL, and Chien KR. Multipotent embryonic isl1 + progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. *Cell* 127: 1151–1165, 2006.
76. Mummery C. Genetic selection of cardiomyocytes from human embryonic stem cells. *Mol Ther* 15: 1908–1909, 2007.
77. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, and Field LJ. Haematopoietic stem cells do not transdifferentiate

into cardiac myocytes in myocardial infarcts. *Nature* 428: 664–668, 2004.
78. Nagaya N, Fujii T, Iwase T, Ohgushi H, Itoh T, Uematsu M, Yamagishi M, Mori H, Kangawa K, and Kitamura S. Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. *Am J Physiol Heart Circ Physiol* 287: H2670–H2676, 2004.
79. Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, and Yamanaka S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol* 26: 101–106, 2008.
80. Nelson TJ, Ge ZD, Van Orman J, Barron M, Rudy-Reil D, Hacker TA, Misra R, Duncan SA, Auchampach JA, and Lough JW. Improved cardiac function in infarcted mice after treatment with pluripotent embryonic stem cells. *Anat Rec A Discov Mol Cell Evol Biol* 288: 1216–1224, 2006.
81. Nelson TJ, Martinez-Fernandez A, Yamada S, Perez-Terzic C, Ikeda Y, and Terzic A. Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. *Circulation* 120: 408–416, 2009.
82. Niagara MI, Haider H, Jiang S, and Ashraf M. Pharmacologically preconditioned skeletal myoblasts are resistant to oxidative stress and promote angiomyogenesis via release of paracrine factors in the infarcted heart. *Circ Res* 100: 545–555, 2007.
83. Noiseux N, Gnecchi M, Lopez-Ilasaca M, Zhang L, Solomon SD, Deb A, Dzau VJ, and Pratt RE. Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. *Mol Ther* 14: 840–850, 2006.
84. Nussbaum J, Minami E, Laflamme MA, Virag JA, Ware CB, Masino A, Muskheli V, Pabon L, Reinecke H, and Murry CE. Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. *FASEB J* 21: 1345–1357, 2007.
85. Nygren JM, Jovinge S, Breitbach M, Sawen P, Roll W, Hescheler J, Taneera J, Fleischmann BK, and Jacobsen SE. Bone marrow derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat Med* 10: 494–501, 2004.
86. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, and Schneider MD. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci USA* 100: 12313–12318, 2003.
87. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, and Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 322: 949–953, 2008.
88. Oyama T, Nagai T, Wada H, Naito AT, Matsuura K, Iwanaga K, Takahashi T, Goto M, Mikami Y, Yasuda N, Akazawa H, Uezumi A, Takeda S, and Komuro I. Cardiac side population cells have a potential to migrate and differentiate into cardiomyocytes *in vitro* and *in vivo*. *J Cell Biol* 176: 329–341, 2007.
89. Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, and Daley GQ. Disease-specific induced pluripotent stem cells. *Cell* 134: 877–886, 2008.
90. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, and Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 451: 141–146, 2008.
91. Perin EC, Silva GV, Assad JA, Vela D, Buja LM, Sousa AL, Litovsky S, Lin J, Vaughn WK, Coulter S, Fernandes MR, and Willerson JT. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. *J Mol Cell Cardiol* 44: 486–495, 2008.
92. Prigione A, Fauler B, Lurz R, Lehrach H, and Adjaye J. The senescence-related mitochondrial/oxidative stress pathway is repressed in human induced pluripotent stem cells. *Stem Cells* 28: 721–733, 2010.
93. Rota M, Kajstura J, Hosoda T, Bearzi C, Vitale S, Esposito G, Iaffaldano G, Padin-Iruegas ME, Gonzalez A, Rizzi R, Small N, Muraski J, Alvarez R, Chen X, Urbanek K, Bolli R, Houser SR, Leri A, Sussman MA, and Anversa P. Bone marrow cells adopt the cardiomyogenic fate *in vivo*. *Proc Natl Acad Sci USA* 104: 17783–17788, 2007.
94. Satin J, Kehat I, Caspi O, Huber I, Arbel G, Itzhaki I, Magyar J, Schroder EA, Perlman I, and Gepstein L. Mechanism of spontaneous excitability in human embryonic stem cell derived cardiomyocytes. *J Physiol* 559: 479–496, 2004.
95. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Assmus B, Tonn T, Dimmeler S, and Zeiher AM. Intracoronary bone marrow derived progenitor cells in acute myocardial infarction. *N Engl J Med* 355: 1210–1221, 2006.
96. Segers VF and Lee RT. Stem-cell therapy for cardiac disease. *Nature* 451: 937–942, 2008.
97. Siminiak T, Kalawski R, Fiszer D, Jerzykowska O, Rzezniczak J, Rozwadowska N, and Kurpisz M. Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: phase I clinical study with 12 months of follow-up. *Am Heart J* 148: 531–537, 2004.
98. Singla DK. Embryonic stem cells in cardiac repair and regeneration. *Antioxid Redox Signal* 11: 1857–1863, 2009.
99. Singla DK, Lyons GE, and Kamp TJ. Transplanted embryonic stem cells following mouse myocardial infarction inhibit apoptosis and cardiac remodeling. *Am J Physiol Heart Circ Physiol* 293: H1308–H1314, 2007.
100. Singla DK and Sobel BE. Enhancement by growth factors of cardiac myocyte differentiation from embryonic stem cells: a promising foundation for cardiac regeneration. *Biochem Biophys Res Commun* 335: 637–642, 2005.
101. Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, Giacomello A, Abraham MR, and Marban E. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation* 115: 896–908, 2007.
102. Smits PC, van Geuns RJ, Poldermans D, Bountioukos M, Onderwater EE, Lee CH, Maat AP, and Serruys PW. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up. *J Am Coll Cardiol* 42: 2063–2069, 2003.
103. Sommer CA, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, and Mostoslavsky G. Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. *Stem Cells* 27: 543–549, 2009.
104. Stadtfeld M, Brennand K, and Hochedlinger K. Reprogramming of pancreatic beta cells into induced pluripotent stem cells. *Curr Biol* 18: 890–894, 2008.

105. Stadtfeld M, Nagaya M, Utikal J, Weir G, and Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science* 322: 945–949, 2008.
106. Stamm C, Kleine HD, Choi YH, Dunkelmann S, Lauffs JA, Lorenzen B, David A, Liebold A, Nienaber C, Zurakowski D, Freund M, and Steinhoff G. Intramyocardial delivery of CD133 + bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies. *J Thorac Cardiovasc Surg* 133: 717–725, 2007.
107. Strauer BE, Brehm M, Zeus T, Bartsch T, Schannwell C, Antke C, Sorg RV, Kogler G, Wernet P, Muller HW, and Kostering M. Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease: the IACT Study. *J Am Coll Cardiol* 46: 1651–1658, 2005.
108. Sun Y. Myocardial repair/remodelling following infarction: roles of local factors. *Cardiovasc Res* 81: 482–490, 2009.
109. Tada T and Tada M. Toti-/pluripotential stem cells and epigenetic modifications. *Cell Struct Funct* 26: 149–160, 2001.
110. Takahashi K, Narita M, Yokura M, Ichisaka T, and Yamanaka S. Human induced pluripotent stem cells on autologous feeders. *PLoS ONE* 4: e8067, 2009.
111. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, and Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131: 861–872, 2007.
112. Takahashi K and Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126: 663–676, 2006.
113. Tallini YN, Greene KS, Craven M, Spealman A, Breitbach M, Smith J, Fisher PJ, Steffey M, Hesse M, Doran RM, Woods A, Singh B, Yen A, Fleischmann BK, and Kotlikoff MI. c-kit expression identifies cardiovascular precursors in the neonatal heart. *Proc Natl Acad Sci USA* 106: 1808–1813, 2009.
114. Tamaki T, Akatsuka A, Okada Y, Uchiyama Y, Tono K, Wada M, Hoshi A, Iwaguro H, Iwasaki H, Oyamada A, and Asahara T. Cardiomyocyte formation by skeletal muscle-derived multi-myogenic stem cells after transplantation into infarcted myocardium. *PLoS ONE* 3: e1789, 2008.
115. Tse HF and Lau CP. Therapeutic angiogenesis with bone marrow—derived stem cells. *J Cardiovasc Pharmacol Ther* 12: 89–97, 2007.
116. Uemura R, Xu M, Ahmad N, and Ashraf M. Bone marrow stem cells prevent left ventricular remodeling of ischemic heart through paracrine signaling. *Circ Res* 98: 1414–1421, 2006.
117. Urbanek K, Cesselli D, Rota M, Nascimbene A, De Angelis A, Hosoda T, Bearzi C, Boni A, Bolli R, Kajstura J, Anversa P, and Leri A. Stem cell niches in the adult mouse heart. *Proc Natl Acad Sci USA* 103: 9226–9231, 2006.
118. Urbanek K, Rota M, Cascapera S, Bearzi C, Nascimbene A, De Angelis A, Hosoda T, Chimenti S, Baker M, Limana F, Nurzynska D, Torella D, Rotatori F, Rastaldo R, Musso E, Quaini F, Leri A, Kajstura J, and Anversa P. Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. *Circ Res* 97: 663–673, 2005.
119. Wang Y, Haider HK, Ahmad N, Xu M, Ge R, and Ashraf M. Combining pharmacological mobilization with intramyocardial delivery of bone marrow cells over-expressing VEGF is more effective for cardiac repair. *J Mol Cell Cardiol* 40: 736–745, 2006.
120. Wernig M, Meissner A, Cassady JP, and Jaenisch R. c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* 2: 10–12, 2008.
121. Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, Soldner F, Broccoli V, Constantine-Paton M, Isacson O, and Jaenisch R. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc Natl Acad Sci USA* 105: 5856–5861, 2008.
122. Won Kim H, Haider HK, Jiang S, and Ashraf M. Ischemic preconditioning augments survival of stem cells via miR-210 expression by targeting caspase-8-associated protein 2. *J Biol Chem* 284: 33161–33168, 2009.
123. Xu N, Papagiannakopoulos T, Pan G, Thomson JA, and Kosik KS. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. *Cell* 137: 647–658, 2009.
124. Yamanaka S. Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. *Cell Prolif* 41 Suppl 1: 51–56, 2008.
125. Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell* 1: 39–49, 2007.
126. Yau TM, Kim C, Li G, Zhang Y, Fazel S, Spiegelstein D, Weisel RD, and Li RK. Enhanced angiogenesis with multimodal cell-based gene therapy. *Ann Thorac Surg* 83: 1110–1119, 2007.
127. Yoon YS, Park JS, Tkebuchava T, Luedeman C, and Losordo DW. Unexpected severe calcification after transplantation of bone marrow cells in acute myocardial infarction. *Circulation* 109: 3154–3157, 2004.
128. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, and Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318: 1917–1920, 2007.
129. Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, Palecek SP, Thomson JA, and Kamp TJ. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res* 104: e30–e41, 2009.

Address correspondence to:
Dr. Muhammad Ashraf
Department of Pathology
University of Cincinnati
Cincinnati, OH 45267-0529

E-mail: ashrafm@uc.edu

Date of first submission to ARS Central, June 14, 2010; date of final revised submission, August 9, 2010; date of acceptance, August 9, 2010.

Abbreviations Used

ES cells = embryonic stem cells
F-box protein 15 = Fbx15
GFP = green fluorescence protein
HDAC = histone deacetylase
iPS cells = induced pluripotent stem cells
Isl1 = Islet-1
Klf4 = Kruppel like factor-4
miRs = micro RNAs
Myc = myelocytomatosis
Oct4 = Octamer 4
Sox2 = *Sry* box containing gene 2