Cell Stem Cell
Review

Regeneration Next: Toward Heart Stem Cell Therapeutics

Emil M. Hansson,[1] Mark E. Lindsay,[1,2] and Kenneth R. Chien[1,3,*]
[1]Cardiovascular Research Center, Massachusetts General Hospital, Charles River Plaza/CPZN 3200, 185 Cambridge Street, Boston, MA 02114-2790, USA
[2]Division of Pediatric Cardiology, Department of Pediatrics, Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, MD 21205, USA
[3]Harvard Stem Cell Institute, Cambridge, MA 02138, USA
*Correspondence: krchien@partners.org
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Stem cell biology holds great promise for a new era of cell-based therapy, sparking considerable interest among scientists, clinicians, and their patients. However, the translational arm of stem cell science is in a relatively primitive state. Although a number of clinical studies have been initiated, the early returns point to several inherent problems. In this regard, the clinical potential of stem cells can only be fully realized by the identification of the key barriers to clinical implementation. Here, we examine experimental paradigms to address the critical steps in the transition from stem cell biology to regenerative medicine, utilizing cardiovascular disease as a case study.

Few would argue with the sentiments of Charles Spurgeon (1834–1892), the leading preacher of Victorian England, who professed that "every generation needs regeneration." In our modern era of stem cell biology, these words have taken on a new meaning, especially for the millions of patients afflicted with chronic diseases for which there is neither a cure nor further available treatment options. In this regard, the almost monthly major advances in stem cell biology have created an aura of excitement and hope in both the scientific and medical communities, resulting in a sense of urgency to move new developments quickly into the clinical setting. This translational dream has been further boosted by the announcement earlier this year that the United States' Food and Drug Administration (FDA) are considering the approval of the world's first clinical trial using human embryonic stem cell (ESC)-derived cells in an attempt to find a remedy for spinal cord injuries (Couzin, 2009).

With respect to cardiovascular disease, literally hundreds of clinical studies have examined the potential therapeutic effects of heart stem cell therapy. A quick Google search yields over 2.5 million separate listings for heart stem cell therapy, with many overseas centers already offering routine treatment for advanced forms of heart failure often for sums ranging upwards of 50,000 US$ per treatment (reviewed in (Lau et al., 2008)). At the same time, the bulk of the clinical data to date suggests that these are relatively early days for cell based therapy for heart disease, with a number of negative, marginal, and transient effects recorded in larger scale double-blind placebo controlled trials. Ironically, the major import of these studies is in pointing out specific clinical roadblocks, including the identification of the optimal cell type, ideal in vivo delivery system, novel approaches to promote the conversion to fully functional, mature cardiac muscle, integrated vascularization, and the appropriate alignment and electrical integration with the recipient myocardium (for review, see (Chien, 2008; Chien et al., 2008). Given the above, the question arises as to exactly where we are on the translational road map from cardiovascular stem cell biology to heart stem cell therapy. At this moment, are we headed in the right direction, taking a tangential course, reaching a fork in the road, or simply "lost in translation" (Chien, 2004)? If the latter is the case, how do we get back on track and what are the critical issues that must be addressed to correct our course? In this review, we utilize studies of regenerative cardiovascular therapy as a paradigm to identify critical issues for stem cell therapeutics in other organ systems, with the long range view that a common core set of problems must be solved to unlock the potential of stem cell biology for regenerative therapy.

Scalability and Heart Stem Cell Therapy

Cardiovascular disease is the leading cause of death in the world today (Lopez et al., 2006). Specifically, myocardial infarction is the leading cardiovascular cause of mortality, and in cases where it does not lead to sudden death, it frequently injures a large enough proportion of the cardiomyocytes of the heart to diminish its contractile capacity below a critical threshold. This damage leads to heart failure, a condition where almost half of the affected patients die within one year from the onset of symptoms - a grimmer prognosis than in many forms of cancer (Jessup and Brozena, 2003). Effective treatment of heart failure is the holy grail of regenerative cardiology, and the conceptual framework regarding therapy is quite simple - stem cells would be used in a replacement setting, thereby replenishing tissue lost as a result of disease. There is no question that this approach is a highly meritorious area of investigation, but, at the same time, there are some important barriers that need to be addressed to allow stem cell-mediated repair to become clinical reality.

One frequently underestimated challenge in regenerative medicine is the sheer number of cells that needs to be replaced. The most common cause of heart failure is a myocardial infarction caused by an occlusion of the left anterior descending coronary artery, supplying the left ventricle of the heart with freshly oxygenated blood. The outcome is a loss of cardiomyocytes, accompanied by the formation of a fibrous scar, resulting in decreased pumping capacity of the heart. In a typical myocardial infarct it is estimated that one billion cardiomyocytes are lost

(Laflamme and Murry, 2005). To put this staggering number of cells in perspective, it is approximately equivalent to one hundred standard 10 cm tissue culture dishes of cells. Although an improvement of cardiac function can be achieved without replacing all lost cells, it is evident that stem cell-derived treatment of heart failure will have to be based on a cellular system permitting the isolation of sufficient number of cells to generate hundreds of millions of cardiomyocytes upon transplantation for a single patient. In considering the prevalence of heart failure world wide, and its recent exponential increase, the challenge of the scalability of heart stem cell therapy, as well as that of other tissues, is a considerable, and often overlooked, challenge (Kirouac and Zandstra, 2008). The clinical therapeutic paradigm will have to be sufficiently robust, scalable, and effective to be adopted widely.

Sources of Autologous Cell-Based Therapy for Cardiac Disease: Blood and Skeletal Muscle Progenitors?

The first experimental attempts to use regeneration to restore cardiac function in an injured myocardium focused on two extra-cardiac sources of cells: progenitors from skeletal muscle and bone marrow. Although both these sources are clearly not authentic heart stem cells, they address the problem of an accessible, scalable cell type, i.e., an autologous stem cell source capable of generating a sufficient number of cells to potentially match the cell loss after myocardial infarction.

The first approach was to transplant satellite cells, skeletal muscle-specific stem cells (''myoblasts''), to the injured heart, with the hope that the cardiac microenvironment would promote the transdifferentiation of myoblasts into cardiomyocytes with a resultant increase in contractile capacity. Unfortunately, experiments in rodents showed that even though satellite cells transplanted to infarcted hearts of syngenic hosts survived and matured to fully differentiated skeletal muscle fibers, there was no evidence of conversion to a cardiomyocyte fate (Reinecke et al., 2002). Furthermore, the engrafted cells did not exhibit electromechanical coupling with the cardiomyocytes of the host myocardium, but functioned in isolation from the native cardiac tissue (Rubart et al., 2004). However, several groups reported a positive effect on heart function in rodent (Taylor et al., 1998) and larger animal (Ghostine et al., 2002) models of myocardial infarction. The mechanistic basis for such a beneficial effect is not fully understood, but may be due to an increased stiffness of the ischemic area of the ventricular wall from the simple increase in cell mass, or the release of paracrine factors from the grafted cells that somehow provide a positive effect for the injured heart. Despite the lack of mechanistic understanding regarding their mode of action, a phase I clinical trial that transplanted satellite cells to the hearts of patients with chronic heart failure was initiated eight years ago (Menasche et al., 2001). A subsequent, more in-depth study (randomized, double blind, placebo controlled, with several centers participating) failed to show improvement in several key parameters of cardiac function after a follow-up period of six months (Menasche et al., 2008). What might explain this disappointing result, given the encouraging data obtained using animal models? Clearly, the mouse offers many advantages as a model system, including transgenesis, many different genetic backgrounds, and a comparatively low cost. However, several important differences are equally clear when human and mouse cardiovascular systems are compared, including parameters such as heart rate, oxygen consumption, properties of the coronary arteries, and response to input from innervation and circulating regulatory peptides (Dixon and Spinale, 2009). Furthermore, most injury models used commonly in mice do not capture all aspects of the pathobiology of human disease, and frequently result in a thin fibrous cap/aneurysm where any cell of interest might have a benenficial effect by simply augmenting the chamber wall mass and secondarily improving function by decreasing wall stress. Consequently, a word of caution is warranted when extrapolating data from mouse models to human patients, and moving to a larger animal model for validating data obtained in mice is necessary. In the cardiovascular field, candidate models include sheep, dog and pig models of cardiac pathology (Dixon and Spinale, 2009). The physiology of the cardiovascular system in these model organisms more closely resemble the human. However, as evident from the above, even encouraging data from experiments in larger animal models do not necessarily translate to a successful outcome in clinical trials. An inescapable conclusion is that positive therapeutic effects of cell-based therapy in small and large animal models of myocardial infarction may not be predictive of clinical success. In short, there is a need for better validated animal model systems for cardiac regenerative therapy.

An alternative strategy to using skeletal muscle has been to transplant bone marrow cells to the injured heart. Over a decade ago, several research groups reported that populations of autologous stem cells, including bone marrow stem cells, appeared to possess a considerably higher degree of developmental potential than previously appreciated (reviewed in (Morrison, 2001)). Upon transplantation, such bone marrow-derived cells were reported to give rise to differentiated progeny in several solid organs, including the heart. Given the relative accessibility of bone marrow, this finding spurred researchers to study the fate of bone marrow stem cells upon transplantation in rodent models of myocardial infarction, reporting a robust transdifferentiation of injected bone marrow cells to cardiomyocytes (Jackson et al., 2001; Orlic et al., 2001). Enthusiasm in the field was further boosted by a report examining postmortem tissue from patients that had received a heart transplant. In female donor hearts transplanted to male recipients, Y-chromosome-positive cardiomyocytes were detected, indicating cellular chimerism of the transplanted heart (Quaini et al., 2002). Similar findings, albeit with a much lower frequency of cardiac chimerism, were reported by other groups, including one study examining cardiac tissue in patients receiving bone marrow transplants (Deb et al., 2003). However, several groups have since contested the original claims that bone marrow cells can efficiently transdifferentiate to a cardiomyocyte fate (Balsam et al., 2004; Murry et al., 2004). Subsequent studies have documented that what originally was interpreted as transdifferentiation may indeed have been the result of cell fusion events, where transplanted cells fuse with differentiated cardiac cells in the recipient (Alvarez-Dolado et al., 2003; Nygren et al., 2004; Terada et al., 2002). It is widely accepted by the stem cell community at large that cell fusion events, such as these, do not generate new differentiated progeny in other organ systems, and by analogy it is unlikely that new cardiomyocytes are being generated by fusion

Table 1. Selected Randomized Trials with At Least 50 Patients

Trial	No.	Cell Type	Absolute LVEF% Δ	LVEDV Δ	LVESV Δ
REPAIR-AMI (Schachinger et al., 2006)	204	BMC	+2.5 (4 mon)	N.S. (4 mon)	N.S. (4 mon)
ASTAMI (Lunde et al., 2006)	100	BMC	N.S. (6 mon)	N.S. (6 mon)	N.R.
Janssens et al. (Janssens et al., 2006)	67	BMC	N.S. (4 mon)	N.S. (6 mon)	N.S. (6 mon)
BOOST (Wollert et al., 2004)	60	BMC	+6.0 (6 mon); N.S. (18 mon)	N.S. (6 mon); N.S. (18 mon)	N.S. (6 mon); N.S. (18 mon)
Meluzin et al. (Meluzin et al., 2008)	60	BMC	+6.0 (3 mon); +7.0 (12 mon)	N.S. (3, 6, 12 mon)	N.S. (3, 6, 12 mon)
TOPCARE-AMI (Schachinger et al., 2004)	59	BMC	+8.3 (4 mon); +9.3 (12 mon)	N.S. (4 mon)	−10 cc (4 mon)
MAGIC (Menasche et al., 2008)	97	SMB	N.S. (6 mon)	N.S. (6 mon)	−8.3 cc/m^2 (6 mon)
Chen et al. (Chen et al., 2004)	69	BMSC	+14.0 (3 mon); +13.0 (6 mon)	−38 cc (3 mon)	−18 cc (3 mon)
MAGIC CELL-3-DES (Kang et al., 2006)	82	PBSC	+5.1 (6 mon for AMI)	N.S. (6 mon)	N.S. (6 mon)
van Ramhoorst et al. (van Ramshorst et al., 2009)	50	BMC	+3 (3 mon)	N.S. (3 mon)	N.S. (3 mon)

The following abbreviations are used: BMC, bone marrow stem cell; SMB, skeletal myoblast; BSMC, bone marrow mesenchymal stem cell; PBSC, peripheral blood stem cell; LVEF% Δ, left ventricular ejection fraction, percent difference; LVEDV Δ, left ventricular end diastolic volume, difference; LVESV Δ, left ventricular end systolic volume, difference; mon, months follow up after treatment; AMI, acute myocardial infarct; N.S., not significant; and N.R., not recorded.

events. Taking this unexpected finding into account, previously published reports may have to be re-evaluated, and there is currently no consensus on whether bone marrow cells have the capacity to transdifferentiate into the cardiac lineage upon transplantation. Nonetheless, clinical trials using bone marrow infusion in cases of acute myocardial ischemia as well as chronic heart failure have been initiated, with the first pilot study reported seven years ago (Strauer et al., 2002). The outcomes of these trials have been somewhat conflicting, with some trials showing a beneficial effect, whereas others have failed to see statistically significant differences compared to the control group. Again, any positive effect from such treatment has been attributed to a poorly understood paracrine effect, possibly by direct effect on the myocardium or by contributing to increased vascularization of the heart. Studies have identified a number of putative paracrine factors that might be responsible, including VEGF, FGF, IGF, and SDF (Gnecchi et al., 2005; Kinnaird et al., 2004; Uemura et al., 2006).

Taken together, clinical trials using bone marrow and satellite cells (summarized in Table 1) indicate that both these sources of cells may have some modest effects when administered to the failing heart. At the same time, the effects clearly cannot be ascribed to cardiac regeneration. The fact that the injection of many cell types have been reported to transiently improve cardiac function in animal model systems following myocardial infarction, including, adipose tissue stromal cells (Li et al., 2007), bone marrow (Orlic et al., 2001), circulating putative endothelial progenitors with features of circulating monocytes (Kocher et al., 2001), skeletal myoblasts (Taylor et al., 1998), and even undifferentiated embryonic stem cells themselves (Min et al., 2002), suggests that the simple short term improvement in cardiac function cannot be taken as direct evidence of cardiac regeneration per se. Moreover, a portion of the effect may relate to effects of decreasing wall stress by increasing the tissue mass in a thinning myocardial wall, an anatomic effect that is independent of a real regenerative effect. Accordingly, a parallel search for identifying populations of stem cells with innate cardiomyogenic potential, i.e., authentic endogenous cardiac progenitor cells seems warranted.

Mobilization of Endogenous Cardiac Progenitor Cells

Promoting the mobilization of a putative endogenous pool of cardiac progenitors in the adult human heart would represent an alternative to replacing lost cardiomyocytes by transplantation. In this manner, the concept would be to activate the endogenous heart regenerative machinery, a process akin to boosting erythrocyte levels with erythropoietin. In this paradigm, the potential problems associated with the surgical procedure, in vitro culturing of cells for grafting, immunological incompatibility, and so forth, are avoided.

Cardiac regeneration has been documented in at least two vertebrate species, newt and zebrafish, where cardiac injury elicits a regenerative response characterized by cell cycle reentry and dedifferentiation of cardiomyocytes (Oberpriller and Oberpriller, 1974), or mobilization of a tissue-specific, undifferentiated progenitor population (Lepilina et al., 2006; Poss et al., 2002), respectively. In contrast, the notion that cardiac tissue in mammalian species would have any potential for regeneration has been very controversial. However, three recent publications provide compelling evidence that there is a finite level of cardiomyocyte turnover in the mammalian heart. Lineage tracing in a mouse mutant with an X-linked mutation revealed a striking regenerative capacity in the fetal heart, indicating that healthy cardiac cells can increase their rate of proliferation and thereby compensate for a 50% loss of cardiac tissue (Drenckhahn et al., 2008). By combination of a cardiac injury model with genetic fate mapping, it was shown that the adult mouse heart also displays some regenerative capacity, albeit to a lesser extent (Hsieh et al., 2007). Through use of radiocarbon dating of human postmortem cardiac tissue, a recent elegant study

documents endogenous regenerative capacity even in the human heart (Bergmann et al., 2009). As in the mouse, a decline in regenerative capacity was observed with increased age, in that cardiomyocyte turnover ranged from a rate of 1% per year in young adults to 0.5% in the elderly. Importantly, although the endogenous regenerative potential of the adult mammalian heart appears to be quite limited, and clearly is not sufficient to cope with the widespread death of cardiomyocytes seen in a myocardial infarction, these findings indicate either an unexpected degree of self-renewal of adult cardiomyocytes, or a potential source of endogenous stem cells with cardiogenic potential. In this regard, published studies from several groups identify minute numbers of putative cardiac stem cells in the adult heart, as defined phenotypically by cell-surface markers such as c-kit (Beltrami et al., 2003), Sca-1 (Oh et al., 2003), or other characteristics (Hoechst dye exclusion [Martin et al., 2004]). These cells were found to proliferate and generate new cardiomyocytes in culture, and in some cases even improve cardiac function upon transplantation (reviewed in Martin-Puig et al., 2008).

Unfortunately, there are several caveats that currently preclude the ultimate utility of adult, cardiac-specific stem cells in regenerative medicine. First of all, to date, there is no consensus on how to identify these cells, and their role as authentic endogenous heart progenitors will rest upon documenting that they are recruited to the site of injury, undergo expansion, and subsequently convert to authentic cardiomyocytes. This achievement will ultimately require conditional lineage tracing in the setting of cardiac injury, and such a tracing is a technically feasible but challenging experimental paradigm that has not yet been fully accomplished with any cell type. Different molecular markers have been suggested as identifiers of adult cardiac stem cells, but the expression of these proteins vary from study to study, do not overlap, and have not always been reproducible. Second, the biology of such putative adult cardiac progenitor cells is hitherto largely unknown. The molecular mechanisms promoting their self-renewal and differentiation into the different lineages of the heart have not been identified, and lineage-tracing experiments providing evidence that they contribute to the generation of new cardiac cells in the adult are lacking. Although mobilization of endogenous cardiac stem cells after injury may be an important strategy for treating heart failure in the future, in the short term it is very difficult to envision whether a sufficient level of mobilization would be capable of meeting the numeric demand for cell-replacement therapy after myocardial infarction.

Lineage Tracing and the Direct Identification of Authentic Endogenous Heart Progenitors

As a result of the challenges that currently prevent the harnessing of endogenous cardiac progenitors, the focus of the field is beginning to shift to stem cells with proven cardiac potential, converging on the developmental biology of cardiogenesis. Recent advances in developmental biology have been used for identification of cardiac stem cells in the embryonic and fetal heart. During embryogenesis, two anatomically distinct groups of cells of mesodermal origin form the first and second heart fields (FHF and SHF, reviewed in Buckingham et al., 2005; Srivastava, 2006). These fields, which can be distinguished spatially as early as day E7.5 of mouse gestation, give rise to either the majority of the left ventricle and parts of the atria or the right ventricle plus parts of the atria and proximal part of the great arteries, respectively. To date, there is no specific marker of progenitor cells of the FHF, although expression of Nkx2.5 identifies progenitor cell populations within both heart fields of the developing heart (Wu et al., 2006). In the SHF, a growing body of evidence shows that cardiac progenitor cells express the LIM-homeodomain transcription factor Islet1 (Isl1) (Cai et al., 2003; Laugwitz et al., 2005; Moretti et al., 2006). Isolated Isl1-expressing cells can be purified, expanded, and differentiated to all cell lineages of the heart. The molecular cues that regulate this population are under investigation. To this end, Isl1 positive cardiac progenitor cells have been expanded in culture by treatment with secreted ligands of the Wnt/β-catenin pathway (Qyang et al., 2007). In addition to FHF and SHF progenitors, recent work has identified epicardial progenitor cells, expressing the transcription factors Wt1 and Tbx18, as yet another multipotent cell type important for proper formation of a fully functioning heart (Cai et al., 2008; Zhou et al., 2008a). Progeny from these epicardial progenitor cells cover the surface of the heart, and differentiated cardiomyocytes derived from these precursors also appear to integrate into the myocardium of all four chambers of the heart. Finally, some cells in the heart are of nonmesodermal origin. During embryogenesis, neural crest cells delaminate from the neural tube and migrate ventrally, some of which reach and populate the developing heart, intermixing with cardiac progenitor cells of mesodermal origin. Cardiac neural crest progenitor cells, identified by their expression of the transcription factor Pax3, contribute to the outflow tract of the developing heart (Conway et al., 1997; Li et al., 1999), reviewed in (Stoller and Epstein, 2005). The mature heart is a mosaic of cells from these different progenitor populations (Figure 1).

Thus, in contrast to the situation in adult hearts, the biology of cardiac progenitor cells in the developing embryo is comparatively well understood, with an established marker for multipotent progenitor cells, identified molecular cues controlling their expansion, and a well defined lineage tree showing their capacity to give rise to all cellular lineages of the heart. However, there are obvious ethical barriers to the use of such cells for transplantation. Access to embryonic cardiac tissue is only possible through destruction of embryos, and fetal tissue will not be available in sufficient amounts to supply the required number of cells.

In sum, for transplantation purposes, it would be ideal to focus on a source of cells that combines the scalability of bone marrow or satellite cells with the proven cardiogenic potential and established cardiac lineage tree of cardiac progenitor cells present during embryonic development.

ESCs, iPSCs, and Reprogramming

With this in mind, embryonic stem cells (ESCs) have emerged as one of the most promising sources of cardiac cells for transplantation purposes. Human ESCs (hESCs), first isolated 1998 by James Thomson and co-workers from the inner cell mass of preimplantation embryos (Thomson et al., 1998), are pluripotent cells capable of differentiating into virtually every cell type, including cells of the heart (Kehat et al., 2001). In the decade following the isolation of hESCs, protocols to differentiate these cells into cardiomyocytes have been refined (for review, see

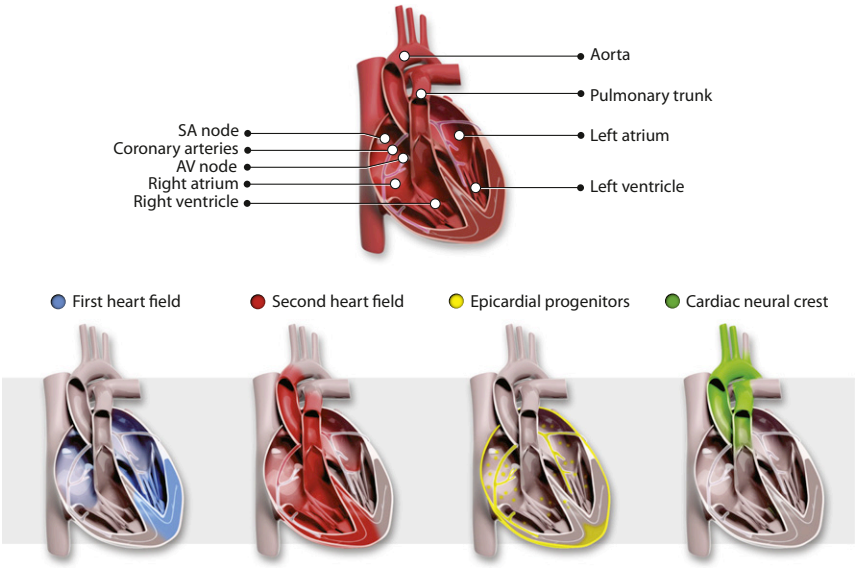

Figure 1. The Heart Is a Mosaic of Cells from Different Progenitor Populations

The heart is composed of cells derived from at least four different populations of progenitor cells—the first heart field progenitor cells (blue), the second heart field progenitor cells (red), the epicardial progenitor cells (yellow), and the cardiac neural crest progenitor cells (green). Progeny from these progenitor pools reside in distinct but partly overlapping areas in the mature heart.

Passier et al., 2006). Several groups have successfully isolated cardiomyocytes or cardiac progenitor cells from differentiating ESCs grown either in three-dimensional clumps termed embryoid bodies or 2D cultures treated with various extracellular proteins that increase the yield of cardiac cells. Importantly, the ESC-derived cardiomyocytes not only share molecular markers with primary cardiomyocytes, but ultrastructural (electron microscopy), electrophysiological (action potential measurements), and mechanical (determination of contractility) studies of the ESC progeny indicate that they also exhibit all hallmarks of cardiomyocytes. Of potential importance, ESC-derived cardiomyocytes have been shown to exhibit a phenotype reminiscent of fetal, rather than adult, cardiomyocytes (Kehat et al., 2001; Mummery et al., 2003). The reason for this phenomenon is not known but deserves further investigation. Given the observed differences between fetal and adult cardiomyocytes, ESC-derived cardiomyocytes with features of adult cells would probably be preferable for clinical transplantation purposes. Nonetheless, ESC-derived cardiomyocytes have already been used in transplantation experiments in rodent models of cardiac disease (reviewed in Passier et al., 2008). These results show that hESC-derived cardiomyocytes can couple electromechanically with cardiomyocytes of the host, and beneficial effects in myocardial infarction have been reported 4 weeks after transplantation (Laflamme et al., 2007). However, in a study with a longer follow up, no effect on cardiac function could be documented 12 weeks after transplantation (van Laake et al., 2007). Thus, the long-term effects of ESC-derived cardiomyocytes to injured myocardium need to be evaluated further.

Recently, a novel way of generating stem cells from differentiated cells has been described. This technique, pioneered by Shinya Yamanaka and colleagues, relies on the reprogramming of fully differentiated cells to ESC-like cells, known as induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006). iPSCs exhibit the two key features of ESCs, in that they can be expanded over many passages in vitro and give rise to cells of all three germ layers, both under appropriate in vivo and in vitro differentiation conditions. Originally established for mouse embryonic fibroblasts with a genetic selection strategy for identifying reprogrammed cells, the basic iPSC derivation protocol has been refined by many groups, permitting reprogramming of human cells (Park et al., 2008; Takahashi et al., 2007; Yu et al., 2007), reprogramming without genetic selection (Blelloch et al., 2007; Meissner et al., 2007), and chemicals enhancing reprogramming efficiency (see Yamanaka, 2009 for review). This body of work has propelled iPSC technology to the forefront of experimental regenerative medicine. Being derived from adult cells, iPSCs bypass the ethical issues regarding the use of embryonic human tissue to cure disease, and immunocompatibility is not an issue because the starting material, skin fibroblasts, can be obtained from the patient. However, there are currently caveats with the iPSC reprogramming procedure that need to be addressed before this elegant technology can be put to clinical use. One important aspect is that the original protocol for reprogramming of human cells to iPSCs relies on the use of viruses integrating into the genome of cells undergoing the reprogramming process. Clinical trials in gene therapy have shown that integration of viruses in tumor-suppressor genes may give a selective advantage and thereby promote malignancy when transplanted to the patient (Hacein-Bey-Abina et al., 2003; Ott et al., 2006). Moreover, given that some of the virally encoded genes are oncogenes that may be reactivated after transplantation, it is clear that protocols permitting reprogramming without the use of viruses are essential before iPSCs can become a clinical tool. Very recently, it has been shown that human iPSC derivation can be achieved with transposon (Kaji et al., 2009; Woltjen et al., 2009), episomal (Yu et al., 2009), and direct protein delivery (Kim et al., 2009) systems, and it will be interesting to compare whether iPSCs generated by these methods are equivalent to iPSCs generated with integrating viruses. Furthermore, the long-term performance of differentiated cells derived from iPSCs needs to be critically assessed. In this regard, a detailed comparison between cardiomyocytes derived from iPSCs and cardiomyocytes derived from ESCs is required before iPSCs can be considered for regenerative therapy in cardiology.

Even with an efficient method for nonviral iPSC generation of human cells in place, and even if iPSCs prove to be functionally equivalent to human ESCs, there is still one potentially important drawback with the iPSC technology—the time iPSC derivation, with stringent criteria for pluripotency and capacity to differentiate to the lineage of interest, requires (reviewed in Maherali and Hochedlinger, 2008), which would at least be relevant for

situations in which patient-specific lines are sought. This requirement is in sharp contrast to banked hESCs, which would be relatively easy to expand, store, and distribute in an off-the-shelf paradigm. For use of iPSCs in a similar manner, banks of pretested pluripotent cell lines would have to be established.

Although still in its infancy, it appears likely that cellular reprogramming may provide important tools for translational scientists aiming at generating a specific cell type for cell therapy. In addition to the iPSC technology, where fully differentiated cells are reprogrammed to the fully undifferentiated ESC-like state, and subsequent differentiation of such cells can give the desired cell type, one can envision more direct ways of reprogramming cells. In a pioneering experiment conducted more than 20 years ago, Harold Weintraub and colleagues showed that forced expression of the myogenic transcription factor MyoD in cultured fibroblasts caused such cells to adopt the myocyte fate (Davis et al., 1987). Thus, it seemed plausible that at least some cells in the body may have a more plastic developmental identity than traditionally thought. Recently, Douglas Melton and colleagues have taken this concept one step further. By first establishing the transcriptional code for generation of endocrine, insulin-producing beta cells in the pancreas during embryonic development (Zhou et al., 2007) and thereafter transducing exocrine cells with viruses expressing these factors (Zhou et al., 2008b), they showed that cellular reprogramming can be achieved not only in the Petri dish but also in vivo. More importantly from a translational point of view, it also established that such reprogramming can be beneficial for the organism, in this case by improving the glucose-controlled secretion of insulin in a mouse model of diabetes mellitus through generation of new beta cells (Zhou et al., 2008b).

It remains to be seen how broadly applicable the ''transdifferentiation by reprogramming'' approach will be. Even if cells in other organs such as the heart may be less susceptible to transdifferentiation, it is possible that modifications of the iPSC protocol may be beneficial. For example, one could envision reprogramming exogenous cells such as fibroblasts to a relatively restricted mesodermal or cardiac-restricted progenitor stage, rather than all the way to pluripotent iPSCs. This approach would make subsequent differentiation and purification procedures considerably less challenging and minimize the risk of transplanting unwanted and potentially dangerous contaminating cells. Furthermore, identification of the factors controlling lineage decisions of progenitor cells can facilitate the generation of defined populations of fully differentiated progeny. In this regard, forced expression of intrinsic fate determinants has proven to be an efficient method for generating dopaminergic neurons from ESCs and neural progenitor cells (Andersson et al., 2006), and more recently similar approaches have been shown to be fruitful also in the cardiac lineage (David et al., 2008; Takeuchi and Bruneau, 2009).

Purification

Two critical steps will be needed to determine whether progenitor cells or differentiated cardiomyocytes will be preferable for transplantation purposes and how to purify this population from a heterogenous culture of differentiating ESCs or iPSCs. To date, several published reports describe transplantation of differentiated cardiomyocytes derived from human ESCs to rodents (for review, see Passier et al., 2008). Alternatively, using a pure population of restricted cardiac progenitor cells may yield a transplanted population with a higher expansion capacity, thereby potentially increasing substantially in number after successful engraftment. This benefit may represent an important advantage given the large number of cells that are to be replaced. Another advantage is that cardiac progenitor cells are able to form endothelial cells, which might result in improved vascularization of the resulting graft. However, controlling the generation of mature, fully differentiated cells from grafted progenitors will be both important and challenging, and more basic research to uncover the molecular mechanisms underlying cardiac progenitor cell differentiation is warranted.

Isolation and expansion of desired progenitor cells, such as cardiomyocytes or cardiac progenitor cells, have generally been accomplished by transgenic labeling of the desired cell type, followed by antibiotic selection, or by purification with cell-sorting techniques. Although highly efficient and of immense importance for research purposes, genetic manipulation of the starting cellular material is generally not considered compatible with the production of clinical-grade cells. The identification of cell-surface markers, enabling antibody-based identification and purification of cells for transplantation, will be an important step in bringing stem cell-based cardiology closer to the clinical setting. On the other hand, it is paramount to exclude all unwanted, more primitive cells from the material to be transplanted, and it remains to be proven whether antibody-based protocols for isolation of a cell population entirely devoid of contaminating ESCs can be developed (Kiuru et al., 2009). It is possible that some forms of genetic labeling will have to be accepted to ensure the purification of a clinically safe population of cells.

Delivery

Recent clinical studies have also clearly identified the need for improved in vivo cell delivery systems to the heart and other tissues. Of the solid organs in which cell therapy has been considered, the heart is unique in that repeated and frequent movement of the organ, resulting in changing pressures and geometry inside the heart, is paramount for life. Thus, it is not trivial to transplant cells to an exact location in the heart and make them stay there. Experiments in rodents have shown that delivery of cells to infarcted myocardium is inefficient. In one published report, as few as 15% of transplanted cells stayed in the heart, whereas the rest of the transplant leaked out either through the injection site or into the recipient's circulation (Muller-Ehmsen et al., 2002). In addition, retention of cells at the site of injection varied widely between hosts, making it very difficult to predict the size of the heart graft following transplantation.

The challenge of poor graft retention may be circumvented by minimizing cell leakage from the injection site, such as by embedding cells in a viscous polymer prior to transplantation (Laflamme et al., 2007). Another attractive possibility would be to engineer contractile cardiac tissue ex vivo into a multilayered graft that can both provide support to the failing heart and be handled surgically as a single piece during transplantation. Efforts in tissue engineering with neonatal cardiomyocytes have provided patches of cardiac tissue that integrate

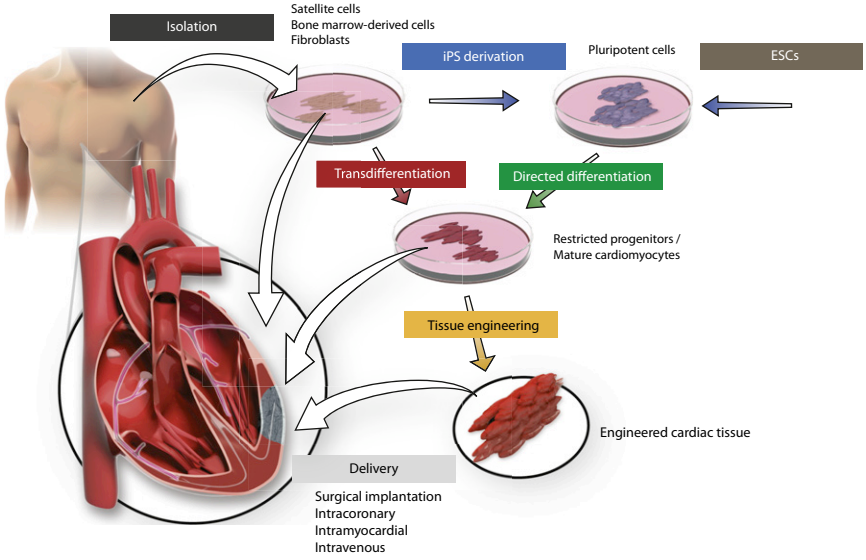

Figure 2. Experimental Approaches to Cardiac Cell Therapy

Clinical trials have been conducted with satellite cells and preparations of bone marrow cells, that have been delivered to the failing heart. Such treatments have failed to increase the number of cardiomyocytes in the heart. Conceptually this would be possible to achieve by purifying cells of the cardiac lineage from differentiating ESCs or iPSCs, or by direct reprogramming of somatic cells to the relevant cell type, and thereafter transplanting such cells to the heart of the patient.

electromechanically with native cardiomyocytes and improve cardiac function when grafted to infarcted rat hearts (Zimmermann et al., 2006). Moreover, recipient blood vessels derived from the surrounding tissue grew into the graft, ensuring the proper supply of oxygenated blood. This is an important finding, given that the size of the implant precluded a sufficient supply of nutrients by passive diffusion from surrounding blood vessels alone. Other reports of successful tissue engineering of interest for regenerative cardiology include the possibility of generating anisotropic sheets of cardiomyocytes on thin film substrates ex vivo (Feinberg et al., 2007). In sum, these approaches illustrate the feasibility and power of tissue engineering in regenerative medicine and the use of purified cardiomyocytes or cardiomyocyte-restricted precursor cells derived from ESCs as a cellular source. In the future such experiments may enable the generation of stem cell-derived grafts for cardiac surgery, which may complement other strategies to restore cardiac function through cellular therapy (summarized in Figure 2).

Cell Survival and Suppression of Rejection

Yet another challenge associated with cell transplantation is the fact that the majority of transplanted cells that manage to stay in the heart will not survive. In fact, it has been estimated that almost nine out of ten transferred cells will eventually die (Laflamme and Murry, 2005; Muller-Ehmsen et al., 2002). Several methods to increase cell survival have been described, including heat shock of the cells prior to transplantation, forced expression of survival factors in the transplanted cells, and exposure of the transplant to soluble prosurvival factors (Laflamme et al., 2007). Although these interventions do seem to increase cell survival, there is clearly room for additional improvement. We still do not have a complete understanding of the mechanisms causing the massive cell death of transplanted cells, and it is likely that insights into these processes will provide novel approaches to increase the survival of cells transplanted to the myocardium.

Additionally, ESCs are foreign to the body and may cause immunological reactions directed against the graft. This risk can be overcome by immunological typing of the cells prior to grafting and with immunosuppressive therapy. Patient-specific iPSCs offer, in contrast to established ESC lines, an autologous source of pluripotent stem cells, thereby bypassing all issues regarding immunological incompatibility. On the other hand, in cases where heart failure is due to a mutation resulting in cell-autonomous dysfunction of cardiomyocytes, such as hereditary forms of cardiomyopathy, cardiac cells derived from patient-specific iPSCs will carry the same disease-causing mutation. In such cases, correction of the disease allele will be required prior to transplantation. The feasibility of this approach has already been shown in a mouse model of sickle cell anemia, in which iPSC derivation, correction of the disease-causing mutation by gene targeting in vitro, and subsequent transplantation of hematopoietic progenitor cells derived from such "corrected" iPSCs cured the disease (Hanna et al., 2007).

Grafting, Integration and Alignment of Transplanted Cells in the Recipient Heart

To enhance the pumping capacity of the failing heart, transplanted cells must not only remain in the heart after transplantation and survive, they must also integrate into the native cardiac tissue. How individual differentiated cells come together to form the three-dimensional structure of the heart is the perhaps most poorly understood area of cardiogenesis. During embryonic development, cardiomyocytes elongate, align, and orient in a linear fashion. In addition, cardiomyocytes are subsequently coupled to each other in an end-to-end fashion by intercalated discs. These specialized structures, containing gap junction proteins, facilitate the spreading of the electrical impulse from one fiber of cardiomyocytes to another. Moreover, layers of fibers are aligned to form an anisotropically oriented basket weave of muscle. Thus, the distinct cellular architecture of contractile tissue permits two key features of the physiology of the ventricular myocardium: rapid and coordinated spreading of the electrical impulse controlling the frequency of the heart beat, and a coordinated contraction resulting in pumping of blood from the heart throughout the tissues of the organism. To improve cardiac function, transplanted cells have to align, engraft, and couple with the myocardium of the host in an ordered fashion. How this organization is orchestrated is not known, but published transplantation experiments show that integration does occur, raising the probability that extracellular matrix or resident cells in the adult heart provide directional

cues that guide the alignment and integration of transplanted cells. Strikingly, a recent publication demonstrated that a preparation of cardiomyocytes repopulated a decellularized heart ex vivo and aligned to form a beating myocardium (Ott et al., 2008).

Pathology of the heart results in disorganization of cardiac tissue and disruption of its intricate electromechanical properties. Fibers of cardiomyocytes are lost or become separated by fibrous scars, and individual fibers lose directional orientation and emanate at an angle from correctly, anisotropically oriented fibers. In addition, cell types not normally present in the heart may invade the cardiac tissue in response to injury. For example, the onslaught of a myocardial infarction results in recruitment of several hundreds of millions of leukocytes and lymphocytes, secreting important mediators of inflammatory and immune responses. Furthermore, the infarcted area may still be in a relatively hypoxic state due to the injured vessels responsible for supplying the area with oxygen. It is in such a molecularly inhospitable mileu that transplanted cells are expected to graft, proliferate and differentiate, integrate, survive, and increase the pumping capacity of the heart. Not surprisingly, it has been documented that transplanted ESC-derived human cardiomyocytes do not graft as efficiently into infarcted heart tissue compared to healthy myocardium (Laflamme et al., 2007), arguing for a loss of cues driving their survival, alignment, and electromechanical integration in the injured myocardium. How to deliver oxygen and nutrients to the grafted cells will be a very important issue, and it will be key to understand how blood vessels present in the heart can be coaxed to invade the layer of transplanted cardiomyocytes. As discussed above, engineering larger grafts of myocardium ex vivo for surgical implantation may prove to be an alternative option to injection of single-cell suspensions into the damaged myocardium. Given that larger grafts will need more oxygen and nutrients than what can passively diffuse from the host circulation, it may prove fruitful to develop vascular structures ex vivo to be delivered in concert with the rest of the graft.

Side Effects

All forms of medical treatment are accompanied by the risk of unwanted side effects. Stem cell-based therapy is no exception, and there are several potential side effects associated with transplantation of cardiac progenitor cells to the ailing heart. The most obvious, immediate concern for any transplant of ESC/iPSC-derived populations is the risk of teratoma formation. Teratomas are tumors composed of a random mixture of different cell types derived from contaminating, undifferentiated ESCs inadvertantly present among the transplanted cells (Lensch et al., 2007). Although teratomas are benign, it is self evident that a growing mass of random tissue inside the ventricular myocardium will be detrimental to cardiac function (see Laflamme and Murry, 2005 for review). Thus, as discussed above, a sorted population of developmentally restricted, cardiac-specific cells, devoid of any contaminating undifferentiated ESCs, is paramount for a positive outcome when using ESC-derived cells therapeutically.

In addition to the risk of teratomas, there are other conceivable side effects more specific to the heart. Here, the problem is not defective differentiation but rather defective function of the grafted cells caused by improper electromechanical integration with the myocardium of the host. This defect generally manifests as dysfunction in contraction. Arrhythmias may occur when the transplanted cells are integrated into the cardiac syncytium but exhibit uncoordinated electrical activity out of sync with cells of the recipient heart. The result could be ectopic pacemaker activity in the ventricular tissue, causing dysynchronous electrical stimulation and therefore inefficient mechanical contraction. Another potential side effect is dyskinesia, in which the grafted cells are not integrated into the rhythmic contraction of the host myocardium but survive after transplantation as an isolated segment of cardiac cells, exhibiting its own frequency of contraction. The graft will thereby perform independently of, and in the worst scenario work against, the cardiac tissue of the recipient. Thus, although principally different, the outcome in both cases will be dysfunction of an already impaired heart. It is important to note that an early clinical trial in which skeletal myoblasts were transplanted to postischemic hearts resulted in arrhythmia in several patients (Menasche et al., 2003).

Toward Regenerative Cardiovascular Medicine for Pediatric Heart Disease: Challenges and Opportunities

Congenital heart disease is the most common birth defect, affecting one out of every one hundred live births. Many lesions are life threatening and require surgical correction, often within the first week of life. The challenges faced by the care of patients with congenital heart disease are altogether different from acquired adult heart disease. Surgical restoration of normal biventricular circulation is necessary in many malformations. During the repair of many defects, it is necessary to implant exogenous material in the heart from an extracardiac source. For closure of atrial or ventricular septal defects, several different materials are often used including autologous pericardium, bovine pericardium, or Gore-tex. These materials are ideal for this purpose because they serve as a barrier to blood flow but do need to functionally couple to the surrounding tissue. On the contrary, other conditions require more extensive extracardiac material whose current properties are not ideal and offer an opportunity for stem cell-based tissue engineering solutions. Additionally, stem cell technology is expanding our understanding of the etiology of complex congenital heart disease by advancing the creation of human and murine in vitro models of cardiac development.

With lineage tracing, an early primordial ISL1 human heart progenitor cell has been recently identified in a human ESC system, which gives rise to a well-characterized family of downstream multipotent SHF heart progenitor cells that then generate diverse lineages (Bu et al., 2009). In the mouse, there is a rapid transition from Isl1 expression to migration and conversion to differentiated progeny in cells of the SHF. At later embryonic stages, few Isl1-expressing cells can be found in the heart, including the developing outflow tract. Moreover, Isl1-expressing cells generally coexpress markers such as Flk-1 and Nkx2.5, possibly reflecting a rapid fate restriction, whereas human cardiogenesis is a much longer process. ISL1-expressing cells that do not express other markers such as FLK-1 or NKX2.5 are found in the developing heart including the outflow tract, which may represent the upstream precursor for the family of multipotent progenitors in the SHF lineage (Bu et al., 2009). Acquisition of the fully differentiated state takes longer for human

Table 2. Comparison of Outflow Tract Development in Humans and Mice

	Mouse	Human
Gestational length	3 weeks	9 months
Time for outflow tract formation	days	weeks
Size of adult heart	1 × 0.5 ×0.3 cm	13 × 8 × 5 cm
Weight of adult heart	110 mg	300 g

During early embryogenesis, the size differences between the human and mouse embryo are relatively modest. From a relatively similar size when beating commences, the difference in size between human and mouse hearts increase rapidly during later stages of embryonic and postnatal development, implying a higher degree of progenitor proliferation in human cardiogenesis.

cells, and the presence of a family of intermediate, lineage-restricted progenitors presumably reflect the need for cellular expansion due to the orders of magnitude in size that distinguish adult mouse and human hearts (Table 2). Additionally, the identification of ISL1-expressing intermediates in the outflow tract during later-stage human cardiogenesis suggests the renewal of this population may play a pivotal role in expanding the tissue mass of diverse lineages. These same compartments are known to be defective in multiple forms of severe human congenital heart disease. If defective renewal of these cells is at fault in these cases, a "stem cell paradigm" may be responsible for the onset of an important subset of outflow tract diseases. In this regard, many of the known monogenic causes of human congenital heart disease are known to affect cell-fate decisions in the islet lineage, and further direct testing of this concept is warranted (Figure 3). One important translational outcome of these studies is the clear identification of a purified and clonable source of human heart progenitors that can serve as in vitro models of human complex congenital heart disease. Future studies extending the work into the context of iPSCs might offer the possibility of generating tissue engineering solutions to generate grafts that can then be populated by in vitro-derived cells.

An example of the potential for tissue-engineering applications is surgical replacement or reconstruction of the semilunar valves and proximal great arteries of the heart. In some congenital lesions such as pulmonary atresia, truncus arteriosis, and pulmonic replacement after Ross procedure, de novo reconstruction of the right ventricular outflow tract and pulmonary valve is necessary (Figure 4). Surgeons have a variety of options but two of the most commonly used right ventricular to pulmonary artery (RV-to-PA) conduits are frozen cadaveric human tissue (homograft) or valved bovine jugular vein (Contegra Xenograft). Despite the many successful outcomes these types of materials have provided, there is additional room for improvement. Unlike native vessels, grafts have no capacity for growth, necessitating upsizing as the hemodynamic needs of the child increase. Additionally, these grafts represent immunologic foreign material and are attacked by the patient, leading to accelerated sclerosis, calcification, and often obstruction of these conduits and the conduit valve. A typical child needing an RV-to-PA conduit in infancy will need at least one to two additional surgeries, with full cardiopulmonary bypass, to upsize the conduit before age 20, not to mention numerous catheterization procedures aimed at balloon dilating or stenting open these conduits.

An ideal conduit material would be appropriately scaled to the patient, have capacity for growth, and evade immune surveillance. Recent advances in stem cell biology allow researchers to imagine the construction of replacement grafts composed of material grown from the patient. Pioneering researchers have made great strides toward this outcome by using animal models and cells from animal and human umbilical cord donor and more recently, with bone marrow-derived cells (Hoerstrup et al., 2002; Mettler et al., 2008; Shieh and Vacanti, 2005; Shinoka et al., 1998). Alternatively, ESC or iPSC technology could be used to produce pluripotent cells and then direct differentiation of these cells into appropriate cardiovascular lineages (Figure 4). The cells generated via this approach could be not only the correct cell type (smooth muscle) but additionally of proper embryonic lineage (SHF) to assure functionality. In cases involving genetic defects, strategies aimed at fixing any known genetic defects within patient-derived iPSCs would need to be employed. Applying this therapeutic paradigm will require tissue-engineering

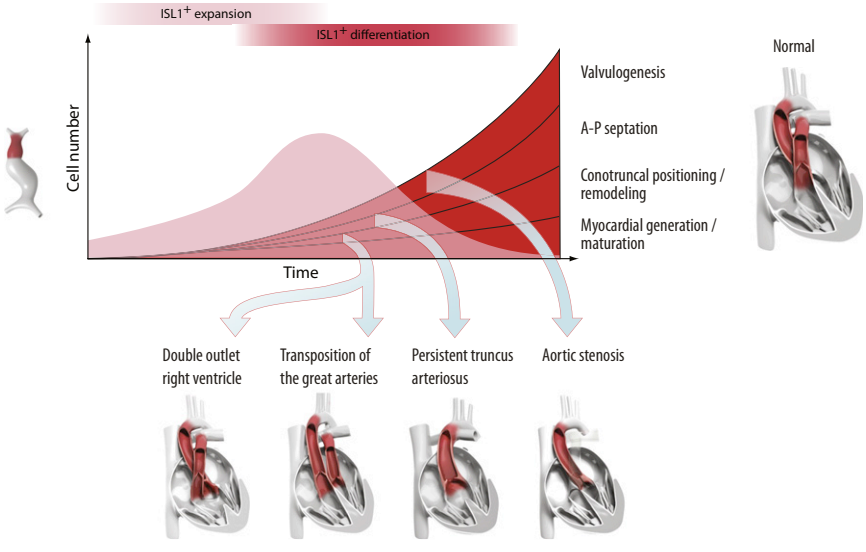

Figure 3. Schematic Illustration of Cardiac Outflow Tract Development and Its Relation to Congenital Heart Disease

The outflow tract is derived largely from SHF progenitor cells with more distal contributions from cardiac neural crest. Disturbances in the proliferation and differentiation of ISL1-expressing progenitor cells disrupt developmental morphogenic functionality and cause malformations of the outflow tract seen in patients with congenital heart disease (blue arrows). Several genes, when mutated, are known to cause these prototypical outflow tract malformations: double outlet right ventricle (NKX2.5, THRAP2, and CHD7), transposition of the great arteries (NKX2.5, GDF1 and CHD7), persistent truncus arteriosus (NKX2.5, TBX1, NOTCH1 and NOTCH2, JAGGED1, GDF1, THRAP2, and CHD7), and aortic stenosis (NOTCH1). A-P, aorto-pulmonary.

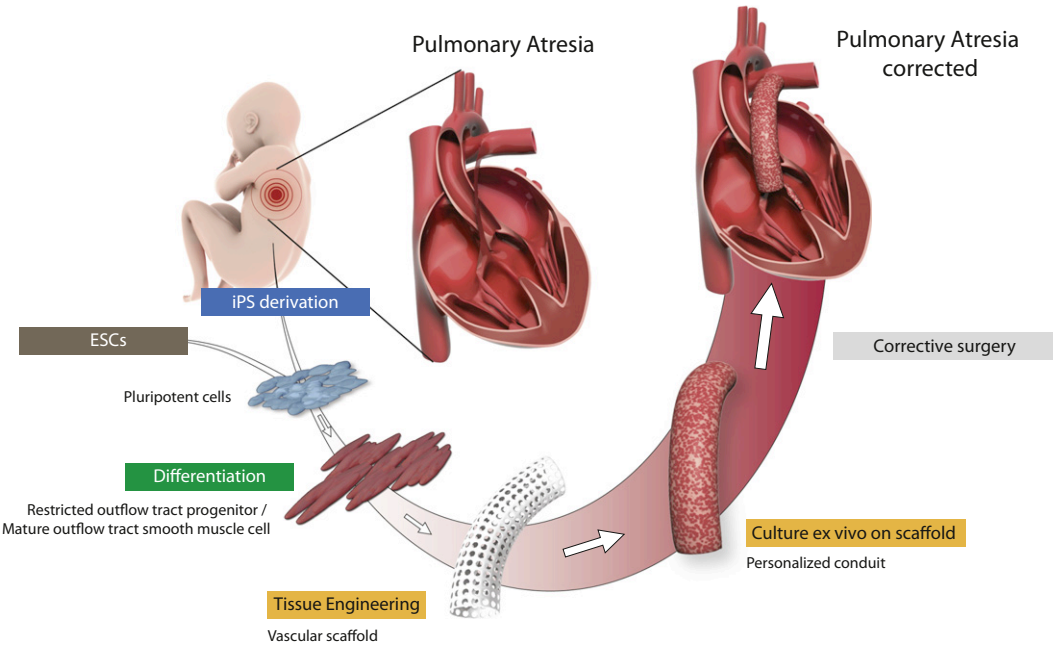

Figure 4. Ex Vivo Construction of ESC-Derived RV-PA Conduit
Implantation of a conduit to substitute for an atretic great artery is needed for several different congenital lesions including pulmonary atresia. Combining advances in ESC or iPSC technology to derive SHF-specific vascular smooth muscle cells with described tissue-engineering solutions could provide proper lineage-derived conduits with growth capacity.

solutions to provide seeded cells with relevant form and function. For a conduit graft, a vascular scaffold with or without valve apparatus will be needed, and this scaffold can then be populated by in vitro-derived cells. This type of scaffold could be engineered either de novo (Shieh and Vacanti, 2005) or by the decellularization-recellularization of tissue from human cadavers or other large mammals as an underlying matrix scaffold, as demonstrated with rat heart (Ott et al., 2008).

Importantly, although the discussion above is focused on comparatively rare congenital defects, the same bioengineering challenges are also applicable to more commonly acquired cardiovascular diseases. Tissue-engineering attempts will become increasingly common as the ability to make patient-specific cells from defined embryologic lineages improves and becomes more efficient. In short, advances in stem cell biology have helped transition dreams of certain medical applications from science fiction-like concepts toward realistic discussions of engineering organs ex vivo for specific disease interventions.

Lessons from the Bone Marrow

Overall, it seems clear that the aim to bring stem cells to clinical practice in the field of cardiology is a noble, worthy, but also challenging if not daunting goal. However, a comparison with the path taken in hematology, the field of clinical medicine in which stem cell biology already has had an enormous impact, may be both instructive and enlightening. Stem cell therapy in the form of bone marrow transplants have been used clinically for more than half a century, with the first successful procedure conducted by E. Donnall Thomas in the 1950s. Here, Thomas cured a patient with leukemia by first subjecting him to total-body irradiation and thereafter injecting bone marrow from the patient's identical twin, who did not have leukemia (Thomas et al., 1959; Thomas et al., 1957). At first glance, this initial indication for bone marrow transplant—cancer, characterized by uncontrolled cellular proliferation—is very different from heart failure, in which the loss of cardiomyocytes typically causes disease. However, the cancerous cells were killed by the irradiation procedure, and the role of the transplanted bone marrow was to replace the recipient's own bone marrow, which was lost during the radiation therapy. Subsequently, bone marrow transplantation was used also in disorders characterized by a deficiency of a particular hematopoietic cell type. The first non-cancer bone marrow transplants took place in 1968, this time to cure a boy with severe combined immunodeficiency disorder by transplant with marrow from his sister (Gatti et al., 1968).

However, in the first two decades, the hematology field was hampered by graft-versus-host disease (GVHD), a phenomenon known already prior to Thomas's pioneering work. It was recognized that during GVHD, grafted cells produce an immune reaction against host tissues (Billingham and Brent, 1957). So that GVHD could be avoided, bone marrow had to be obtained from a close relative, ideally an identical twin as in Thomas's initial transplant procedure. However, it was the unraveling of our core understanding of the control of the human immune system that represented the breakthrough required for bone marrow transplantation to become standard clinical therapy. As the field of immunology progressed, manifested by the identification of the major histocompatibility complex and how it regulates immunological reactions (chronicled in Marx, 1980), the molecular basis of GVHD was ultimately elucidated. This finding in turn opened up the possibility of reducing the risk of GVHD by performing HLA haplotype matching between donor

and recipient and paved the way for successful bone marrow transplants in which donor and recipient were unrelated, which first occurred in the 1970s (reviewed in Thomas et al., 1975). Since then, advances in immunosuppressive therapy have been important in further minimizing the risk of GVHD, and autologous bone marrow transplant has become an option for several diseases.

The chain of events outlined above can serve as an inspiring template for researchers in the fields of translational stem cell biology and regenerative medicine. To establish bone marrow transplantation as a clinical treatment, pioneers of the field had to identify ways to improve grafting (irradiation of the host, thereby escaping an immune response to the graft by host cells) and the most common side effects (HLA haplotyping to ensure immunologically matched transplants to avoid GVHD). Similarly, by identifying problems that need to be solved and addressing them in appropriate model organisms in a tenacious, innovative, and rigorous fashion holds great promise for the burgeoning field of regenerative medicine for heart disease and many other chronic conditions. For stem cell biology, regeneration is next, and an exciting future belongs to a next generation of physicians and scientists working together as a team on this central problem in modern human biology and medicine.

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