Potential of stem-cell-based therapies for heart disease

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The use of stem cells to generate replacement cells for damaged heart muscle, valves, vessels and conduction cells holds great potential. Recent identification of multipotent progenitor cells in the heart and improved understanding of developmental processes relevant to pluripotent embryonic stem cells may facilitate the generation of specific types of cell that can be used to treat human heart disease. Secreted factors from circulating progenitor cells that localize to sites of damage may also be useful for tissue protection or neovascularization. The exciting discoveries in basic science will require rigorous testing in animal models to determine those most worthy of future clinical trials.

Rarely has anything so energized scientists and the lay public alike as the enormous potential of stem-cell biology to treat human disease. The ability to mobilize endogenous progenitor cells in organs or to introduce and differentiate exogenous stem cells for tissue repair could have an impact on many diseases, including those affecting the brain, skeletal muscle, pancreas and heart. Regenerative therapies could be particularly beneficial for heart disease — the number one killer in adults and the leading non-infectious cause of death in children[1]. Cardiomyocytes do not seem to enter the cell cycle after birth, and consequently the heart has almost no regenerative capacity after injury. The long-held dogma has been that the heart cells with which you are born are the ones with which you die.

However, exciting new findings in the past 5 years have caused us to re-evaluate the potential of protective or regenerative cardiac therapies. Like the brain, the heart seems to have reservoirs of progenitor cells that may not be sufficient to replace the acute loss of a large number of cells, but may be able to replace a slow apoptotic loss of cells over a lifetime. Here, we examine the current basic and clinical science that is forming the foundation of future approaches to cardiac regeneration.

Cardiac and ES-cell-derived progenitor cells

Throughout the myocardium, pools of cardiac progenitor cells (CPCs) may participate in the continual replacement of apoptotic cardiomyocytes at a low basal level. Unlike terminally differentiated cardiac cells, CPCs are small cells that do not express cardiac markers and that can self-renew and proliferate. Several seemingly different but overlapping populations of progenitor cells (such as Sca-1- (ref. 2), c-Kit- (ref. 3) and Abcg2- (ref. 4) expressing side populations) can be induced to activate cardiomyocyte-specific genes *in vitro*; however, this effect has been also observed in mesenchymal stem cells, which do not fully differentiate into functional heart cells[5]. In addition, Sca1- or c-Kit-expressing cells may differentiate into cardiomyocytes *in vivo*, contributing to repair of the damaged heart after acute myocardial infarction, but their potential is limited, in part, by the small number of endogenous CPCs. Attempts to mobilize and to expand endogenous progenitor cells by introducing growth factors hold promise but remain controversial[6]. It is likely that the activity of endogenous CPCs will have to be augmented, through knowledge of the mechanisms of normal progenitor expansion and determination during embryonic development, before these cells will contribute substantially in the extreme setting of infarcted hearts.

Because embryonic stem (ES) and progenitor cells resemble early fetal cells that are adopting discrete lineages, elucidating the early developmental events of cardiogenesis has been instructive for understanding and manipulating CPCs. Pluripotent stem cells maintained through transcriptional regulators (such as NANOG, OCT4 and SOX2; ref. 7) are directed to differentiate into the mesoderm lineage by key transcription factors, including MESP and the T-box protein brachyury[8,9] (Fig. 1). Subsequent determination of mesoderm progenitors mimics the embryonic developmental potential of two distinct fields of cells that give rise to the heart. Often referred to as the first and second heart fields, cells in these regions express unique markers of progenitor cells[10]. For example, the LIM-domain-containing transcription factor islet1 (ISL1) is involved in the differentiation of second heart field cells[11], whereas the homeodomain-containing transcription factor NKX2.5 is a marker of both heart fields[10]. Most interestingly, remnant second heart field cells may not only be able to differentiate into many cell types, but also persist in the postnatal heart[12] (Fig. 1). The pool of potential CPCs might be involved in continual maintenance of the heart by differentiating into several types of cardiac cell, including muscle, conduction and vascular cells, although the precise lineage potential of distinct subtypes remains to be determined (Fig. 2). It will be important to identify specific markers of the primary heart field to locate progenitor cells postnatally and even into adulthood.

Developmental markers of the primary and secondary heart fields may be useful in enriching ES cells for CPCs. When allowed to grow in clusters called 'embryoid bodies', mouse and human ES cells can differentiate into many lineages, including beating cardiomyocytes. Numerous approaches to increase the number of CPCs in this system have been devised, including the addition of retinoic acid or dimethyl sulphoxide, and the inhibition of BMP signalling[13]. Modulation of WNT signalling may also be useful[14]. In addition to key signalling and transcriptional events that may direct the cardiac lineage, the discovery of microRNAs that are muscle-specific (for example, miR-1 and miR-133) and involved in differentiation of CPCs in the embryo[15,16] raises the interesting possibility that microRNAs could be useful in initiating or sustaining the

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cardiogenic programme. Finally, improvements in chemical libraries and high-throughput screens make it possible to screen for small molecules that could regulate the proliferation or differentiation of CPCs[17].

In summary, numerous studies are underway to isolate and modify the behaviour of CPCs. Such pursuits are essential because the direct introduction of undifferentiated ES cells into the heart results in the formation of teratomas[18]. The different lines of investigation and approach described above provide hope that we may be able to regulate the commitment, proliferation and differentiation of ES or non-ES cells into cardiomyocytes, and to harness these cells for therapeutic purposes.

Other types of progenitor cell

Advances in understanding the basic biology of stem cells have been balanced by mixed and controversial results from translational and clinical studies. Early studies in mice suggested that bone-marrow-derived mesenchymal stem cells (BMSCs) could differentiate into cardiomyocytes[19], and thus the introduction of BMSCs after myocardial infarction might induce the repair of damaged myocardium. More recent studies with genetically marked cells indicate that BMSCs do not transdifferentiate into cardiomyocytes[20–22]. It remains possible that BMSCs do confer some beneficial effects, possibly by secreting paracrine factors that are cardioprotective or angiogenic[23].

On the basis of early mouse work suggesting myogenesis, numerous clinical trials with autologous BMSCs were begun in individuals with acute myocardial infarction or ischaemic myocardium[24]. Early anecdotal reports suggested some benefit, but subsequent randomized trials yielded mixed results. Several small studies reported a statistically significant increase in cardiac pumping ability and coronary perfusion, and a decrease in infarct size. At least one larger recent trial, however, has not shown a clinical benefit[25]. Similarly, a randomized trial based on earlier mouse data[26], in which granulocyte colony-stimulating factor was used to mobilize endogenous BMSCs after infarction, did not show improvement in cardiac function[27].

If BMSCs do confer physiological benefit, then they do so by an unknown mechanism, raising concern for ongoing or pending clinical trials, many of which initially assumed that BMSCs can differentiate into new cardiomyocytes. Although the focus has now shifted to understanding the potential non-cell-autonomous effects of BMSCs on hypoxic myocardium, much remains to be learned. Secreted angiogenic factors and/or activation of pathways that promote cell survival might protect and rescue hypoxic myocardium, thereby limiting damage to tissue and improving cardiac function[23]. If paracrine factors are the key agents, isolating and delivering such factors at high concentrations or engineering BMSCs to secrete larger amounts could result in more significant protection[28]. Interestingly, thymosin β4, which is secreted in very large quantities by BMSCs[28,29], is cardioprotective after acute myocardial infarction[30] and induces angiogenesis in mice[31,32]. Future large-animal and clinical trials of thymosin β4 and other secreted factors hold promise and may obviate the need for cell-based therapy for the at-risk hypoxic myocardium.

The road ahead

Future research on the clinical applications of stem cell biology for human heart disease will be based on advances in understanding cell lineage decisions and the regulation of pluripotency and differentiation in cardiac and vascular cells. Approaches for regenerating damaged muscle and for treating children who lack specific subsets of cardiac lineages will face many hurdles. The ability not only to guide and expand stem cells into the cardiac lineage but also to repress alternative fates will be crucial to avoid differentiation into cell types that may be harmful to cardiac homeostasis. Methods for safe delivery, migration and proper integration of stem cells will need to be perfected to avoid complications and abnormal electrical coupling that could lead to arrhythmias, as has been experienced with introduction of skeletal muscle into the myocardium[33]. Finally, it will be essential to solve the immunological issues surrounding rejection if non-autologous sources of stem cells

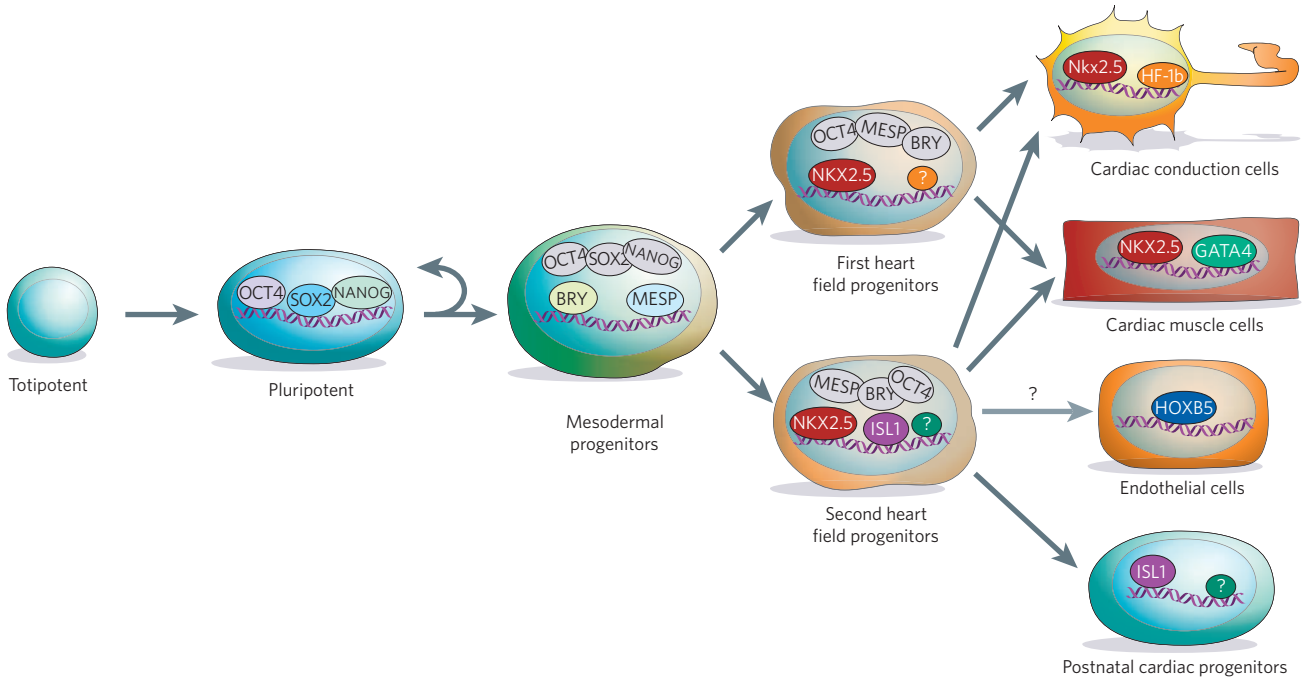

Figure 1 | Differentiation of embryonic cells into the cardiac lineage. Transcriptional regulation of early embryonic cells by factors such as NANOG, OCT4 and SOX2 maintains pluripotency. Decreased activity of pluripotency factors is accompanied by increased activity of lineage-specific transcriptional activators such as brachyury (BRY) and MESP in the mesoderm lineage. Mesodermal cells that begin to differentiate into cardiac cells segregate into two distinct populations of cardiac progenitor cells (CPCs), marked by the transcription factor NKX2.5. Those CPCs that also express the transcription factor ISL1 are similar to cells derived from the embryonic second heart field, whereas those without ISL1 expression may be more like first heart field cells. These progenitors may be able to differentiate into several types of cardiac cell, and ISL1-expressing cells uniquely give rise to niches of postnatal CPCs. Some transcription factors involved in lineage decisions are indicated. GATA4, GATA-binding protein 4; HF-1b, trans-acting transcription factor 4; HOXB5, homeobox B5.

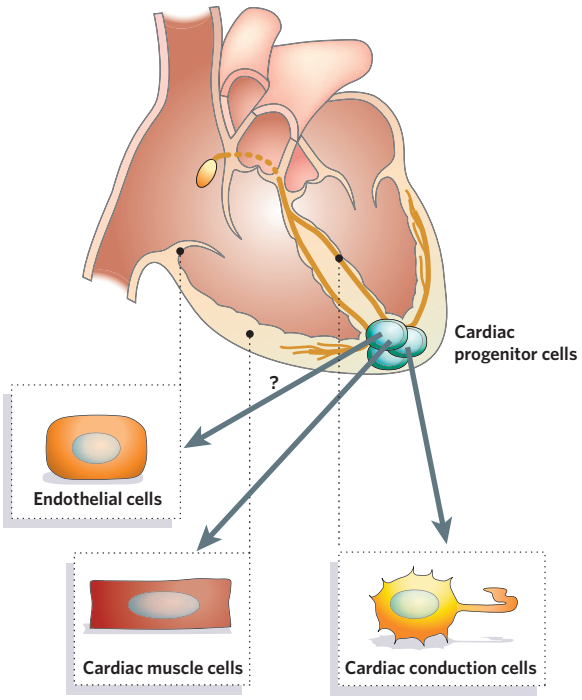

Figure 2 | Potential of postnatal cardiac progenitor cells (CPCs). Niches of CPCs in the postnatal heart may have the potential to differentiate into endothelial cells for vessel formation, cardiac muscle cells for contractility, and cardiac conduction cells for coordinated electrical activity of the heart, although precise lineage potentials of distinct subsets remain to be determined.

are used. In this respect, technologies to develop individual-specific stem-cell lines through somatic-cell nuclear transfer[34] or cell fusion[35] may allow engineered stem cells containing the individual's own genetic material to be used both for treatment and for studies of pharmacological efficacy.

Although there are clearly many obstacles to overcome, it is significant that a roadmap of the derivation and use of stem cells for human heart disease is now conceivable. The coming years will undoubtedly bring new developments and technologies that will require rigorous scientific evaluation and prudent judgment, balancing healthy scepticism with eagerness driven by sobering clinical needs. ■

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