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Lessons for cardiac regeneration and repair through development

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Abstract

Cell-based regenerative strategies have the potential to revolutionize the way cardiovascular injury is treated, but successful therapies will require a precise understanding of the mechanisms that dictate cell fate, survival, and differentiation. Recent advances in the study of cardiac development hold promise for unlocking the keys for successful therapies. Using mouse models and embryonic stem cells, researchers are uncovering cardiac progenitor cells in both embryonic and adult contexts. Furthermore, the signaling molecules and transcriptional regulators that govern these cells and their behavior are being revealed. In this review, we focus on the recent advances in these areas of cardiac developmental research and their impact on the expanding field of regenerative medicine.

Keywords

Regenerative medicine; Heart development; Signaling; Transcription factors; Embryonic stem cells

Re-“developing” regenerative medicine

Heart disease is one of the most serious challenges for modern medicine. Improvements in acute treatments have increased the number of patients who survive myocardial infarctions. However, infarcts remain a significant cause of mortality and morbidity, owing to the limited regenerative capacity of the mammalian heart, and contribute to progressive heart failure [1]. For years, clinicians have imagined using cell-based regenerative therapies to repair damaged cardiac tissue through transplantation. Early attempts at such treatments utilized a number of different cell types, including myoblasts and cells from the bone marrow [2–4]. While some of these treatments have shown measurable improvements in cardiac function, the transplanted cells failed to transdifferentiate into cardiac muscle [5–9] and, in some cases, did not electrically integrate into the heart [10], leading to arrhythmias [11].

Ideally, cells from the cardiac lineage should be used to regenerate damaged cardiac tissue. Indeed, implantation of cardiomyocytes has been beneficial in animal and disease models [3, 4], and embryonic stem cells (ESCs) or induced pluripotent stem (iPS) cells (Box 1)

represent a theoretically unlimited source of these cells. Nevertheless, true regenerative therapy for heart disease is still years away and depends upon an expanded understanding of the principles dictating initial cardiac genesis during development. Identifying and generating the appropriate cell types, controlling their behavior *in vitro* and *in vivo*, and predicting how these cells will respond to a myocardial infarct are major hurdles for regenerative cardiac therapy and are best addressed with a strong foundation in cardiac development.

Here, we explore the rapidly changing field of cardiac regenerative therapy in the context of cardiac development. We briefly highlight the current state of the field and then discuss cardiac development. We review recent studies from mouse models and ESCs that have identified potential regenerative cell types and defined the molecular cues that regulate their specification and differentiation. Finally, we comment on how these advances might inform and improve future regenerative strategies, and we raise important questions for the field moving forward.

Current strategies for cardiac regeneration

Much has been made of the potential of ESCs to generate disease-relevant cell types [12]. Approaches using ESCs for transplantation of functional cardiomyocytes in animal models have begun to show promise. Multiple studies have demonstrated engraftment of mouse ESC (mESC)-derived cardiomyocytes into a recipient rodent heart, and importantly, these treatments significantly improved contractile function [13,14]. Initial studies with human ESCs (hESCs) are also promising, owing to fairly high engraftment rates and graft sizes that improved with time [15]. This was achieved through heat shock exposure of the implanted cells, which improves cardiomyocyte survivability upon implantation, and donor cardiomyocyte proliferation within the infarct [15]. In addition, the grafts showed donor and graft-mediated angiogenesis [15]. In some studies, grafts showed successful electrical coupling with the surrounding tissue [16,17], allowing them to serve as biological pacemakers. These studies involved healthy, non-infarcted hearts, so it was unclear how successful these approaches would prove in the hostile niche of a myocardial infarct.

Recent studies, focused on using hESC-derived cardiomyocytes to repair injury models, have had some success. Transplants of cardiomyocyte-enriched hESC cultures engraft infarcts less efficiently in rat injury models; the success rate in one study was less than one in five [18]. Other complications, such as persistent non-cardiac cysts at the site of engraftment, have been observed. These complications could be largely reversed by directing cardiac differentiation, which improved purity, and co-injecting a cocktail of pro-survival compounds [18]. Importantly, transplanted hESC-derived cardiomyocytes improve cardiac function in multiple studies using both infarcted rats and engineered cardiac tissue as models [18–21].

Yet, significant questions remain. The long-term benefits of the engraftments are uncertain. In one study, grafts yielded only short-term functional improvement for up to three months [22]. In addition, the mechanism by which these treatments improve functional output is still unclear. Revascularization of infarct and not graft size might be responsible for the observed benefits [23]. Thus, while initial studies are promising, clinical treatments need to be more efficient and effective. Research focused on understanding how these tissues form during embryonic development provides an avenue to inform and improve regenerative strategies.

Identifying and understanding cardiac precursors

Success of regenerative strategies will depend heavily on the identification of an ideal cell type. Although recent studies have used cells committed to the cardiomyocyte lineage,

cardiac progenitor cells (CPCs) are an enticing alternative; they might allow the generation of more integrated functional tissues, composed of multiple cardiovascular cell types, within infarcts. Despite the considerable knowledge about cardiac development, surprisingly little is known about its earliest stages and cellular precursors. These have become areas of intense scientific research.

The identification and isolation of CPCs from ESC cultures is a major roadblock in the use of these cells for regenerative strategies. However, multiple groups have now reported the isolation of CPCs from both mouse embryos and ESC cultures [24–28] (Figure 1a). These studies used different markers to identify each population. In one study, expression of the vascular endothelial growth factor receptor, Flk1, was found to mark first a hematopoietic population followed by a cardiogenic population in differentiating mouse embryoid bodies [24]. A second group identified cardiogenic clones coexpressing Flk1 and the transcription factors Nkx2–5 and Islet-1 (Isl1) [25], and later reported the Isl1 lineage marked cardiogenic cells in human ESC cultures [28]. Additionally, a third group demonstrated that expression of the receptor tyrosine kinase c-kit marked a progenitor subpopulation of Nkx2–5-expressing cells [26]. Interestingly, in humans low expression of the Flk1 ortholog KDR and lack of c-kit ($KDR^{low}/c\text{-kit}^{-}$) marks a cell population with cardiogenic potential [27]. Thus, the relationship between these different precursor populations (whether they represent distinct lineages, a single lineage at distinct differentiation stages, or a single population visualized with distinct markers) remains to be determined (Figure 1b). Importantly, general features of CPCs have been established by these studies. CPCs are highly proliferative and can give rise to multiple cellular lineages, including cardiomyocytes, smooth muscle cells, and endothelial cells [24–28]. These characteristics suggest that CPCs are a desirable cardiovascular cell type for therapeutic use.

Within the mammalian embryo, the heart derives from bilateral fields of cardiac precursors that form during gastrulation and migrate to the anterior pole [29]. These cells contribute to diverse regions and cell types of the heart. Recently, our understanding of early cardiogenic fields expanded with the discovery of a second heart field [30]. The first and second heart fields appear to contribute to distinct regions of the heart, [31, 32] and only the second heart field expresses Isl1 during differentiation [33]. Thus, precursors within the two fields seem to give rise to distinct lineages and differentiate according to divergent transcriptional programs, though recent work suggests first heart field precursors may also transiently express Isl1 [34–35]. The duality of heart genesis raises questions about cell type choice for regenerative strategies. Specifically, do precursors from the fields behave differently in therapeutic contexts? And if so, which pool is most desirable?

These questions are beginning to be addressed. Using a genetically-labeled fluorescent reporter system, multiple cardiogenic populations with different relationships to the two heart fields have been isolated from mouse embryos and ESC cultures via fluorescence-activated cell sorting [36]. The propensity to generate cardiomyocytes as well as the type of cardiomyocytes formed by each population were different [36]. Thus, the identification and isolation of specific cardiac precursor populations, perhaps using cell surface markers unique to each population, and the characterization of functional differences among these precursors will be important for advancing regenerative strategies.

Signaling inputs for cardiovascular stages of differentiation

In the developing embryo, cardiac precursors are specified by a tightly controlled signaling environment. Hence, understanding how different signaling pathways dictate cardiac precursor specification and subsequent behavior is critical for generating these cells *in vitro* and predicting how they will behave post-transplant. In many developmental systems,

Nodal/Activin/TGF- β , bone morphogenetic proteins (BMPs), and fibroblast growth factors (FGFs) promote cardiogenesis [37]. While non-canonical Wnts, which are β -catenin independent, have been shown to promote cardiac specification, canonical Wnts, which signal through β -catenin, were believed to function largely as inhibitors of cardiogenesis [38,39] (Box 2). Recent findings, however, challenge this dogma.

Recent findings have demonstrated that canonical Wnt signaling plays distinct time-specific roles during cardiogenesis, regulating the specification, maintenance, and differentiation of CPCs distinctly. According to one study, canonical Wnt activity blocks the specification of CPCs, promotes CPC proliferation, and stops the differentiation of CPCs into cardiomyocytes during mESC differentiation [40]. Similarly, more beating embryoid bodies were observed when the Wnt pathway was activated during the early stages of differentiation; at later stages, Wnt suppresses beating or promotes cardiac gene expression, depending on the study [41–43]. These differences could be the result of differences in culture conditions and the timing of Wnt activation. Taken together, however, these results demonstrate divergent time-dependent roles for canonical Wnt signaling.

In vivo evidence for a positive role for canonical Wnts in cardiac progenitor proliferation exists as well. In the developing mouse, expression of a stabilized form of β -catenin markedly increases the proliferation of Isl1-expressing cardiac progenitors [40, 41], whereas β -catenin null embryos have hypoplastic structures derived from the second heart field [41]. Thus, canonical Wnts play positive, as well as repressive, roles in heart formation during embryogenesis.

Multiple signaling pathways integrate and cross-regulate during cardiac development. By specifically activating and blocking their activity, Activin/Nodal and Wnt activity were shown to be essential for the specification of mesoderm in ESC cultures, whereas BMPs promote mesoderm formation through a Wnt/Activin/Nodal-dependent mechanism [44]. Interestingly, Activin/Nodal, BMP, and Wnt signaling are important for specifying subsets of mesoderm [44]; thus, it is likely that multiple signaling inputs regulate the earliest steps of cardiogenesis through mesoderm specification and allocation (Figure 2a). Once cardiogenic mesoderm is specified, Notch regulates CPC expansion through an interaction with Wnt/ β -catenin signaling [45]. Understanding the interplay between signaling pathways will be necessary to define the normal steps in cardiac development and, ultimately, to guide cells toward a desired therapeutic endpoint.

Driving differentiation with cardiogenic factors

Transcription factors (TFs) govern most developmental gene expression programs. The power of TFs to establish cellular identity has been recently exemplified by cellular reprogramming, in which the ectopic expression of TFs in somatic cells can generate many cell types. Examples include the generation of skeletal muscle cells from fibroblasts [46], the complete reprogramming of somatic cells into iPS cells [47–48] (Box 1), and the conversion of mesoderm-derived fibroblasts into normally ectoderm-derived neurons [49], demonstrating that the right combination of TFs can completely alter the state of a differentiated cell. These approaches demonstrate the possibility for directed trans-lineage reprogramming.

In the heart, a large group of TFs cooperate to control cardiac gene expression [29, 50]. These include members of the Nkx, Mesp, Gata, Islet, Tbx, Mef2, and Hand families. Similar to signaling pathways, understanding the TF networks that control cardiac development could yield effective means for generating desired therapeutic cell types *in vitro* as well as mobilizing or transdifferentiating cells *in vivo* either after injury or disease onset. Numerous cardiac factors might have this potential.

Mesp1, a basic helix-loop-helix TF, is transiently expressed in mesodermal populations that contribute to the majority of the heart [51]. Owing to its expression within early cardiogenic mesoderm, it is an enticing candidate for a critical regulator of the cardiac transcriptional program. Several groups have overexpressed Mesp1 to probe its function in cardiogenesis. Mesp1 overexpression induces greater numbers of mesodermal-derived precursors [52], an earlier advent of beating [53], more beating areas [53–54], and increased expression of cardiac markers [52–54] during mESC differentiation. In addition, ectopic expression of Mesp1 in *Xenopus* tadpoles produces ectopic contracting tissue [54]. Together, these data suggest this factor might promote transdifferentiation into cardiac precursors (Figure 2b). Although these experiments are promising, the mechanism by which Mesp1 functions as a cardiogenic factor is uncertain, and additional research is necessary to fully define its role.

Cardiogenic transcriptional regulators can induce mesodermal derivatives to transdifferentiate into functional myocytes within the mouse embryo. The combination of factors that is effective in this process reveals important aspects of the biology of mammalian cardiac transcriptional regulation. Ectopic expression of three factors, Tbx5, Gata4, and Baf60c, drives cardiogenesis in non-cardiogenic posterior mesoderm as well as amniotic mesoderm [55] (Figure 2c). Baf60c, a component of the conserved SWI-SNF-like Brg1/Brm-associated factor (BAF) chromatin-remodeling complex, might allow BAF complexes to recognize cardiac regulatory elements and facilitate TF binding by altering the local chromatin environment. Indeed, Gata4 and the BAF complex ATPase Brg1 were detected at cardiac promoters only when coexpressed with Baf60c [55]. Understanding the cooperative roles that TFs and chromatin regulators play during cardiac differentiation will likely be pivotal in driving therapeutic cardiac cell fates.

Understanding the determinants of the cardiac transcriptional programs has raised the distinct possibility of direct transdifferentiation of adult cell types into functional cardiomyocytes. The induction of cardiac tissue from embryonic mesoderm helps define the minimal inputs required for cardiac differentiation, but does not necessarily provide a therapeutically useful approach to generate new cardiomyocytes. Recent work in the mouse demonstrates that cardiogenic transcription factors can reprogram fibroblasts into functional cardiomyocytes [56]. The combination of Gata4, Tbx5, and Mef2c direct reprogramming of cardiac fibroblasts or tail-tip fibroblasts into cells that express cardiac markers, form sarcomeres, have a transcriptional profile that closely resembles that of cardiomyocytes, and demonstrate spontaneous calcium flux, beating, and action potentials that resemble adult cardiomyocytes [56] (Figure 2d). Two of the factors, Gata4, and Tbx5, are part of the combination that direct mesoderm to cardiomyocytes [55], indicating a likely common mechanism of action. Importantly, it was found that the reprogramming of fibroblasts to cardiomyocytes does not proceed via dedifferentiation to a precursor state, but rather appears to be a direct conversion [56]. These findings indicate that it is feasible to reprogram somatic cells into cardiomyocytes, providing an exciting potential for regenerative strategies that could involve conversion of resident fibroblasts to replace cardiomyocytes lost to ischemic injury.

Postnatal progenitor cell types

The activation of regenerative precursor cells resident within mature hearts is a promising though challenging therapeutic strategy, providing that such cells could be readily identified. Isl1-expressing CPCs have been identified in mammalian postnatal hearts, including human [57]. These cells expand *in vitro* and give rise to bona fide cardiomyocytes that express sarcomeric proteins and generate action potentials. Isl1-positive progenitors have been isolated soon after birth, but thus far, have not been found in significant numbers in more mature hearts, such as those that would be most in need of cardiac repair. However, it is

enticing to imagine mobilizing these resident progenitors soon after infarct to initiate repair. Further studies will determine if these progenitors are present during adulthood and if the same signals that regulate embryonic cellular potential also regulate the potential of precursors in more mature hearts.

In addition, multiple studies have identified other putative adult cardiac progenitor cells using various markers. $c\text{-kit}^+ \text{Lin}^-$ cells were identified in adult rat hearts and demonstrated robust self-renewal and differentiation potential for cardiac lineages [58]. These progenitors also demonstrated high efficacy for regenerating cardiomyocytes in injury models [58]. Another group identified the presence of sca-1^+ cells that could differentiate into cardiomyocytes upon treatment with 5'-azacytidine, a compound known to promote cardiogenesis [59]. Hoechst dye-effluxing marked side population (SP) cells were also identified in the developing and adult heart [60]. Side populations mark stem/progenitor cells in other organ systems, and cardiac SP cells can proliferate and generate cardiomyocytes *in vivo* [60]. Interestingly, these progenitor populations appear to be exclusive of one another, suggesting the existence of multiple progenitor cell types capable of cardiac differentiation. While promising, little independent confirmation of these cell types is available, and much more needs to be known about the developmental origins of these progenitors, as well as their efficacy in treating myocardial infarcts.

The epicardium is an epithelial layer that surrounds the heart and contributes to the coronary vasculature and cardiac fibroblasts during development [61]. In zebrafish, epicardial cells contribute the ventricular wall during adult-stage growth and injury repair, although the epicardium contributes little if any new cardiomyocytes during regeneration [62,63]. Mammalian hearts display limited regenerative activity, but the regenerative potential of the mammalian epicardium is not well understood. Epicardial cells may contribute to the heart and generate cardiomyocytes during mammalian development [64, 65], although this has been debated [66]. The possibility of the epicardium functioning in a regenerative role in the adult mammal has captivated developmental biologists and clinicians alike. Additional research will determine if the reparative capacity exists and to what extent it can be exogenously stimulated.

Recent observations in zebrafish suggest still another possible mechanism to cardiac regeneration. It was found that zebrafish regenerate amputated myocardium by dedifferentiating existing myocardium, and subsequently can repopulate the scar tissue that first replaces the amputated tissue [67, 68]. If controlled dedifferentiation could be induced in mammalian myocardium, this could prove a useful strategy for regeneration.

Regeneration and development: moving forward

In the past several years, our understanding of how the heart develops has expanded tremendously, from identifying embryonic and postnatal cardiac progenitors to deducing how signal and transcriptional networks work to form and control these cell types. As a result, multiple regenerative strategies for the heart now appear feasible, including the transplantation of cardiac precursors, the mobilization of endogenous progenitors, and the transplantation of transdifferentiated cardiomyocytes (Figure 3). Looking forward, several remaining questions deserve attention.

First, are cardiac progenitors the best cell type for transplantation after infarct? Although recent findings support this possibility [21], studies are needed to determine the types of mature cells present within engraftments and whether grafts fuse seamlessly with resident cardiac tissue for true regeneration.

Additionally, we must better understand the signaling and transcriptional determinants of cardiac differentiation. Armed with this knowledge, researchers should be able to generate more pure populations of transplantable cardiac cell types from ESCs with greater facility. Furthermore, these pathways will likely reveal key nodes that mediate cell survival, proliferation, and differentiation; targeting these nodes within the infarct could provide the best routes for optimizing transplantation effectiveness or maximizing endogenous regenerative capacity.

Finally, the finding that fibroblasts can be directly reprogrammed into induced cardiomyocytes (iCMs) suggest that somatic cell reprogramming might prove the most desirable approach. Directly reprogramming somatic cells, which are plentiful, creates the option to generate large numbers of patient-specific transplantable cells, so as to avoid immune rejection. iPS cells also present an opportunity to generate patient-specific cardiac tissue; however, iPS generation can take several months from reprogramming to validation. Direct transdifferentiation from somatic cell to functional cardiomyocyte might allow for more rapid transplant after injury, when the infarct is more plastic to remodeling.

In addition, direct reprogramming might be possible *in vivo*; plentiful resident cell types at the site of injury could be transformed into functional heart tissue without the need for transplantation. Indeed, cardiac fibroblasts, which are found throughout the heart and are highly prevalent within infarcts, are alluring targets for such an approach.

Few fields have enjoyed the rapid growth of enthusiasm seen recently in cardiac regenerative medicine. Despite this, the long-term therapeutic potential of regenerative strategies remains uncertain. What is clear, however, is that if these approaches are to be successful, they will require the close collaboration between developmental biologists and translational researchers. The recent past has seen major advancements owing to such synergies, and developmental insights will continue to prove critical if we are to elicit true regeneration of the heart.

Box 1: The induction of pluripotency

In the mid-1960s, it was demonstrated that somatic nuclei derived from *Xenopus laevis* could be transplanted into enucleated oocytes to generate tadpoles and fertile adult frogs [69]. These results suggested that oocytes contain key regulatory factors that can instruct nuclei to adopt pluripotent plasticity and demonstrated that cells did not experience an irreversible modification of the genetic material during differentiation as previously presumed, but rather could be reprogrammed to a more naïve developmental state.

The advent of induced pluripotent stem (iPS) cells has further revolutionized our understanding of this process. iPS cells are somatic or non-pluripotent stem cells that are reprogrammed into a pluripotent state by introduction of exogenous factors that are critical regulators of this state. Multiple methods now exist for the generation of iPS cells, and many donor cell types have been used [70], but the standard approach involves viral introduction of several factors (Oct3/4, Sox2, c-myc, and Klf4) and selection of pluripotent colonies using colony morphology, selectable markers, and/or fluorescent reporters. Mouse IPS cells demonstrate all the hallmarks associated with pluripotent ES cells, including expression of pluripotency markers, the ability to generate derivatives from all three germ layers *in vitro* and in teratomas, and even demonstrate full developmental potential by tetraploid aggregation [47,71,72]. Indeed, entire mice can be generated from the genetic material originally found in a fibroblast.

The success of translating iPS technology to humans has generated a fervor within the scientific community over the potential therapeutic use of these cells [70]. Patient-specific pluripotent cells can be generated and potentially used in transplantations, which would

eliminate the problem of immune rejection that may accompany the transplantation of hESC derivatives. In addition, disease-specific iPS cells can be used to model human diseases in a dish by generating disease-affected cell types in difficult-to-study diseases, such as amyotrophic lateral sclerosis (ALS). Such studies could go a long way towards uncovering disease mechanisms, and may be useful in screening drug candidates. Indeed, numerous clinically-relevant applications for human iPS cells exist. Many studies are underway to improve the efficacy of iPS generation and limit the potential safety complications that might accompany using these cells for human cell replacement treatments.

Box 2: Discovering the Signaling Inputs for Cardiogenesis

Prior to the use of embryonic stem cells in parsing the time-specific roles of signaling pathways during the many steps of cardiac differentiation, identification of the key signaling inputs in cardiogenesis was determined by careful embryological studies of multiple model organisms. Early studies demonstrated that the endoderm and neural tissue played inductive and repressive roles during cardiogenesis in the newt, respectively [73]. Gradually, these relationships were identified in many other vertebrate species [74–76]. This was followed by the search for the molecules that bestow these inductive and repressive properties to these embryonic tissues.

The most likely candidate for an inductive signal from the endoderm are the BMPs. Studies in the developing chick showed expression of multiple BMPs within the anterior endoderm and demonstrated that ectopic activation of BMP signaling could activate cardiac genes in non-cardiogenic mesoderm [77]. Conversely, inhibition of BMP signaling by antagonists led to reduced cardiogenesis in the chick [77,78]. These studies were supported by work in frogs that demonstrated that reduced BMP signaling through overexpression of dominant-negative receptors led to reduced or abnormal cardiogenesis *in vivo* [79]. Finally, *Bmp2* mouse mutants were shown to have various abnormalities in heart development [80], consistent with a conserved role for BMP signaling in cardiac induction.

The cardiac inhibitory properties on the neural tube have been largely attributed to canonical Wnt signaling. In chick, canonical Wnts, such as Wnt1 and Wnt3a, inhibit cardiogenesis in explanted paraxial mesoderm in a similar manner to co-cultured neural tissue [81]. Importantly, inhibition of Wnt leads to ectopic cardiogenesis in the chick embryo and relieves neural tube-mediated repression of cardiogenesis in explants [81–82]. Similar studies were conducted in frog and showed that Wnt inhibition could ectopically induce cardiogenesis in non-cardiogenic ventral marginal zone mesoderm [83]. Studies in the mouse embryo demonstrated that loss of β -catenin leads to ectopic cardiac differentiation [84].

Multiple, additional signaling pathways involved in cardiogenesis were originally identified using the power of experimental embryology, including Fgfs, activin/nodal, and non-canonical Wnts [37,38]. The pioneering work performed identifying the myriad pathways involved in cardiac specification and development has laid the framework for the recent advances described in this review. These traditional approaches, used in combination with *in vitro* developmental models like ES cells, provide a powerful means for dissecting the developmental roles of signaling in the heart, as well as in other systems.

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Figure 1.

Precursors of the mouse heart. Cardiac precursors have been identified and isolated from mouse embryos and ESC cultures (**a**). Multiple different precursors have emerged, including Flk1⁺ [24], Isl1⁺/Nkx2-5⁺/Flk1⁺ [25], and Nkx2-5⁺/c-kit⁺ [26] cell populations. These cells demonstrate multipotency for resident cell types of the heart, including cardiomyocytes, smooth muscle cells and endothelial cells. The interrelationship between these different precursors and how they contribute to the heart *in vivo* is still unclear (**b**). These populations might consist of similar or identical precursors that appear distinct because they were isolated using different approaches (**top**). In contrast, they might represent completely distinct lineages that independently contribute to the heart (**center**) or discrete stages of differentiation within a common cardiac lineage (**bottom**).

Figure 2.

Molecular regulators of cardiac differentiation. Multiple signaling inputs are important for cardiac differentiation, and each have distinct effects at each developmental stage (**a**). BMPs, canonical Wnts, and Activin/Nodal all promote early mesodermal specification. Wnts also have both positive and negative effects on cardiac differentiation. Wnts block the specification of CPCs, but promote their expansion; Wnts repress the differentiation of these progenitor cells. Transcription factors regulate cardiac differentiation (**b–d**). Overexpression of *Mesp1* increases cardiac differentiation, most likely by promoting the specification of cardiogenic mesoderm and CPCs from mesodermal cell types. Overexpression of *Tbx5*, *Gata4* and *Baf60c* transdifferentiates mesodermal cells into cardiomyocytes, and overexpression of *Tbx5*, *Gata4*, and *Mef2c* can reprogram cardiac or skin fibroblasts from the adult into cardiomyocytes.

Figure 3.

Strategies for regenerative cardiac treatment. Current approaches for cell-based regenerative heart therapy focus on utilizing ESC-derived cardiomyocytes for direct implantation onto an infarcted area (yellow asterisk) (**a**). Multiple novel approaches might prove more effective. Multipotent cardiac progenitors might be directly implanted into an injured heart or guided to differentiate *in vitro* followed by transplantation of the mature cardiac cell types (**b**). Alternatively, inductive signals or chemical agonists could mobilize resident progenitors within the heart or surrounding epicardium to regenerate injured areas, bypassing the need for transplantation (**c**). Cardiogenic factors such as transcription factors and chromatin regulators might be used to transdifferentiate cells *in vitro* into cardiomyocytes (**d**), making the generation of patient-specific cardiomyocytes feasible and improving the quantity and efficiency of cardiomyocyte generation *in vitro*. These factors or chemical agonists might also be applied directly to the heart to transdifferentiate residual cardiac fibroblasts into functional heart muscle.

Figure 4 within Box 1.

Generation of iPS cells. In both humans and mice, somatic cells such as fibroblasts can be reprogrammed into induced pluripotent stem cells through introduction of reprogramming factors. In the mice, iPS cells have been used to generate differentiated cell types from all germ layers as well as entire mice. For human iPS cells, differentiated cells can be used for transplantation and other medically important applications.