Published in final edited form as:

Nat Med. 2014 December ; 20(12): 1386–1393. doi:10.1038/nm.3764.

An emerging consensus on cardiac regeneration

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Abstract

Cardiac regeneration is a rapidly evolving and controversial field of research. The identification some 12 years ago of progenitor cells that reside within the heart spurred enthusiasm for cell-based regenerative therapies. However, recent evidence has called into question both the presence of a biologically important stem cell population in the heart and the ability of exogenously derived cells to promote regeneration through direct formation of new cardiomyocytes. Here, we discuss recent developments that suggest an emerging consensus on the ability of different cell types to regenerate the adult mammalian heart.

The long-held view that cardiomyocytes in the mammalian heart are postmitotic and hence live as long as the organism itself was first called into question over two decades ago¹. Prior to this report, pathological examinations of the mammalian heart indicated an abundance of mitotic cells before birth, but their rapid disappearance soon after birth suggested terminal differentiation and minimal ongoing renewal of cardiomyocytes. Although studies have reported DNA replication in cardiomyocytes during aging or after injury, most failed to evaluate actual cytokinesis as direct evidence of bona fide proliferation and new cardiomyocyte formation^{2,3}. Thus, a solid quantitation of the number of cardiomyocytes in the heart that are regenerated over time or after injury has eluded the field^{4,5}.

Loss of cardiomyocytes from the heart as a result of myocardial infarction (MI) or as part of progressing heart failure is a major cause of morbidity and mortality⁶. After MI it is clear that the human heart has very limited regenerative capacity because scarred areas appear to persist indefinitely. However, it is possible that the scar itself precludes effective regeneration from surrounding cardiomyocytes or progenitor cells, as smaller areas of injury that lack scarring appear to more effectively regenerate in animal models¹. Importantly, the

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COMPETING FINANCIAL INTERESTS

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adult human heart was reported to contain mitotic nuclei after pathological or physiological stress, suggesting some level of potential cardiomyocyte renewal^{1,7,8}.

Although various cell sources underlying endogenous cardiac regeneration have been proposed, some findings were not confirmed by independent studies, and disputes over methodological issues have hampered the formation of a consensus on the issue. In this Perspective we will discuss the most recent evidence suggesting that the heart does indeed have a finite capacity for regeneration and the current theories as to the dominant cellular source of cardiomyocyte renewal. We will also discuss ongoing and planned strategies for cardiac regeneration therapies and associated issues that remain unresolved.

Does adult cardiac regeneration occur?

Although there is still controversy as to the extent to which the adult mammalian heart can regenerate, the early neonatal mouse heart clearly regenerates, showing essentially complete recovery after an extensive injury event⁹. Lower vertebrates also have the capacity to regenerate large portions of their adult hearts after injury. When zebrafish or newts are subjected to an apical resection procedure, they can completely regenerate the lost myocardium¹⁰⁻¹². The exact factors that regulate the regenerative response are slowly emerging, and immune cells seem to be important through their release of paracrine factors^{13,14}. Interestingly, a recent mosaic lineage-tracing study in adult zebrafish showed that only a small number of cardiomyocytes are responsible for the addition of the majority of new cardiomyocytes in response to injury¹⁵. Although the fact that so few cells gave rise to all newly formed cardiomyocytes is somewhat surprising, it is also encouraging, as theoretically only a small number of cardiomyocytes or progenitor cells may be needed to regenerate large areas of the adult heart. These findings in lower organisms and early mouse neonates suggest that generation of new cardiomyocytes from existing cardiomyocytes might be possible within the adult human heart. Indeed, recent studies have identified genes whose products that can coax adult cardiomyocytes to behave more like their neonatal counterparts and traverse the cell cycle¹⁶. Although these genes may not be ideal targets for therapy, such studies are beginning to uncover basic mechanisms that may eventually lead to targeted therapies for cardiac regeneration at the level of the cardiomyocyte itself.

Although recent findings in lower vertebrates and neonatal mice are encouraging, cardiac regeneration in the adult mammalian heart is much harder to assess. The most common approach to identify cardiomyocytes with cell cycle activity, which suggests renewal, has been labeling with DNA nucleosides such as tritiated thymidine, EdU and BrdU. These agents allow measurement of DNA synthesis and have been used to estimate the level at which new cardiomyocytes are generated in the heart. However, the majority of proliferative cells in the adult heart are not cardiomyocytes, so even the best histological techniques can give equivocal results^{2,17}. There are a number of other challenges related to assessing cardiomyocyte renewal (**Box 1**). Hence, it has been extremely difficult to reliably assess the level of cardiac renewal in the healthy adult heart and even more difficult to quantify the level of regeneration after MI injury given the large invasion of proliferating inflammatory cells that occurs.

Box 1**DNA synthesis labeling to assess cardiac regeneration**

Identification of proliferating cells has typically relied on detection of exogenously added DNA nucleosides that integrate into newly formed DNA. Using this approach, most studies find that less than 1% of adult ventricular cardiomyocytes incorporate DNA nucleosides in the uninjured heart over a year's time. Interpretation of these low rates of DNA synthesis in adult cardiomyocytes is further complicated by the fact that adult cardiomyocytes can undergo DNA synthesis without karyokinesis. This is known as endoreduplication, and it increases DNA content per nucleus (the cells become polyploid); even the number of nuclei can increase per cardiomyocyte^{100,101} (see illustration). An alternative to labeling of DNA synthesis is measurement of cytokinesis, which is a true indicator of new cardiomyocyte formation. However, this remains a major technical challenge for the field⁶⁰. Adding to these challenges, specific identification of cardiomyocyte nuclei on the basis of histological techniques is difficult given the vast number of noncardiomyocyte cells (fibroblasts, endothelial smooth muscle cells and immune cells) that readily replicate and are also present in the heart¹⁰². Such issues can lead to an overestimation of cardiomyocyte renewal in the heart, especially after an injury when proliferative immune cells infiltrate the heart and, in some cases, fuse with cardiomyocytes^{19,20}.

Recent studies, however, have used new strategies to more accurately quantify cardiomyocyte regeneration. For example, to reduce errors in sampling cardiomyocyte nuclei when measuring DNA synthesis, a transgenic mouse model that expresses β -galactosidase (LacZ) specifically in all cardiomyocyte nuclei was used. This revealed renewal rates of just below 1% per year (as a percentage of all cardiomyocytes) when used in conjunction with BrdU or ³H-thymidine incorporation². These numbers may be higher in the atria of the heart and may also increase in some regions of the heart after MI. More recently, an *in vivo* cellular renewal experiment using multi-isotope imaging mass spectrometry in mice suggested a roughly similar yearly rate of renewal of 1% from existing cardiomyocytes (**Fig. 1a**)⁵. These results complement an unusual study in human post-mortem hearts, which used ¹⁴C labeling of cardiomyocyte nuclei to estimate regeneration. This method exploits the fact that ¹⁴C levels rose worldwide due to nuclear testing during the Cold War. The analysis estimated a cardiomyocyte renewal rate of 0.5–1% per year in adult humans⁴. Thus, although reports exist in the literature suggesting a much higher rate of endogenous cardiac regeneration, we believe the recent experiments discussed above are technically more definitive, collectively suggesting a renewal capacity of no more than 1–2% per year. To what extent these rates might increase after injury is not clear. In mice, such a low turnover rate is likely of little physiological consequence, but in humans, with a much longer lifespan, this low level regenerative capacity may be quite important.

Cellular sources of adult cardiac regeneration

The DNA-labeling strategies described above did not determine the cellular source of regeneration in the adult or injured heart. Although in lower vertebrates and the early neonatal mouse heart cardiomyocytes appear to be the source for regeneration, it remains unknown to what extent this same process occurs in the adult mammalian heart or whether other cell sources also contribute to renewal (**Table 1**). Indeed, noncardiomyocyte cellular sources, such as cardiac progenitor cells (CPCs), can also contribute new cardiomyocytes to the heart. To determine the cellular source of regeneration, cell transplantation studies and genetic lineage tracing based on molecular markers have been used. Here we will discuss cell types that have been reported to contribute to cardiac renewal and their physiologic relevance.

Bone marrow derived cells

In 2001, a high-profile report showed 68% regeneration of the infarcted myocardium in rodents 9 days after injection of Lin⁻c-kit⁺ bone marrow cells into the injured area¹⁸. This result was remarkable given the vast extent of remyocardialization of the heart that was observed and that only 9 days was needed for the transdifferentiating bone marrow cells to traverse cardiac cellular commitment, differentiation and functional maturation. Subsequent reports failed to replicate these findings, instead reporting that bone marrow c-kit⁺ cells did not have cardiomyogenic potential, no matter how the cells were isolated or treated¹⁹⁻²¹. Thus, we believe it fair to conclude that the majority of studies have since disproven bone marrow-derived Lin⁻c-kit⁺ cells as having any direct cardiomyocyte regenerative potential. These cells may however provide paracrine factors to stimulate either endogenous CPCs or cardiomyocytes themselves to proliferate at some finite level²²⁻²⁴.

Cardiac progenitor cells

c-kit protein expression marks stem cells in bone marrow and might also do so in other organs. A report in 2003 demonstrated the existence of endogenous Lin⁻c-kit⁺ cardiac progenitor cells (CPCs) in the heart itself^{25,26}. These resident CPCs were reported to regenerate approximately 70% of the adult rodent heart after expansion in culture followed by injection back into recipients with MI injury²⁵. However, subsequent studies by two independent laboratories were unable to demonstrate that c-kit⁺ CPCs taken from the adult rodent heart and cultured *ex vivo* had the ability to differentiate into cardiomyocytes^{27,28}. Therefore, although it currently remains unknown whether cardiac resident c-kit⁺ CPCs function as true endogenous stem cells for cardiomyocyte replenishment, our assessment of the literature is that these cells are not true cardiomyocyte stem cells but can give rise to cardiomyocytes on rare occasion, as will be discussed below.

Endogenous murine CPCs expressing stem cell antigen-1 (Sca-1), another marker present on murine hematopoietic stem cells, were also shown to be present in the heart and to be capable of some level of transdifferentiation into new cardiomyocytes or, as an alternative explanation, by fusion with existing cardiomyocytes within the heart after intravenous administration²⁹. These findings were potentially supported by a recent attempt to perform genetic lineage tracing of Sca-1⁺ endogenous CPCs³⁰. This study used a transgenic mouse in

which the mouse Sca-1 promoter was used for lineage tracing to show that endogenous Sca-1⁺ cells can give rise to smooth muscle cells, endothelial cells and cardiomyocytes. However, these results may be partially flawed because the original study that described the Sca-1–encoding transgene showed that many more cells express the transgenic construct than expected based on known sites of Sca-1 protein expression³¹. This suggests a level of ectopic expression for this Sca-1 promoter–dependent lineage-tracing strategy, potentially owing to the absence of specific enhancer elements in the transgenic construct or owing to insertional effects of the transgene itself. Hence, the true extent to which these Sca-1 lineage–traced CPCs contribute new cardiomyocytes to the heart remains uncertain. Moreover, recent lineage-tracing studies using endothelial drivers of Cre recombinase showed at least transient expression of Sca-1 on perivascular cells that eventually became cardiomyocytes, suggesting that Sca-1–expressing cells in the heart may simply be endothelial progenitors³². Importantly, there is no orthologous counterpart of Sca-1 in humans, thereby precluding isolation and therapeutic strategies based on Sca-1 as a cellular marker in humans.

An important lesson from these Sca-1 lineage-tracing experiments is that the molecular marker used must mimic endogenous expression as closely as possible. One way of improving reliability is the use of targeted knock-in strategies to direct Cre recombinase expression from the endogenous marker locus. However, even a knock-in strategy does not guarantee reliable lineage tracing if not performed correctly. For example, a targeted knock-in of a tamoxifen-inducible *cre* gene at the *Kit* allele (encoding c-kit) was recently described and assessed in the immune system and intestine³³. Careful analysis of these mice showed that lineage tracing was not possible for any of the known bone marrow–derived c-kit⁺ cells other than mast cells³⁴. Thus the knock-in approach used by this group appears to have partially disrupted proper expression of the inducible *cre* gene from the *Kit* allele^{33,34}. Moreover, even after careful validation of these knock-in models, care must be employed in extrapolating findings in mice with a lifespan of 2 years to humans with a lifespan of 75 years.

With respect to c-kit⁺ CPCs from the heart, we recently targeted the endogenous murine *Kit* locus to drive expression of the gene encoding Cre recombinase or a tamoxifen-regulated Cre protein (MerCreMer) for genetic lineage tracing of c-kit⁺ progenitor cells *in vivo*³⁵ (**Fig. 1b**). With the genetic strategy we employed, the mice showed 100% overlap between expression from the targeted allele and all the known sites of c-kit protein expression. Using these mice, the results confirmed that c-kit⁺ cells within the heart can indeed differentiate into bona fide cardiomyocytes as previously suggested, but at a calculated yearly rate of approximately 0.05% in the adult mouse, which is unlikely to be meaningful from a physiologic perspective. However, the c-kit⁺ lineage did give rise to abundant endothelial cells in the heart, suggesting that these precursors may be dedicated vascular endothelial progenitor cells, similar to the Sca-1–expressing progenitor cells discussed earlier^{35,36}.

The emerging consensus is that whereas c-kit⁺ and Sca-1⁺ resident CPCs can differentiate into cardiomyocytes at some finite level, rigorous genetic strategies suggest that neither endogenous c-kit⁺ nor Sca-1⁺ CPCs are capable of regenerating the heart with new contracting myocardium to a physiologically meaningful extent. However, since endogenous

CPCs can give rise to all cardiac cell types, understanding the molecular mechanisms at play may lead to ways of diverting these CPCs away from an endothelial program and toward a cardiomyocyte cell fate.

Side population cells

Side population (SP) cells are another group of presumed endogenous cardiac progenitors, originally described in the bone marrow³⁷. SP cells express Abc transporter proteins (*Abcg2* and/or *Abcb1* genes) that can export the DNA dye Hoechst, which allows these cells to be identified using flow cytometry. Similarly to bone marrow-derived c-kit⁺ cells, bone marrow-derived SP cells were first investigated for their cardiac regenerative potential in a cellular transplantation experiment after MI injury in mice³⁸. The injected cells appeared to differentiate into cardiomyocytes and endothelial cells, although the total numbers of cardiomyocytes generated were rather low. Fusion between injected cells and residing cardiac cells was not assessed but was probably a contributing factor given the known fusogenic properties of bone marrow-derived cells^{19,38,39}. Following these initial reports using bone marrow SP cells, cardiac-resident SP cells were identified and shown to transdifferentiate into cardiomyocytes, endothelial cells and smooth muscle cells⁴⁰⁻⁴³. The therapeutic potential of these cardiac SP cells has also been tested in cell transplantation experiments in mice, where they appear capable of homing to the injured heart and differentiating into the main cardiac lineages⁴³. There is also some overlap reported between the SP marker *Abcg2* and the Sca-1 and c-kit surface protein markers⁴⁴, suggesting that the rare cardiomyogenic activity of endogenous cardiac SP cells could be more closely aligned with a subpopulation of CPCs that is Sca-1⁺, c-kit⁺ or both. Further studies using these three marker proteins may help to identify the specific population of CPCs that is most easily coaxed into the cardiomyocyte lineage.

Other cardiac progenitor cells

Another potential source of cardiac regeneration is cells that remain undifferentiated after cardiac development. During cardiac development, there are distinct progenitor populations that can be isolated either from the first or second heart fields or other developmentally defined areas⁴⁵. These developmental progenitors are not adult CPCs such as the c-kit⁺ or Sca-1⁺ cells discussed above but are instead committed mesenchymal precardiomyocytes that are positive for Nkx2.5 or Isl1 (ref. 46). During embryonic development, such cells are the true cardiac progenitors that give rise to all lineages found in the heart⁴⁷. Some Isl1⁺ cells persist in the heart postnatally and retain this precardiomyocyte program, but these cells are already committed and so are distinct from uncommitted c-kit⁺ or Sca1⁺ CPCs^{46,48}.

Similarly, whereas epicardially derived cells differentiate into cardiomyocytes during development⁴⁹, how many exist in the adult heart and to what extent they are capable of generating new cardiomyocytes are still under investigation. A recent paper used Wt1 as a lineage-tracing marker to suggest epicardially derived cells can have regenerative activity after MI, although the extent of new myocardium formed was not reported⁵⁰, and another group was unable to replicate these findings with the same mouse model⁵¹. The main difference between these disparate studies was timing of administration of the regenerative mediator thymosin β 4 in relation to the induction of MI⁵². Other epicardial cells (Wt1⁻)

might similarly be reactivated to give rise to cardiomyocytes, although this appears to be mostly a developmental process⁵³. Thus, our view is that epicardially derived cells serve as a source of new cardiomyocyte formation in the developing heart, but not at physiologically meaningful levels in the adult heart (such as after MI injury). Further investigation is needed to understand the nature of these epicardial cells in the adult heart and their regenerative capacity.

Cardiomyocytes

Although various progenitor cells certainly have the ability to generate cardiomyocytes at low levels, the cardiomyocyte itself appears to be the major cellular source underlying ongoing cardiomyocyte renewal in the adult mammalian heart^{5,54}. This is consistent with data from neonatal mice, adult zebrafish and newts, where large areas of the heart regenerate after an acute resection or infarction procedure due to cardiomyocyte proliferation^{9,10,12,55}. A recent study in adult mice assessed cardiomyocyte renewal from existing cardiomyocytes by combining genetic lineage tracing with ¹⁵N-thymidine labeling, followed by imaging mass spectrometry (**Fig. 1a**). Although this strategy is similar to DNA labeling with ³H-thymidine (**Box 1**), combining this technique with lineage tracing suggested that the majority of renewed cardiomyocytes arose from existing cardiomyocytes⁵. Despite these results, which we consider definitive, they are in contrast to an earlier report that suggested noncardiomyocytes as a major source of new cardiomyocyte formation in the heart after injury using a similar lineage-tracing approach⁵⁶. Finally, a recent paper using interchromosomal genetic recombination⁵⁷ as a strategy to label cardiomyocytes showed generation primarily from existing cardiomyocytes⁵⁴. Although this strategy does not allow for verification of labeling efficiency, a cardiomyocyte renewal level of ~0.01% was reported over an 18-month time frame and, surprisingly, this percentage did not increase after MI. We believe these studies show in a definitive way that cardiomyocytes are the primary source of the limited rates of new cardiomyocyte production in the adult heart.

Box 2

Challenges in cardiac regenerative therapy

Endogenous repair

There are still many uncertainties related to cell therapy. It is not clear whether certain cell types have benefits over other cells (**Table 1**). Furthermore, the delivery method used, such as catheters to provide cells directly to the heart, or intravenous injection, which requires homing of cells to the heart, are important considerations that may have a direct impact on cellular engraftment. As engraftment rates of injected cells are extremely low, especially in the long term after injection (>8 weeks in mouse models), the prevailing hypothesis is that the injected cells may create a local milieu that could stimulate regeneration from endogenous cells, CPCs or cardiomyocytes. What factors constitute this regenerative milieu are unclear, but it could include paracrine effectors such as interleukins, angiogenic growth factors or thymosin β 4, as well as extracellular matrix components.

Maturation

An important topic of study for cell-based therapies includes the maturation of newly formed cardiomyocytes. At present, newly formed cardiomyocytes that are generated by cellular strategies initially display fetal-like characteristics and immature functional and electrical properties that can lead to arrhythmia. Hence, it will be important to find ways of generating fully mature cardiomyocytes with adult gene expression patterns resulting in well-organized sarcomeres, highly structured t-tubules and adult ion channel expression to ensure maximal force generation with less risk of arrhythmias. Previous studies showing complete remyocardialization and restoration of cardiac function of injured areas of the heart by CPCs in less than 2 weeks we believe are inconsistent with the known biology of cardiomyocyte development.

Although cardiomyocytes in the heart appear to be the best cellular source for ongoing renewal, they nonetheless are highly refractory to cytokinesis and hence are still a relatively inefficient cellular source for regeneration. This lack of cytokinesis is probably multifactorial, with one possibility being a genetic block, as overexpression of certain cyclins or deletion of genes encoding retinoblastoma protein family members can enhance cardiomyocyte cell cycle activity^{58,59}. Alternatively, the rigid sarcomeric structure of an adult mammalian cardiomyocyte may prevent the formation of a contractile ring or some other process critical for cytokinesis⁶⁰. A recently published mouse model used GFP-tagged anillin to better quantify cardiomyocyte cytokinesis, which appeared to occur mainly in mononucleated cardiomyocytes^{61,62}. Although 75–95% of murine cardiomyocytes are binucleated, in humans only 35% are multinucleated, potentially suggesting a higher regenerative capacity in humans^{62,63}. Additional studies have uncovered mechanisms that promote proliferation in cardiomyocytes⁶⁴, but these go beyond the main focus of this Perspective. As cardiomyocytes appear to be the most important cellular source for new cardiomyocyte generation, further study of the regulation of cardiomyocyte proliferation and cytokinesis is warranted.

Implications for cardiac regeneration therapies

Although the emerging consensus is that endogenous renewal in the adult heart is largely due to proliferation of existing cardiomyocytes, some low-level renewal activity from CPCs is also clearly present. Future therapeutic strategies to regenerate the heart may include stimulation of cardiomyocyte proliferation or inducing cardiomyocyte differentiation from CPCs. Other strategies to add more cardiomyocytes to the injured heart are currently being developed or have already been tested in patients and are discussed below.

Bone marrow–derived stem cell therapies

Although a finite level of endogenous regeneration exists in the adult heart, the current therapeutic strategies all use exogenously added cells to stimulate regeneration and improve cardiac function. The first clinical trials involving transplantation of circulating or bone

marrow-derived mononuclear progenitor cells after MI were not designed to regenerate the heart but instead were probably targeted at revascularization or ventricular remodeling⁶⁵. However, in 2001, injected bone marrow cells were reported to dramatically regenerate the myocardium in rodents¹⁸, creating the expectation for human clinical trials that such an approach might completely revitalize the heart with new cardiomyocytes instead of only improving angiogenesis²². Although an initial randomized clinical trial showed some functional improvement with injected bone marrow cells (**Table 1**), and 5-year follow-up data confirmed the sense that there may be some benefit to cell therapy, the results are still debated because survival was not different in this small-scale REPAIR-AMI trial (204 patients). Moreover, a randomized clinical trial with survival as a primary endpoint has only recently started (BAMI, NCT01569178, enrolling 3,000 patients)^{66,67}.

Many trials have since been performed with a variety of cell types, and although some initial trials showed functional improvement, other studies did not replicate these findings, and, more concerning, the most positive studies also contained the biggest number of discrepancies in the course of the clinical trial^{22,68}. These issues notwithstanding, not a single trial has been published with overall survival as an endpoint, which is clearly the most important parameter to consider in deciding whether progenitor cell therapy should be widely adopted as a mainstay treatment option in the future.

Many investigators currently agree that, although cellular injections might have some (temporary) beneficial effects, the injected cells themselves do not persist in the heart, nor do they substantially transdifferentiate into new cardiomyocytes⁶⁹. More recent studies have suggested that the effects of cellular injections (of any sort) are probably mediated through some unknown paracrine effect⁷⁰ (**Box 2**). Furthermore, a number of different studies have shown that injected cells may activate endogenous progenitor cells to have a regenerative influence, presumably mediated by paracrine effectors^{71,72}. Why these endogenous progenitor cells are not maximally activated by the initial cardiac injury event and associated local inflammatory response is perplexing. Moreover, to what extent cell therapy provides any benefit after MI and even whether these treatments should continue is still a fiercely debated topic with no clear consensus in sight^{23,68,73}.

Cardiac progenitor cell-based therapies

In addition to transplantation of bone marrow cells, injection of autologous cultured CPCs appears to also be safe, as described in two recent small-scale trials^{74,75} (**Table 1**). The first trial used autologous c-kit⁺ CPCs isolated from cardiac tissue harvested at the time of coronary artery bypass grafting⁷⁴. After expansion of these cells in culture, they were injected into patients through the arterial bypass graft. The main goal of the study was safety, so although the authors showed a potential positive effect on cardiac function and remodeling, the number of randomized patients was too small, hence why an additional trial is planned. Indeed, a future study with many more patients has been proposed that will also combine c-kit⁺ CPCs with bone marrow-derived mesenchymal stem cells to maximize regenerative potential⁷⁶.

A second clinical trial employed cardiosphere-derived cells, which are cells generated from cardiac tissue explants by specialized culturing conditions^{75,77,78}. This trial was also not

powered to detect differences in cardiac function, but no safety issues emerged and there were positive signs suggesting reduced scar size or improved perfusion. However, the overall beneficial effect on function, morbidity and survival will have to await future larger trials.

For all clinical trials conducted to date, engraftment of transplanted cells remains very low, and so the potential benefits are probably due to paracrine effects as opposed to transdifferentiation of injected cells into cardiomyocytes^{69,70} (**Box 2**). What constitutes this paracrine effect, and whether it could be mimicked using a specific pharmacological treatment to avoid the use of transplanted cells altogether, remains to be determined.

Other cell-based therapies

Another future therapeutic approach employs cardiomyocytes derived from allogeneic human embryonic stem cells (ES cells)⁷⁹ or induced pluripotent stem cells (iPS cells)^{80,81} (**Table 1**). Both approaches have been investigated in animal models of MI. One study used predifferentiated cardiomyocytes as a patch rather than a single-cell suspension to allow immediate contractile augmentation. The aim of using a patch is to prealign the cardiomyocytes within an extracellular matrix so they can immediately contribute contractile force after adherence to the native myocardium⁸². An alternative approach involves direct injection of ES cell–or iPS cell–derived cardiomyocytes into the injured myocardium⁸³.

Finally, another future strategy to generate more cardiomyocytes in the heart is direct conversion of endogenous fibroblasts, which are abundant in scarred myocardium, into cardiomyocytes⁸⁴ (**Table 1**). This approach was recently described in mice and has been replicated by multiple groups^{85–89}. However, it remains uncertain how efficient this strategy will prove to be in patients, and converting endogenous CPCs into cardiomyocytes could be a more successful approach. Refinement of the fibroblast conversion cocktail is ongoing, as are efforts to translate the findings to human fibroblasts^{90–92}. To date, fibroblast conversion to beating cardiomyocytes in cell culture is relatively inefficient. Although definitive quantification of conversion *in vivo* is difficult, it is associated with improvement in cardiac function^{88,93,94}. Importantly, fibroblast, ES cell– and iPS cell–based approaches have not yet been attempted in patients, as safety concerns remain⁹⁵.

Despite the promising results observed in animal models with the approaches discussed above, where new contracting cardiomyocytes or contractile patches of myocardium are generated, a number of critical issues need to be resolved before these strategies would be considered safe for human trials. For example, newly formed cardiomyocytes may not directly couple to the existing myocardium, and they initially have an immature phenotype that may lead to arrhythmias^{83,96} (**Box 2**). Other major issues include the inability to ensure that every ES cell–or iPS cell–derived cell in a treatment suspension has differentiated beyond a level of pluripotency that could cause cancer or a teratoma⁹⁷ and immunological issues that could arise if allogeneic cells are used^{83,98,99}.

Conclusion

On the basis of accumulated evidence over the past 10 years, largely using animal models, a consensus has emerged that cardiomyocytes themselves are the most prominent source of ongoing cardiomyocyte renewal in the adult mammalian heart. Although endogenous CPCs can give rise to new cardiomyocytes, this occurs at very low levels, which is unlikely to be of physiological relevance. Moreover, initial enthusiasm to regenerate the heart by transplant of autologous or allogeneic progenitor cells and bone marrow cells has dampened because the clinical benefits observed to date have been modest at best. Other strategies to increase cardiomyocyte transdifferentiation from various cell sources as well as better understanding of how to employ myocardial patches from ES or iPS cells should therefore be sought out. Despite the more recent data from animal models suggesting that CPCs may not be the main endogenous source of cardiac regeneration, the very fact that the heart has some regenerative capacity suggests researchers may be able to expand this potential using CPCs or similar cell therapeutic approaches. This will require full elucidation of the molecular mechanisms in play, and exploiting regulatory effectors to enable more directed transdifferentiation or functional integration from select cell sources. In this Perspective we have critically discussed shortcomings in the field, nonetheless we believe that cardiac regeneration is a critical field of study that will undoubtedly result in therapeutic benefits as more research unfolds.

ACKNOWLEDGMENTS

This work was supported by grants from the US National Institutes of Health (to J.H.v.B and J.D.M.). J.D.M. is an investigator of the Howard Hughes Medical Institute.

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Figure 1.

Genetic lineage tracing approaches to assess cardiomyocyte renewal. **(a)** Inducible α -myosin heavy chain (α -MHC) promoter-driven MerCreMer transgene crossed with a transgene reporter of LacZ-loxp-GFP (ZEG) that was used for labeling of adult cardiomyocytes (green cells, ~85% labeled) combined with ^{15}N -thymidine labeling of DNA synthesis (red nuclei) followed by imaging mass spectrometry indicated a low rate of cardiomyocyte renewal of 1% per year from preexisting cardiomyocytes during normal aging (as examined in sham or control mice)⁵. After MI injury, ~3.2% new cardiomyocytes are generated from preexisting cardiomyocytes in the area adjacent to the infarct⁵. **(b)** Inducible c-kit promoter-driven GFP labeling in the postnatal mouse heart shows multilineage potential of c-kit⁺ progenitor cells (green cells) with low ability to generate fibroblasts and smooth muscle cells and an even lower ability to generate cardiomyocytes (yearly rate calculated to be 0.05%)³⁵. In contrast, a high preponderance of endothelial cells are derived from c-kit⁺ progenitors³⁵.

Table 1

Cellular sources for therapeutic cardiac regeneration

Cell type	Commonly used markers	Origin	Regenerative potential	Therapeutic considerations	References
Embryonic stem cells	Oct4, Nanog, SSEA4	Isolated from embryonic tissue	High	Preclinical stage, using patch after predifferentiation or direct injection of cells	79,83
Induced pluripotent stem cells	Oct4, Nanog, SSEA4	Isolated from adult tissue	High	Preclinical stage, using patch after predifferentiation	80 – 82
Cardiomyocytes	TnT, TnI, Myh6, Myh7	Heart	Moderate	No current strategy	5,9,54
Fibroblasts	CD90, Colla2, vimentin, Postn	Heart	Limited	Preclinical stage, regenerative potential dependent on transdifferentiation efficiency	84 – 92
Cells from cardiospheres	CD105, CD29	Cardiac biopsy	Limited	Clinical trial phases 1 and 2, exogenous cell therapy improved cardiac function in animal models	75,77,78
Mesenchymal stem cells	CD105, CD117	Bone marrow	Limited	Clinical trial phase 2, exogenous cell therapy with minimal improved cardiac function, limited engraftment	72,76
Bone marrow progenitors	CD117, CD34	Bone marrow	Limited	Clinical trial phase 3, exogenous cell therapy with minimal improved cardiac function, limited engraftment	65 – 67
Side population cells	Abcg2, Mdr1 (Sca1 in some studies)	Heart	Limited	Preclinical stage, limited engraftment	40 – 44
c-kit ⁺ cardiac progenitors	CD117, CD45 ⁻	Heart	Limited	Clinical trial phase 2, exogenous cell therapy with limited engraftment	25,27,28,35
Isl1 ⁺ cardiac progenitors	Isl1	Developing heart	Limited	No current strategy	46 – 48
Sca1 ⁺ cardiac progenitors	Sca1, CD31 ⁻	Murine heart	Limited	No current strategy (Sca1 equivalent not in human genome)	29,30,44
Epicardial progenitors	Wt1, Tbx18, CD90, CD44	Developing heart	Limited	No current strategy	49 – 53