Chapter 6
Cellular Approaches to Adult Mammalian Heart Regeneration

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6.1 Introduction

6.1.1 Human Heart Failure

Cardiomyopathies are a major cause of death throughout the world, due in part to the inability of the human heart to significantly regenerate. Improvements in the management of acute myocardial infarction (MI) have led to drastic improvements in short-term mortality rates since the 1960s [1]. However, due to a scarcity of effective long-term therapeutic options, the 5-year survival after diagnosis of heart failure is only 50 % [2]. Thus, heart failure remains an incurable condition and a major cause of death.

The etiology of heart failure is complex, but the syndrome is characterized by cardiac output that is insufficient to meet the metabolic demands of the body. A central complication of heart failure in general is the loss of cardiomyocytes through various cell death mechanisms (reviewed in [3]). In acute myocardial infarction, catastrophic cell death is incurred due to the occlusion of coronary vasculature, which deprives the infarcted region of oxygen and nutrient rich blood. Cardiomyocytes die from both apoptosis and necrosis, though the percent contribution of each death

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mechanism is unclear. Necrotic myocardium is eventually replaced by scar tissue, which lacks the contractile and elastic properties needed for optimal heart function. Ischemic reperfusion is thought to contribute to cell death [4] through inflammation [5], radical oxygen species generation [6], and abnormal calcium handling [7]. Strategies to mitigate peripheral myocardial cell death could potentially be implemented during surgical reperfusion [8, 9]. However, due to the acute lack of blood supply, reperfusion therapy is typically too late to save the dying infarcted myocardium, and fibrotic remodeling follows.

In chronic heart failure, cell death is thought to slowly contribute to deterioration of the ventricular myocardium, thus reducing its ability to effectively contract. This is further complicated in many cases by several aspects of remodeling, such as proliferation of fibroblasts, conversion to myofibroblasts [10], and accompanying alterations in extracellular matrix composition [11]. The re-expression of fetal-specific genes during heart failure has been described by several groups, including a switch from α-myosin heavy chain to β-myosin heavy chain (reviewed in [12]). Metabolic remodeling of cardiomyocytes is also seen in heart failure, such as a shift from fatty acid oxidation to glycolysis (reviewed in [13]). Collectively, these aspects of myocardial remodeling can result in gross morphological changes and associated alterations in tissue mechanics, such as myocardial stiffening, thickening or thinning of the ventricular myocardium, and ventricular dilation, as well as alterations in calcium handling and contractility; all of which can severely affect heart function and feedback on disease progression.

6.1.2 Species Variability in Heart Regeneration

Although adult mammals exhibit an insufficient natural ability to repair damaged myocardium, several lower vertebrates, such as zebrafish, newt, and axolotl, maintain a remarkable regenerative capacity, even in later stages of life. These species-specific differences in regenerative capacity (reviewed in [14, 15]) are an important topic of study in the pursuit of human regeneration. Due to the availability of transgenic models, zebrafish is the best characterized of these species. Mechanistically, genetic lineage tracing experiments show that zebrafish heart regeneration relies primarily on the dedifferentiation and expansion of pre-existing differentiated cardiomyocytes [16, 17]. Poss and colleagues showed this myocardial dedifferentiation involves re-expression of early developmental markers such as *gata4* with an accompanying reduction in myocardial conduction velocity at the injury site [16]. Furthermore, a cryoinjury model demonstrated enhanced cell cycling in a fraction of cardiomyocytes expressing embryonic cardiac myosin heavy chain [18]. Epicardial signaling seems to play a role in the regenerative response to injury [19, 20], but myocyte contributions from epicardial cells directly are apparently limited. The role of a dynamic extracellular matrix was shown to be important in mediating zebrafish heart regeneration [21]. Specifically, fibronectin was upregulated in the myocardium following injury and was required for regeneration. Interestingly, fibronectin deposition in adult mammalian hearts has also been observed post-injury [22, 23], but may signal a fibrotic response in this context [24–26].

Several reports have also demonstrated a strong regenerative ability in adult newt [27–30] and axolotl hearts [31] using various injury models. Due to a lack of lineage tracing transgenic tools in these organisms, the source of new myocardium has not been definitively shown. However, Braun and colleagues showed a reduction in contractile protein expression after injury [32], reminiscent of the cardiomyocyte dedifferentiation observed in zebrafish heart regeneration [16, 17], suggesting a possible common mechanism. Not surprisingly, changes in extracellular matrix protein expression were also shown to accompany adult newt heart regeneration. Of particular interest, tenascin C was found to increase newt cardiomyocyte cell cycle re-entry in vitro [33]. However, evidence for cytokinesis was not shown. Interestingly, matrix production and remodeling enzymes were shown to change along with differentiation of immortalized CPCs in vitro, providing a direct link between the state of cardiomyocyte maturation and extracellular matrix remodeling [34].

Some reports have suggested that accelerated lower vertebrate regeneration is a consequence of cellular plasticity. For example, adult newt cardiomyocytes have been shown to transdifferentiate toward skeletal myocyte or chondrocyte lineages after transplantation into regenerating limb blastema [32]. Conversely, transdifferentiation was not observed during in vitro culture or after transplantation into intact limbs. It would be interesting to see if adult mammalian cardiomyocytes can be transdifferentiated by amphibian blastema; this would indicate a conserved intrinsic regenerative program within vertebrate cardiomyocytes and a non-conserved extrinsic tissue response to injury.

Although adult mammalian hearts do not efficiently regenerate, Olson and colleagues showed in 2011 that neonatal mice (up to postnatal day 7) can regenerate their heart after apical resection [35]. Genetic lineage tracing experiments showed that, similar to zebrafish, the cardiomyocytes are repopulated by pre-existing cardiomyocytes. Immunostaining with anti-Troponin antibodies demonstrated sarcomeric disassembly in myocytes, again suggesting dedifferentiation and expansion of resident cardiomyocytes as a driver of regeneration. Notably, there has been some controversy over the extent of neonatal cardiac regeneration, where it has been suggested that neonatal hearts heal by scarring after apical resection [36]. However, several investigators report the reproducibility of neonatal heart regeneration in an apical resection model and have suggested technical differences as a source of variability [37]. Furthermore, it is not surprising that the severity of injury influences the efficiency of regeneration [38].

Whether or not neonatal hearts exhibit complete regeneration in response to injury, their apparent neomyogenic capacity is a major point of focus that could potentially be used clinically if similar mechanisms can be exploited in the adult myocardium. Thus, it is important to critically evaluate not only the functional recovery after MI, but also the extent of new cardiomyocyte generation in neonatal mice. To that end, cell cycle re-entry of neonatal cardiomyocytes has been thoroughly demonstrated. Soonpaa et al. used tritiated thymidine to demonstrate a spike in S-phase DNA synthesis in neonatal murine cardiomyocytes, beginning near birth and persisting throughout the first week of life [39]. The fraction of binucleated cardiomyocytes increased steadily during this period as the cells lost the ability to complete cytokinesis.

In contrast to S-phase re-entry, the study of cell division is currently more technically challenging. Cytokinesis has traditionally been evaluated using antibodies against cleavage furrow markers such as Aurora B kinase. These techniques can be difficult to interpret with in vivo or in vitro samples, since staining in closely associated non-cardiomyocytes could contribute to false-positive results. This has led investigators to explore alternative methods, such as mosaic analysis with double markers (MADM), to genetically trace divided cardiomyocytes [40]. Interestingly, pulsing of MADM transgenic mice with tamoxifen between postnatal day 2 and 8 revealed that 5 % of labeled MYH6-expressing cardiomyocytes had undergone cytokinesis, giving rise to single labeled (GFP^+ or RFP^+) cells. Due to differential sorting of chromosomes, as well as non-sortable labeling in G0/G1, this figure likely underestimates the actual rate of cytokinesis in labeled cardiomyocytes. Furthermore, it is unclear whether Cre-mediated interchromosomal recombination is unbiased with respect to different cellular states in the heterogeneous cardiomyocyte population. Thus, at this time it is difficult to quantify the actual rate of cardiomyocyte cell division. Nonetheless, it is generally accepted that a significant proportion of neonatal cardiomyocytes have the ability complete cell division and contribute to cardiac regeneration. However, by postnatal day 7, murine cardiomyocytes have mostly exited the cell cycle [39] and lost their ability to regenerate injured myocardium [35].

Interestingly, it has been suggested that altered cardiac circulation accompanies newt heart regeneration, where blood is shunted away from the left ventricle [41]. This is reminiscent of enhanced cardiomyocyte cell cycle and myocardial remodeling in patients with ventricular assist device [42, 43], where a reduction in load may allow partial induction of a regenerative response. It would be interesting to see if neonatal mice exhibit a similar phenomenon during cardiac regeneration. For example, although functional closure of the ductus arteriosus occurs within 3 h post-birth in mice, remodeling takes place over several weeks [41]. Thus, additional studies would be prudent to evaluate the possibility of compensatory shunting of circulation during ventricular regeneration in neonatal mice.

6.1.3 Developments in Induced Heart Regeneration

Despite significant progress in understanding regenerative processes in lower vertebrates and in neonatal mice, it is still unclear how many of these findings can be applied to induce cardiac regeneration in adult mammals. The observation that neonatal mouse hearts can regenerate cardiac injuries is alluring, but there are major differences between neonates and adults with respect to cardiac physiology at the cellular, tissue, and neurohumoral levels. A modest degree of cell cycle re-entry has been observed in adult human and mouse cardiomyocytes [39, 44–46], but evidence for cardiomyocyte cell division in adult mammals is scant. To estimate human cardiomyocyte turnover, Bergmann et al. took advantage of a period of nuclear bomb testing in the 1950s and 1960s, which resulted in a pulse of atmospheric ^{14}C

eventually being incorporated into newly synthesized DNA in human cardiomyocytes [44, 45]. They found that less than 1 % cardiomyocytes were turned over annually in adult humans. Additionally, they showed that DNA content increased in the first 10 years of human life, until most cardiomyocytes were tetraploid [44]. In contrast to mice, most adult human cardiomyocytes are mononucleate [47]. Together, these results indicate that most human cardiomyocytes terminally exit the cell cycle before karyokinesis, whereas mouse cardiomyocytes tend to exit the cell cycle after karyokinesis, but before cytokinesis [48].

Although measurement of cell division in human cardiomyocytes is extremely difficult, recent advances in lineage tracing technology have enabled definitive labeling of divided cardiomyocytes in mice. A recent study using mosaic analysis with double markers [49] showed that approximately 1 % of labeled adult cardiomyocytes had undergone cell division after 2 weeks of daily tamoxifen induction [40]. However, as discussed above, potential bias of interchromosomal recombination could obscure quantification of cell division. Importantly, myocardial infarction prior to labeling did not increase cell division, indicating a lack of regeneration in adult mouse hearts. Still, the immense burden on human health has warranted an abundance of investigations seeking the ultimate feat of cardiovascular medicine: induced adult human heart regeneration.

Numerous strategies have been devised to induce adult mammalian heart regeneration and typically rely on mouse models of myocardial infarction, such as permanent left anterior descending (LAD) artery ligation [50, 51]. Ischemia-reperfusion (IR) models [52] are an even better representation of human myocardial infarction, due to post-MI surgical intervention [8, 9]. Large animal models [53, 54] are useful to translate findings in mice and to test regenerative strategies that are difficult in rodent models due to differences in anatomy, physiology or scalability.

Here, we discuss various therapeutic approaches (summarized in Fig. 6.1) to induce mammalian heart regeneration, including strategies that augment endogenous cardiac regeneration, or supply an exogenous source of cardiomyocyte replacement, consisting of allografts or the re-introduction of modified autologous cells.

6.1.4 Cardiac Progenitor Cells

Attempts to stimulate endogenous heart regeneration and replenish lost cardiomyocytes has been in part motivated by the hypothetical existence of a population of resident or non-resident cardiac progenitor cells (CPCs), which were thought to be a renewable source of committed cardiomyogenic cells. In theory, either autologous or allogeneic CPCs could conceivably be grafted into ischemic injuries to facilitate cardiac regeneration. However, several supposed CPC cell types have ultimately been found to represent at best a very rare contributor to new cardiomyocytes in vivo. For example, *Lin⁻c-kit⁺* CPCs initially showed promise for adult mammalian heart regeneration [55]. However, these cells were later reported to have limited utility in induced adult mammalian heart regeneration, despite their potential to support regeneration in

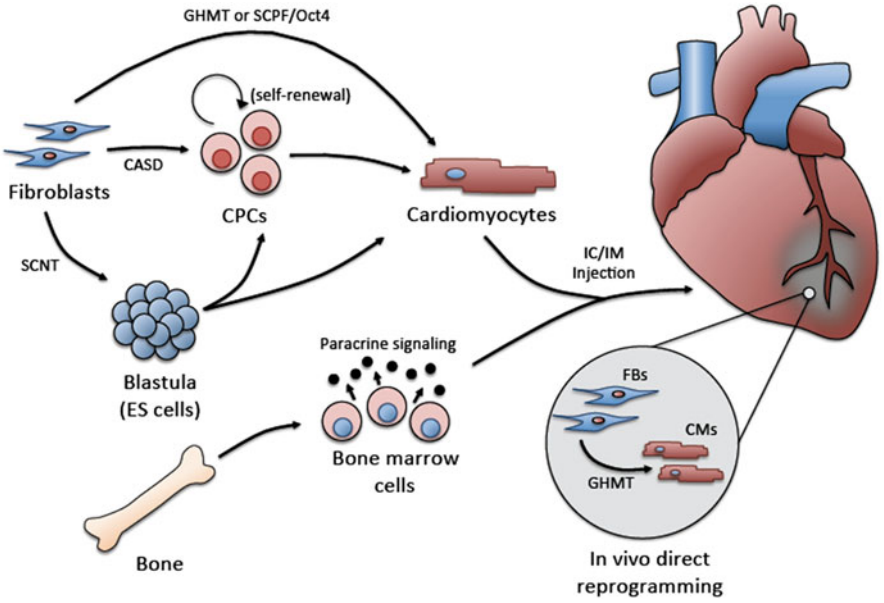

Fig. 6.1 Autologous cellular approaches to cardiac regeneration. Promising sources of autologous patient cells for therapeutic cardiac regeneration include dermal fibroblasts and bone marrow cells, which can be delivered to the infarct via intracoronary (IC) or intramyocardial (IM) injection. Bone marrow cells are thought to act via paracrine effects to encourage regeneration. Fibroblasts can be converted directly to cardiomyocyte-like cells via GHMT or small molecules (SCPF) and Oct4. An expandable population of cardiac progenitors can be created using cell activated and signaling-directed (CASD) lineage conversion. CPCs and cardiomyocytes can also be created via embryonic stem cells created using somatic cell nuclear transfer (SCNT). (*Inset*) In vivo reprogramming can be used to convert resident cardiac fibroblasts into cardiomyocyte-like cells in situ using GHMT factors

neonates [56, 57]. A recent article confirmed the lack of significant direct contribution by cardiac resident c-kit$^+$ progenitors to new cardiomyocytes [58]. Specifically, *c-kit*$^+$ cells did not co-express *Nkx2.5* or sarcomeric proteins at any stage, but were consistently found to co-express the endothelial marker CD31. Furthermore, endothelial-specific *Tie2*-driven expression of Cre completely abolished a *c-kit* driven floxed LacZ reporter. Thus, despite the observation of c-kit$^+$ cells in both the developing and adult heart, they were found to contribute mostly to endothelial cells, rather than cardiomyocytes. As an exogenous cell therapy for heart regeneration [59], it seems likely that any potential benefit of *c-kit*$^+$ progenitor cells to cardiac function would be indirect, for example through paracrine signaling. Other potential endogenous adult murine CPCs have been described, such as *Sca1*$^+$ cells [60, 61]. However ectopic Cre-expression may have confounded initial interpretations of *Sca1*$^+$ CPCs, and the lack of a human ortholog limits the application to human heart failure therapy (reviewed in [62]).

By contrast, *Isl1*$^+$ cells are a true cardiomyocyte progenitor population derived from the second heart field and have been shown to give rise to a majority of cardiomyocytes in the developing mouse heart [63, 64]. Cre-based lineage tracing

experiments showed that by embryonic day 9.75, *Isl1*$^{+}$ progenitor cells generated nearly all cells in the outflow tract and right ventricle, as well as 65 % of the left atria and 20 % of the left ventricle [63]. Moretti et al. showed that *Isl1*$^{+}$ precursors are multipotent and could give rise to smooth muscle and endothelial lineages in addition to cardiomyocytes [65]. They also demonstrated that *Isl1*$^{+}$ cells could be differentiated in vitro from ES cells and propagated on cardiac mesenchyme feeder layers, indicating a potential source of therapeutic progenitor cells for heart failure. A majority of the remaining heart, including the left ventricle, is derived from *Isl1*$^{-}$ progenitors from the primary heart field, characterized by expression of early developmental markers such as GATA4, NKX2.5, and TBX5 (reviewed in [66]).

The persistence of a clinically useful population of resident CPCs in adult mammalian hearts has been an elusive and ongoing pursuit. However, more tangible applications of developmental CPC research in heart regeneration have come through the use of CPC markers to identify potential alternative therapeutic cellular sources of neomyogenesis. Such induced CPCs can now be obtained by pretreatment of ES and iPS cells, as discussed below. Furthermore, the understanding of fetal heart development on the molecular level has led to the discovery of fetal gene re-expression during heart failure [12], which could represent failed attempts to regenerate the adult heart through developmental recapitulation.

6.1.5 Bone-Marrow Derived Cells

Bone marrow-derived cells (BMCs) represent an attractive source of regenerative therapy, since autologous donor tissue can be easily and safely obtained. Initial promise came from an early study that showed 5-azacytidine treatment could induce cardiomyocyte differentiation from immortalized BMCs in vitro [67]. Subsequently, it was shown that autologous BMCs could improve recovery after myocardial infarction in rats [68, 69]. A 2001 study showed a low rate of myocardial engraftment in an ischemia-reperfusion model after bone marrow transplantation of supposed multipotent *CD34*$^{-/low}$, *c-kit*$^{+}$, *sca1*$^{+}$ side population (SP) cells, obtained from *Rosa26-lacZ* donor mice [70]. The purity of the SP cells was high at 91 %, but a even a low rate of contamination by other cell types could confound the interpretation that SP cells themselves give rise to cardiomyocytes. Nevertheless, the observation that bone marrow derived cells could contribute to endothelial cells and cardiomyocytes at all was encouraging for future developments.

Numerous other pre-clinical and clinical studies have investigated the safety and efficacy of bone marrow-derived cell therapy on acute myocardial infarction and heart failure. Results from some individual clinical trials have been positive [71], but large-scale meta-analyses have shown either modest or no benefit on cardiac function or mortality [72, 73]. Looking forward, it will be interesting to see the results of an ongoing large scale phase III clinical trial testing the efficacy of intracoronary delivery of autologous BMCs [74].

6.1.6 Embryonic Stem Cells

Human embryonic stem (hES) cells can be obtained from sperm-fertilized blastocysts [75] or, more conveniently, produced from adult fibroblasts by somatic cell nuclear transfer into oocytes [76, 77]. Being pluripotent, ES cells have the ability to give rise to all three germ layers, including all cell types of the heart. Thus, ES cells are a promising source of cardiomyocyte replacement in the failing heart. However, teratoma formation from direct ES cell injection demonstrates that neither normal nor failing myocardium lacks the developmental signals for faithful differentiation into myocardial lineages [78, 79]. ES cell-derived cardiomyocytes (ES-CMs) can be differentiated from hES cells in vitro by treatment with activin A and BMP4 [80]. In an athymic rat IR model, it was shown that infarcted myocardium could be grafted with hES-CMs by direct cardiac injection [80]. Importantly, a pro-survival cocktail (containing cell adhesion promoting Matrigel, mitochondrial death inhibitors Bcl-KL peptide and cyclosporine A, vasodilator pinacidil, AKT activator IGF-1, and caspase inhibitor ZVAD-fmk) was used to improve graft survival and functional recovery.

Despite the initial excitement for ES-CM treatment, a later study showed that although both allogeneic undifferentiated ES cell and ES-CM treatment provided improvements to ejection fraction in infarcted mouse myocardium, the ES-CM treated groups had an increased risk of cardiac arrhythmia and death [81]. This observation was presumably due to incomplete maturity of *in vitro* differentiated hES-CMs, or alternatively to the mismatch in normal heart rate between human and mouse cardiomyocytes. A subsequent study using an immunocompromised guinea pig cryoinjury model showed engraftment by hES-derived cardiomyocytes with reduced arrhythmia [82]. However, a non-human primate model of the more relevant IR injury again showed significant arrhythmia after engraftment of hES-CMs [83].

These exciting developments in ES-derived myocardial grafts show promise for future heart failure treatments. However, there is a clear need to better understand cardiomyocyte differentiation and to develop protocols to create more mature cardiomyocyte grafts that can recapitulate native pacing. In that light, a recent study showed that 1 year old in vitro differentiated ES-CMs are more similar to mature myocardial tissue in vivo and that the let-7 miR family plays an important role in the maturation process [84]. Furthermore, an earlier study showed that forced expression of connexin 43 improved conduction not only in embryonic cardiomyocyte grafts, but even in skeletal myoblast grafts in infarcted mouse hearts [85].

Despite the use of ES cells as a powerful research tool, and the promising results of preclinical heart regeneration studies, reluctance to enter clinical trials hinges in part on their potential for immune rejection and tumorigenesis [86], not to mention ethical constraints. It will be interesting to see if future developments in autologous ES cell creation [76] and refinements in differentiation and purification protocols will change these perspectives.

6.1.7 Induced Pluripotent Stem Cells

In 2006, Takahashi and Yamanaka reported that adult fibroblasts could be reprogrammed to become induced pluripotent stem (iPS) cells [87]. By forced expression of *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4*, adult mouse fibroblasts became competent for teratoma formation and differentiation into all three germ layers. However, it was still not clear whether the same protocol could be used with human cells. The following year, the same group reported that iPS cells could be generated using human fibroblasts [88]. This was a landmark development in regenerative medicine because it indicated that dispensable autologous adult donor tissue could be used to potentially regenerate any tissue, including the heart.

Although iPS cells theoretically should avoid complications due to immune rejection when using reprogrammed autologous cells, some evidence has suggested otherwise [89]. Furthermore, the tumorigenic risk of retrovirus-reprogrammed cells has led others to pursue chemical or protein-mediated derivation of reprogrammed cells [90, 91]. Still, the pluripotency of iPS cells necessitates a better understanding of differentiation and the development of robust progenitor purification before clinical applications can safely use iPS cells. Nonetheless, iPS cells have become an invaluable research tool and will continue to change the face of regenerative research.

6.1.8 Direct Reprogramming

The discovery of iPS cell reprogramming and the risk of teratoma/tumor formation from the use of pluripotent stem cells quickly led others to pursue alternative approaches to cellular reprogramming. Related approaches were then used to directly reprogram fibroblasts into induced cardiomyocyte-like (iCM) cells without a pluripotent intermediate. The motivation for this type of reprogramming lies in the abundance of fibroblasts in the infarcted myocardium that could serve as a source of new cardiomyocytes. A key observation that led to the discovery of direct reprogramming approaches was the recognition that several core transcription factors (GATA4, HAND2, MEF2C, MESP1, NKX2-5, and TBX5) play a major role in heart development and differentiation. In 2010, a subset of these factors, GMT (GATA4, MEF2C, and TBX5), was used to directly reprogram mouse cardiac and dermal fibroblasts into iCM cells in vitro [92]. Subsequently, in vivo reprogramming was achieved with either GMT or GHMT (GMT+HAND2), yielding improved cardiac function after myocardial infarction in mice [93, 94]. Co-injection of thymosin β4 with GMT reprogramming improved myocardial function after MI [93, 95]. Ding and colleagues showed that small molecules SCPF (SB431542, CHIR99021, parnate, and forskolin) and Oct4 alone could achieve direct reprogramming in vitro [96]. Alternative reprogramming formulations have since been developed, including a microRNA cocktail that effectively converts adult cardiac fibroblasts [97]. Importantly, Olson and colleagues reported a cardiac reprogramming cocktail that works in human cells [98]. Recently, it was shown that Akt1/protein kinase B enhances GHMT conversion efficiency and iCM maturity, including increased polynucleation [99].

In contrast to iPS cells, direct reprogramming offers a source of cardiomyocyte replacement that bypasses the teratoma-competent pluripotent stage. However, more efficient methods to convert and target cardiac fibroblasts need to be developed to move forward in the clinic [100]. In addition, the use of safe vectors or chemical approaches for reprogramming factors would expedite clinical utility of direct reprogramming [96, 100]. Furthermore, despite its promising direction, the tradeoff of reprogramming fibroblasts into cardiomyocytes must still be critically evaluated with respect to the loss of fibroblast function in the failing heart [101]. Perhaps the recent discovery of expandable induced cardiomyocyte-like progenitors [102] will lead to similar strategies that can address concerns of a fibroblast-cardiomycote tradeoff for in vivo conversion.

6.1.9 Dedifferentiated Adult Cardiomyocytes

Dedifferentiation of adult cardiomyocytes can be seen through the re-expression of fetal gene programs in heart failure [12]. Thus, it should not be surprising that adult mammalian cardiomyocytes can dedifferentiate to some degree in culture [103, 104]. Still, evidence for true adult cardiomyocyte cell division, even in the far-removed in vitro environment, is scarce. This suggests that despite varying degrees of dedifferentiation of adult cardiomyocytes in vitro and in vivo, there may exist an inherent block to actually complete cell division. This idea is further supported by the rarity of cardiomyocyte-derived cancers. Nevertheless, rare examples of significantly proliferating adult mammalian cardiomyocytes have been reported, such as rat cardiomyocytes showing high levels of bromodeoxyuridine (BrdU), Ki67 and phosphohistone 3 (PH3) staining in vitro [104]. Recently, the dedifferentiation process of these cultured myocytes was shown to be regulated by epigenomic reprogramming [105].

Fascinatingly, explanted cardiac tissue, cultured under non-adhesive conditions, has been shown to recapitulate a stem cell-like niche that apparently contributes to myocardial repair [106]. The cell preparations derived from such cultures, deemed cardiosphere-derived cells (CDCs) are now being evaluated for the treatment of heart failure in humans. Phase I clinical trials have shown positive results with an increase in viable mass and a reduction in scar size [107, 108]. Interestingly, it was recently shown that exosomes from CDCs may help mediate their regenerative effects [109]. It will be interesting to see how ongoing clinical trials could potentially improve patient outcome [110].

6.1.10 Stimulation of Adult Cardiomyocyte Proliferation

The induction of cardiomyocyte proliferation through cell cycle re-entry and true cell division has been a heavily sought goal of research, with the ultimate goal of adult human heart regeneration through the expansion and replenishment of

endogenous cardiomyocytes. Numerous reports have demonstrated induced re-entry into S-phase by adult mammalian cardiomyocytes, for example by cell cycle activators Cyclin A2 [111] and E2F [112]. Although cytokinetic figures have been observed, robust cardiomyocyte cell division has been difficult to achieve. Immortalization with SV40TAg indicated that it is possible to induce persistent cell division in adult rat ventricular myocytes [113]. However, it is unclear what percentage of adult cardiomyocytes have the capacity to divide without apoptosis even under oncogenic conditions. Since the risk of tumorigenesis precludes serious consideration of SV40Tag in the clinic, the search for regulated stimulation of cardiomyocyte proliferation continues. Various approaches have since been used to increase cardiomyocyte proliferation and enhance MI repair, such as those involving miRNAs [114–116] and neuregulin [117, 118] signaling. The Hippo pathway has recently become an intense subject of investigation in heart regeneration due to its role in organ size control [119]. Modulation of the Hippo pathway has been shown to extend the developmental window of cardiomyocyte proliferation and offer modest improvements when administered after MI in several reports [120–122]. Despite promising results from many of these studies, the major cell cycle blocks in adult mammalian cardiomyocytes are largely not well understood. Furthermore, definitive regeneration in adult mammals is still an active pursuit with room for improvement.

6.1.11 Tissue Mechanics

As mentioned earlier, mechanical stiffness has been associated with reduced ventricular function and progressive heart failure. Recombinant elastin production by transduced endothelial cell transplants reduced infarct size and improved cardiac function after myocardial infarction in rats [123]. This result corroborates observations of progressive heart malfunction as a result of mechanically mediated myofibroblast conversion and runaway fibrosis accompanied by cardiomyocyte cell death (reviewed in [124]). Tissue mechanics has been shown to be important in several aspects of cardiomyocyte biology, such as contractility [125], development [126–128], differentiation [129], and maturation [130]. Recently, a collagen matrix patch containing FSTL1 was used to promote myocardial repair in a porcine myocardial infarction model [131]. It was found that therapeutic effect was influenced not only by the location of FSTL1 secretion, but also by the elasticity of the collagen patch. Thus, it is becoming increasingly clear that tissue/matrix mechanics plays an important role in cardiac disease and remodeling and should be carefully considered in future efforts to induce heart regeneration.

6.1.12 Engraftment

Engraftment of exogenous cells into the heart has been a challenging hurdle to treat heart disease via cellular approaches. The dynamic mechanical demands of the human heart, forcefully pumping at approximately 1 Hz, likely pose a

thermodynamic barrier to cell attachment and integration within the dense extracellular matrix. Not surprisingly, there may be an age-dependence on the success of donor cell engraftment, as shown by higher engraftment of fetal and neonatal rat cardiomyocytes into injured and non-injured adult rat hearts when compared to adult cardiomyocyte engraftment [132]. Despite a higher rate of engraftment for younger donor tissue, engraftment cell survival is typically very low, even for stem and progenitor cell grafts [133]. Nevertheless, an enormous body of work describes various attempts to achieve therapeutic benefit from exogenous cell therapy in heart injury models, as reviewed above. Concurrent developments are underway to increase cell engraftment in the heart and other tissues, including cell adhesive matrices [134, 135] as well as cell pretreatment to increase cardiac homing (reviewed in [136]).

6.2 Conclusions

The field of regenerative biology has made enormous progress in understanding some of the species differences in cardiac regeneration and in the discovery of several therapeutic strategies that have shown some effect on mitigating the effects of human heart failure. However, the ultimate therapeutic endpoint is still out of reach, and further work will be required to obtain a better basic understanding of myocardial biology, including the molecular nature of adult cardiomyocyte cell cycle block, the role of tissue mechanics in heart disease, and the interplay between fibrosis and cardiomyocyte health. Exciting clinical and preclinical developments in cellular and molecular therapies utilizing cardiospheres or miRNA and Hippo signaling could be revealing in the oncoming years. Still, it will be crucial to continue the pursuit of basic discovery in cardiomyocyte biology and the refinement of drug, gene, and cell delivery approaches to maximize progress toward human heart regeneration.

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