

Cardiac Repair and Regeneration

Dominic Lunn^{1,2,#}, Vanessa Wazny^{1,2,#}, Stephen Cutie^{1,2} and Guo N. Huang^{1,2*}

¹Cardiovascular Research Institute and Department of Physiology, University of California, San Francisco, USA

²Eli and Edythe Broad Center for Regeneration Medicine and Stem Cell Research, University of California, San Francisco, USA

#Equal contribution

Abstract

The regenerative capacity of the heart varies drastically across the animal kingdom. Certain species, such as zebrafish and newts, display a remarkable innate ability for heart regeneration. In contrast, heart regeneration in adult mammals is limited. Heart regenerative potential also varies during organismal development. For example, while neonatal mice can regenerate their hearts, this is lost during the first week after birth. Understanding cardiac regenerative pathways will play a critical role in discovering therapeutic approaches to stimulate human cardiac regeneration. In this review, we explore the known strategies to stimulate intrinsic heart regeneration and highlight current cell replacement therapies.

Keywords: Cardiac regeneration; Cardiac repair; Cardiomyocyte; Heart failure

Abbreviations: CM: Cardiomyocyte; PCNA: proliferating cell nuclear antigens; miRNA: microRNA; NGR1: Neuregulin-1; FSTL1: Follistatin-like-protein; iCM: induced CM-like cell; YAP: yes-associated protein 1; DGC: dystrophin-glycoprotein complex; Salv: Salvador; ESC: Embryonic stem cells; CPC: Cardiac progenitor cell.

Introduction

The ability to regenerate the heart varies across both phylogeny and ontogeny. For instance, adult zebrafish possess the remarkable ability to regenerate their hearts following amputation of 20% of the ventricle [1]. Similarly, neonatal mice in their first week of life can regenerate their hearts with minimal scarring after cardiac injury [2]. In contrast, the adult mammalian heart is incapable of regeneration following injury.

The inability of adult mammals to regenerate their hearts is clinically relevant, since the lack of regeneration in humans is a leading cause of morbidity and mortality. One of the primary causes of heart disease is myocardial infarction, resulting in cardiomyocyte (CM) death and decreased cardiac function. After cardiac injury, there is very little self-renewal of the myocardium, and instead deposition of non-contractile scar tissue results in cardiac remodeling, impairing cardiac function, progressively leading to heart failure [3].

The human heart was first identified as a post mitotic organ in 1925 when it was discovered that the increase in heart size occurs through the increase in CM cell size, rather than the increase in CM cell number [4]. This was also confirmed by a lack of mitotic markers in the adult heart [4]. Soon after, mitotic CMs were found in the hearts of children [5]. However, it was not until 1994 that the first convincing evidence emerged of CM proliferative activity in adults, when Quaini et al., detected PCNA, a gene expressed at the G1-S boundary of the cell cycle and required for DNA synthesis/cell proliferation, in myocardial samples from patients with congestive heart failure [6]. By 1998 it was generally accepted that adult mammalian CMs could proliferate, however the biological significance of this event was disputed due to the low rates of CM DNA synthesis observed. In normal adult mice, CMs displayed an estimated annual turnover of 1.095% [7]. A similar turnover rate was displayed in humans when Bergmann et al. (2008) reported that annual CM turnover decreases from 1% to 0.3% between the ages of 20 to 75 in an intuitive study that made use of the elevated levels of carbon-14 produced during the Cold War nuclear bomb testing to determine the age of human CMs by measuring the amount of carbon-14 incorporation into DNA [8]. A recent report even suggests

that human infants possess the intrinsic capacity to repair myocardial damage and completely recover cardiac function. It reports a newborn baby sustaining massive cardiac damage from severe myocardial infarction and then, within weeks, functional recovery was observed which later translated into long-term normal cardiac function [9]. This study suggests a latent regenerative potential of the human heart that is locked away somewhere in the genome. However, the mechanisms and potential translation into therapeutic strategies are yet to be determined. Also in mice, endogenous systems do exist to upregulate the rate of CM proliferation following injury, demonstrated by the roughly 5-fold increase in CM proliferation in the peri-infarct area following injury [10]. However, this remains biologically insignificant and still insufficient for functional cardiac recovery.

Current approaches to treat heart failure involve pharmacological treatments to counteract the maladaptive counter-regulatory mechanisms activated by cardiomyopathy. Although these drug-based strategies improve mortality, they fail to address the causative loss of CMs and vasculature. This review will explore the two most studied approaches for cardiac repair: strategies to augment endogenous CM renewal through regeneration and current CM replacement therapies (Figure 1).

Strategies for intrinsic regeneration

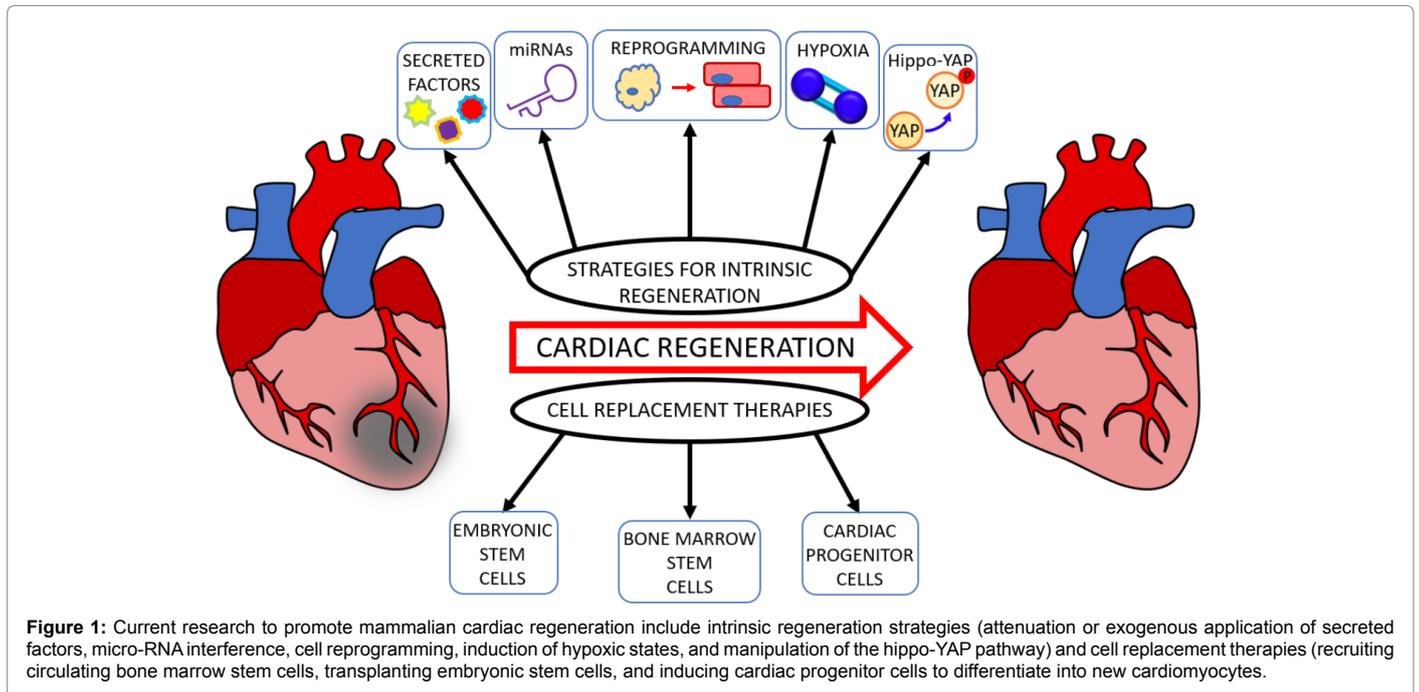
Unlike other tissues in the body, the adult mammalian heart has little/no regenerative potential; however, neonatal mammals have recently been discovered to possess regenerative capacity during early postnatal development [11]. Therefore, evidence is emerging that the mammalian heart may, in fact, possess a level of regenerative capability that may be exploited following cardiac injury. The process of regeneration somewhat resembles organogenesis during embryonic development, requiring control of cell division, differentiation, migration, integration and maturation [12]. However, regeneration

***Corresponding author:** Guo N. Huang, Cardiovascular Research Institute & Department of Physiology, University of California, San Francisco, USA, Tel: 1-415-502-2873; E-mail: Guo.Huang@ucsf.edu

Received December 21, 2017; **Accepted** January 12, 2018; **Published** January 22, 2018

Citation: Lunn D, Wazny V, Cutie S, Huang GN (2018) Cardiac Repair and Regeneration. Cell Dev Biol 7: 193. doi:10.4172/2168-9296.1000193

Copyright: © 2018 Lunn, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.



is further complicated by the need to clear damaged tissue, suppress fibrosis, regulate inflammation and reconstitute a subsection of CMs, extracellular matrix, blood vessels and lymphatics [13].

Secreted Factors and miRNAs

One likely reason why it is difficult to induce significant CM proliferation in adult hearts is the presence of multiple layers of negative feedback blocking mature CM cell-cycle activity. To overcome this, various strategies for inducing intrinsic CM proliferation have been proposed, including pro-proliferative miRNAs and secreted factors.

One such secreted factor, NGR1, has been shown, together with its co-receptor, to trigger mammalian heart regeneration via CM proliferation [14]. In mice, the *Errb2* receptor is downregulated around one week after birth, coinciding with the loss of CM proliferative potential. D’Uva and colleagues demonstrated that the transient induction of an activated form of *Errb2* in juvenile mice could prolong the proliferative window, and could even restore regenerative capacity when induced in adult CMs by facilitating partial dedifferentiation into a more proliferative state [14]. Another factor, *FSTL1*, is a glycoprotein secreted by the epicardium in response to ischemic and infarct injuries. By applying patches loaded with *FSTL1* to the heart following myocardial infarction, one group successfully increased the survival of CMs, reduced scarring, increased vasculature and stimulated CM proliferation at the injury border zone [15].

Much like transcription factor controlled expression of pro-regenerative genes, miRNAs can serve a similar role. miRNAs are single-stranded, non-coding RNA molecules that post-transcriptionally regulate gene expression in a suppressive manner. A variety of miRNAs have been shown to induce proliferation after overexpression both *in vitro* and *in vivo*. In particular combinations of miR-590, miR-199a, and the miR-17-92. Additionally, overexpression of miR-17-92 in adult CMs, originally identified as a human oncogene, protects the heart from myocardial infarction-induced injury [16]. Though CM pro-proliferative strategies offer much encouragement for regeneration, caution must be taken to manage the potential oncogenic effects that could prevail in patients.

Reprogramming cells into CM-like fate

Research into another therapeutic strategy currently in its infancy involves the direct reprogramming of non-myocytes into induced CMs (iCMs). In a proof-of-concept experiment, retroviral delivery of the transcription factors *Tbx5*, *Mef2c*, and *Gata4* reprogrammed murine fibroblasts into iCMs *in vitro* [17]. This was later recapitulated *in vivo* in the context of myocardial infarction, resulting in reduced infarct size and improved cardiac function [18,19]. However, this strategy is currently limited by factors such as iCM efficiency, functionality and mechanical/electrical integration. It also appears as though a different subset of reprogrammable factors is required in humans as opposed to mice [17]. Furthermore, the use of current delivery methods, such as viruses, imposes a risk of oncogenesis that needs to be carefully considered and investigated before any human trials are to begin.

Hypoxia

The ability to tolerate hypoxic conditions is a shared trait among species with cardiac regenerative capacity. Following birth, environmental oxygen levels of the mouse increase from a hypoxic environment (~30 mmHg) to a relatively normoxic environment (~100 mmHg). Likewise, zebrafish dwell in hypoxic aquatic environments, and can tolerate oxygen pressures as little as 15 mmHg [20]. Both the developmental transition from embryonic to post-natal life, and the evolutionary transition from aquatic to terrestrial life, demonstrates a correlation between increased oxygen levels and decreased ability to regenerate the heart. This decline in regenerative potential of the heart is due to the increase in mitochondrial oxidative metabolism and DNA damage, causing CM cell-cycle arrest in comparatively hyperoxic conditions [20]. Recently, Nakada et al. demonstrated that the gradual reduction of inspired oxygen decreased the production of reactive oxygen species, resulting in the promotion of proliferation in adult CMs. Hence, this data demonstrates the potential therapeutic exploitation of hypoxemia to enhance cardiac regeneration [21].

Hippo/YAP signaling pathway

The Hippo signaling pathway is a conserved kinase cascade, originally identified in *Drosophila* imaginal discs acting to limit cell size and tumorigenesis by phosphorylating YAP [22]. Recently this pathway has been implicated during mammalian regeneration [23]. In 2011, work from Heallen et al., revealed that the Hippo pathway inhibits Wnt signaling during cardiogenesis to limit CM proliferation, thus limiting heart size in mammals. Furthermore, embryos deficient in Hippo resulted in elevated CM proliferation, resulting in overgrowth of cardiac ventricular walls and trabeculae [24]. This same effect has also been demonstrated through the forced expression of constitutively active YAP, resulting in the constant activation of the downstream transcription factor, TEAD1, involved in mitogenic pathways [25].

Another identified Yap target, Dystrophin-glycoprotein complex (DGC), is a cardiac muscle transmembrane protein that plays a role in facilitating interactions of the cytoskeleton, membrane and extracellular matrix [26]. Dag1, a DGC component, has been found to bind Yap and therefore inhibit CM proliferation in mice [27]. Furthermore, Hippo and DGC deficient adult mouse hearts displayed excessive proliferation of CMs following cardiac injury [27]. Interestingly, the deletion of Hippo in a Duchenne muscular dystrophy mouse model prevented the heart failure and myocardial fibrosis typical of patients with the disorder [27].

Additional components to the Hippo pathway, such as Salv, have also recently been reported. Following myocardial infarction, the knockdown of Salv in the ischemic heart enhanced the reparative capacity of the heart and recovered contractile function [28]. Consistent with this finding, the increased expression of genes involved in the cardiac cell cycle, cardiac growth and cardiac contractility in the heart specific Salv knock out following myocardial infarction implies enhanced CM proliferation governing the regenerative response.

Since it has been identified that Hippo signaling is upregulated in the human heart following ischemic injury [28], these findings suggest a potential therapeutic strategy of Hippo pathway inhibition following heart failure, in order to improve mortality and cardiac function.

Cell replacement therapies

Another approach for cardiac repair is cell replacement therapies. This involves the transplantation of various cell types onto/into the damaged heart to repair and restore function. Current cell replacement strategies being explored as means for cardiac repair include transplantation of adult and embryonic stem cells (ESCs), mobilization of bone marrow stem cells, and recruitment of endogenous cardiac progenitor cells (CPCs) [29]. ESCs are one of the most successfully transplanted cell types, demonstrating the ability to engraft and electrically integrate into host heart tissue and improve heart function after myocardial infarction [30,31]. However, many issues arise with the use of ESCs, such as ethically obtaining them, graft immune rejection, their potential for teratoma formation and their immature state in comparison to resident CMs which likely reduces their contractile efficiency [32]. Mobilizing circulating bone marrow stem cells with cytokines is another intriguing possibility for promoting cardiac regeneration, either through increasing vasculogenesis or direct transdifferentiation into CMs [29]. However, such interventions have as-of-yet failed to yield significant post-infarct enhancement of cardiac function. CPCs are another common cell type used in cell therapy research, which possess the ability to differentiate into many cell types [33]. Of interest are the *c-kit*+ cells (identified by high expression of CD117). It has been discovered that injection of *c-kit*+ CPCs into

injured hearts mediate the formation of functional blood vessels and CMs in the regenerated myocardium [34]. Following encouraging pre-clinical studies, *c-kit*+ CPCs were tested in human patients with ischemic heart failure. Patient autologous *c-kit*+ CPCs were expanded in culture and grafted onto injured hearts. This revealed a statistically significant 7% improvement in left ventricular ejection fraction and no later adverse effects relating to *c-kit*+ CPCs [35]. Despite promising signs, more mechanistic information needs to be uncovered on CPCs to allow optimized human treatment.

Conclusion

Research in different experimental animal models is revealing an insight into the mechanisms governing cardiac regeneration and has shed light on the barriers restricting cardiac regeneration in humans. To date, the innovation of cell replacement therapies and appreciation of the natural capacity of mammalian heart regeneration has offered an overarching vision for developing new therapies to counteract mortality caused by ischemic heart disease.

References

1. Poss KD (2002) Heart Regeneration in Zebrafish. *Science* 298: 2188-2190.
2. Porrello ER (2011) Transient Regenerative Potential of the Neonatal Mouse Heart. *Science* 331: 1078-1080.
3. Sutton MGSJ, Sharpe N (2000) Left ventricular remodeling after myocardial infarction: Pathophysiology and therapy. *Circulation* 101: 2981-2988.
4. Karsner HT, Saphir O, Todd TW (1925) The State of the Cardiac Muscle in Hypertrophy Atrophy. *Am J Pathol* 1: 351-372.
5. MacMahon HE (1937) Hyperplasia and Regeneration of the Myocardium in Infants and in Children. *Am J Pathol* 13: 845-854.
6. Quaini F, Cigola E, Lagrasta C, Saccani G, Quaini E, et al. (1994) End-stage cardiac failure in humans is coupled with the induction of proliferating cell nuclear antigen and nuclear mitotic division in ventricular myocytes. *Circ Res* 75: 1050-1063.
7. Soonpaa MH and Field LJ (1998) Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Circ Res* 83 15-26.
8. Bergmann O, Bhardwaj RD, Bernard S, Barnabe-Heider F, Walsh S, Zupicich J, et al. (2009) Evidence for Cardiomyocyte Renewal in Humans. *Science* 324: 98-102.
9. Haubner BJ (2016) Functional Recovery of a Human Neonatal Heart after Severe Myocardial Infarction. *Circ Res* 118: 216-221.
10. Senyo SE (2013) Mammalian heart renewal by pre-existing cardiomyocytes. *Nature* 493: 433-436.
11. Porrello ER, Olson EN (2014) A neonatal blueprint for cardiac regeneration. *Stem Cell Res* 13: 556-570.
12. Carlson BM (2005) Some principles of regeneration in mammalian systems. *Anatomical Record - Part B New Anatomist* 287B: 4-13.
13. Xin M, Olson EN Bassel-Duby R (2013) Mending broken hearts: Cardiac development as a basis for adult heart regeneration and repair. *Nat Rev Mol Cell Biol* 14: 529-541.
14. D'Uva G (2015) ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation. *Nat Cell Biol* 17: 627-638.
15. Wei K (2015) Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. *Nature* 525: 479-485.
16. Chen J (2013) Mir-17-92 cluster is required for and sufficient to induce cardiomyocyte proliferation in postnatal and adult hearts. *Circ Res* 112: 1557-1566.
17. Ieda M (2010) Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 142: 375-386.
18. Qian (2012) *In vivo* reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature* 485: 593-598.

19. Mendell JT, Olson EN (2012) MicroRNAs in stress signaling and human disease. *Cell* 148: 1172-1187.
20. Puente BN (2014) The Oxygen Rich Postnatal Environment Induces Cardiomyocyte Cell Cycle Arrest Through DNA Damage Response. *Cell* 157: 565-579.
21. Nakada Y (2016) Hypoxia induces heart regeneration in adult mice. *Nature* 541: 222-227.
22. Pan D (2010) The hippo signaling pathway in development and cancer. *Dev Cell* 19: 491-505.
23. Zhao B, Tumaneng K, Guan KL (2011) The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. *Nat Cell Biol* 13: 877-883.
24. Heallen T (2013) Hippo signaling impedes adult heart regeneration. *Development* 140: 4683-4690.
25. Lin Z (2015) Pi3kcb links Hippo-YAP and PI3K-AKT signaling pathways to promote cardiomyocyte proliferation and survival. *Circ Res* 116: 35-45.
26. Lapidos KA, Kakkar R, McNally EM (2004) The Dystrophin Glycoprotein Complex: Signaling Strength and Integrity for the Sarcolemma. *Circ Res* 94: 1023-1031.
27. Morikawa Y, Heallen T, Leach J, Xiao Y, Martin JF (2017) Dystrophin-glycoprotein complex sequesters Yap to inhibit cardiomyocyte proliferation. *Nature* 547: 227-231.
28. Leach JP (2017) Hippo Pathway deficiency reverses systolic heart failure after infarction. *Nature* 550: 260-264.
29. Krane M, Wernet O, Wu SM (2010) Promises and pitfalls in cell replacement therapy for heart failure. *Drug Discov Today Dis Mech* 7: e109-e115.
30. Min JY (2003) Long-term improvement of cardiac function in rats after infarction by transplantation of embryonic stem cells. *J Thorac Cardiovasc Surg* 125: 361-369.
31. Shiba Y (2012) Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature* 489: 322-325.
32. Wong SSY, Bernstein HS (2010) Cardiac regeneration using human embryonic stem cells: producing cells for future therapy. *Regen Med* 5:763-775.
33. Beltrami AP (2003) Adult Cardiac Stem Cells Are Multipotent and Support Myocardial Regeneration we have documented the existence of cycling ventricular myocytes in the normal and pathologic adult mam. *Cell* 114: 763-776.
34. Bearzi C (2007) Human cardiac stem cells. *Proc Natl Acad Sci USA* 104: 14068-14073.
35. Chugh AR (2012) Administration of cardiac stem cells in patients with ischemic cardiomyopathy: The SCIPIO trial: Surgical aspects and interim analysis of myocardial function and viability by magnetic resonance. *Circulation* 126.