Trends in Cardiovascular Medicine 30 (2020) 338–343

Contents lists available at ScienceDirect

Trends in Cardiovascular Medicine

journal homepage: www.elsevier.com/locate/tcm

Cardiac regenerative therapy: Many paths to repair ☆

Natalie A. Gude, Mark A. Sussman*

SDSU Heart Institute and Biology Department, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182, USA

ARTICLE INFO

Keywords:
Cardiac repair
Cardiac stem cell
Cardiomyocyte
Regenerative therapy

ABSTRACT

Cardiovascular disease remains the primary cause of death in the United States and in most nations worldwide, despite ongoing intensive efforts to promote cardiac health and treat heart failure. Replacing damaged myocardium represents perhaps the most promising treatment strategy, but also the most challenging given that the adult mammalian heart is notoriously resistant to endogenous repair. Cardiac regeneration following pathologic challenge would require proliferation of surviving tissue, expansion and differentiation of resident progenitors, or transdifferentiation of exogenously applied progenitor cells into functioning myocardium. Adult cardiomyocyte proliferation has been the focus of investigation for decades, recently enjoying a renaissance of interest as a therapeutic strategy for reversing cardiomyocyte loss due in large part to ongoing controversies and frustrations with myocardial cell therapy outcomes. The promise of cardiac cell therapy originated with reports of resident adult cardiac stem cells that could be isolated, expanded and reintroduced into damaged myocardium, producing beneficial effects in preclinical animal models. Despite modest functional improvements, Phase I clinical trials using autologous cardiac derived cells have proven safe and effective, setting the stage for an ongoing multi-center Phase II trial combining autologous cardiac stem cell types to enhance beneficial effects. This overview will examine the history of these two approaches for promoting cardiac repair and attempt to provide context for current and future directions in cardiac regenerative research.

The Cardiac Stem Cell (CSC) field

The field of adult mammalian CSC biology and cell therapy emerged nearly twenty years ago [1,2], generating great excitement about the possible existence of resident CSCs and their therapeutic potential for treating heart disease. Original adult CSCs were identified and isolated using criteria established to define hematopoietic stem cells (HSCs) such as expression of c-Kit and Sca1 surface proteins in the absence of hematopoietic lineage markers [2]. The discovery of adult neuronal stem cells and *de novo* neurogenesis in the adult mammalian brain, another presumably non-regenerative organ [3], validated the premise for finding adult progenitors in the heart. A highly active phase of cardiac research ensued amid disagreements over the ability of these cells to transdifferentiate into functional myocardium [4–6]. Despite this controversy, the idea that the adult mammalian heart might possess heretofore unrecognized regenerative potential sparked important novel cardiac research directions and collaborations [7,8], including numerous clinical trials for cardiac cellular therapy. Multiple independent laboratories have identified and characterized bone marrow [9,10] and CSC populations based on an array of properties including stem cell protein expression, dye exclusion [11], growth characteristics in culture [12], or stem cell and cardiac gene expression profiles [13]. Circulating or resident endothelial progenitors [14–17], pericytes [18,19] and epicardial cells [20,21] are a few examples of nonmyocyte cell types investigated for cardiac regenerative potential. Adoptive transfer experiments in preclinical small and large animal models using various candidate stem/progenitor cells have revealed that the cells are exerting beneficial effects, whether by creating new myocardial tissue, stimulating endogenous repair mechanisms, or preserving injured myocardium through secretion of cardioprotective factors [22–25].

CSC controversy

The field of myocardial proliferation has always been controversial, but came under increased scrutiny following disparate claims of cardiomyocyte turnover in adult mammalian heart [26,27] and disagreement regarding the contribution of adult cardiac interstitial cell populations to cardiomyogenesis [28–35]. Studies from multiple laboratories replicating many aspects of the original CSC findings remain unchallenged [36–42], lending credence to discoveries and characterizations enumerated above. Even CSC skeptics acknowledge existence of cardiac c-Kit+ cells and demonstrated

☆ Disclosures: MA Sussman is a founding member of CardioCreate, Inc.

* Corresponding author.
E-mail address: heartman4ever@icloud.com (M.A. Sussman).

https://doi.org/10.1016/j.tcm.2019.08.009
1050-1738/

that they have endothelial lineage potential [28]. Although the very laboratory that launched the adult CSC field came under scrutiny resulting in retractions and expressions of concern for several publications [43], many important discoveries and research directions have their origins in the CSC field, including cardiac exosomes [44–46], the cardiac secretome [47], and the concept that modest endogenous repair mechanisms within the heart can be augmented through potentiation of endogenous repair [48–50] or enhancement of exogenously applied cell therapies [37,51,52]. Important tools and methodologies such as induced pluripotent stem cells (iPSCs) [53], embryonic stem cells (ESCs) [54,55], tissue reprogramming [56–58], stem cell lineage tracing models [28,38,42,59], dual recombinase lineage tracing models [60,61] and gene editing [62,63] have been developed for probing cardiac biology and revealing the molecular mechanisms underlying cardiomyocyte differentiation and heart disease. Tissue engineering approaches combine material sciences with stem cell biology to devise tissue compatible patches as delivery systems for beneficial factors to damaged hearts [64–68]. Finally, genomic and RNA sequencing technologies are uncovering novel molecular relationships among cardiac tissues at the bulk, single cell, and single nucleus levels, highlighting the heterogeneity of cardiac interstitial cells and iPSCs [53,69–73], and molecular dynamics of cardiomyocytes in response to injury [74]. The genesis of myocardial regenerative medicine has roots in the cardiac stem cell field and many important scientific problems remain to be resolved including functional roles of endogenous cardiac interstitial cells. Regardless of whether there is a 'true' cardiac stem cell, the diversity of cardiac interstitial cell subpopulations exhibits a spectrum of cell types and plasticity that continues to defy arbitrarily simple categorizations such as "stem" or "progenitor" cell.

Cardiac aging

Aging is a major risk factor for cardiovascular disease, leading to compromised cardiac function and reparative capacity at the tissue, cellular and molecular levels [75]. Yet most experimental models addressing cardiac failure and repair rely on non-aged animals and tissues. While findings based on these models provide fundamental insights into cardiac therapy, they do not fully capture the health challenges faced by most adult or aging patients requiring treatment for heart disease. Endogenous myocardial survival and repair decline with aging, exhibiting features of cellular senescence and aging, such as a Senescence Associated Secretory Phenotype (SASP), as well as inability to proliferate, self-renew and differentiate into functional tissue. Compromised renewal capacity of aged myocardium presents a significant challenge for cellular therapeutic approaches that rely upon endogenous responses of the host tissue to mediate repair [76]. 'Next generation' cellular therapeutic approaches are confronting the problem of aging with *ex vivo* enhancements such as genetic or bioengineering approaches [77,78]. Modeling cardiac aging at the cellular and organismal levels, along with continued research into mechanisms underlying age-related cellular and molecular dysregulation are all necessary to design effective preventative measures and treatments for cardiac disease in aging patients.

Clinical consequences

The original c-Kit cell therapy model utilizing bone marrow cells [1] prompted an early rush to clinical cardiac cell therapy in part due to easy accessibility of the cells. High patient variability and lack of standardization among these early efforts produced modest overall outcomes, although some patients responded better than others, and unanticipated benefits were observed. Recent meta-analysis of clinical trials for cell-based therapies unrelated to the disputed CSC studies reveals that these treatments are safe and can improve clinical outcomes in patients with refractory angina [79]. Several cardiac cell therapy trials using non-cardiac autologous cells demonstrate varying degrees of improvement [77] but overall, meta-analyses indicate that adult bone marrow therapy improves cardiac patient survival, and infarct repair in humans receiving CSC therapy is better than in large animal preclinical models [80,81]. Regarding autologous cardiac-derived cell therapies, the SCIPIO Phase I clinical trial utilizing cardiac c-Kit+ CSCs derived from patients meeting specific functional criteria established safety, feasibility, and yielded lasting functional improvement in several patients [82]. Adoptive transfer of autologous cardiosphere derived cells likewise proved safe in the CADUCEUS trial [83]. Although Phase II trials for SCIPIO remain unrealized, the CONCERT-HF trial [84] combining autologous cardiac c- Kit+ CSC and mesenchymal stem cells has resumed. In summary, cardiac cell therapy holds promise as a treatment for cardiac disease, but remains limited in terms of observed functional improvement, evidence of actual myocardial regeneration in patients, and increased skepticism from the scientific and clinical communities [85]. Innovative approaches such as the combinatorial cell therapy used in CONCERT-HF, and enhancement strategies to potentiate survival, engraftment and beneficial properties of adult human cardiac progenitors may overcome these limitations [52]. Meanwhile, clinical outcomes for ongoing studies will play an important role in deciding the fate, so to speak, of autologous cardiac cell therapy in the treatment of heart disease.

Myocyte cell division

Myocyte cell cycle and proliferation are longstanding topics of cardiac research gaining renewed attention as part of the effort to understand and promote myocardial repair [86]. Although robust proliferative capacity exists in non-mammalian vertebrates and neonatal rodent hearts, cell division is all but absent in adult mammalian cardiomyocytes. Animals models in which adult cardiomyocyte counts are increased, whether through transgenic overexpression of cell cycle proteins, or manipulation of hormonal or metabolic function, likely mediate their effects by prolonging and enhancing perinatal myocyte proliferation rather than driving cell cycle completion in fully mature myocytes. [50,87–91]. Furthermore, agents that increase proliferation in immature myocytes may manifest alternate phenotypes in adult cells. For example, transgenes promoting cell division in embryonic and neonatal cardiomyocytes exert protective effects in adult cardiomyocytes, contributing more to myocardial preservation than actual regeneration following pathologic insult [92,93]. To complicate matters further, many methods that rely on detection of canonical cell cycle markers as evidence of proliferation do not represent cell division in postmitotic adult cardiomyocytes [94,95]. Cells often increase their DNA content in response to stress or for other non-replicative purposes, such that incorporation of BrdU into chromosomal DNA will not distinguish between S-phase of the cell cycle, DNA repair, or an increase in ploidy unrelated to cellular division [96]. Likewise, phospho-histone H3 labeling, frequently used to denote mitosis, is expressed in non-proliferating cardiomyocytes exposed to hypertrophic stress [97], while Aurora Kinase B can be detected in binucleating cardiomyocytes [98]. Newer studies adding more precise cellular markers such as midbody or IQGAP3 positioning to define cell cycle progression are challenging the claims of actual cardiomyocyte division given that aborted cytokinesis, endoreplication, endomitosis and endo-duplication can generate positive signals for classic cell cycle markers [98,99]. Genetic approaches such as cardiomyocyte- specific Mosaic Analysis with Double Markers (MADM) or hypoxia lineage tracing mouse models detect a very low rate of cardiomyocyte proliferation in

the postnatal mouse heart and following pathological challenge [100,101]. Finally, reports of cardiomyocyte turnover rates in humans vary widely depending on methodology used and interpretation of results [26,102]. Regardless of the detection method, cell division in mature mammalian cardiomyocytes clearly does not occur at sufficient levels to repair cardiac damage. A better understanding of cardiomyocyte cell cycle status, progression and withdrawal is required to identify molecular targets for prompting proliferation in these cells [103].

Perspective

Identification and characterization of a specific cardiac interstitial cell expressing c-Kit receptor tyrosine kinase in the absence of hematopoietic lineage markers launched the field of adult CSC research. Somewhere along the way, c-Kit itself became the lightning rod for controversy surrounding cardiac cell therapy. Stem cell properties including self-renewal, pluripotency and clonogenicity of c-Kit cardiac stem/progenitor cells have been successfully demonstrated by some groups [104] but not others [105] leading to an over-emphasis on the c-Kit marker itself while blurring the distinction between c-Kit as one of many stem cell markers, and cardiac c-Kit biology in general. Mouse models for investigating c-Kit, including genomic c-Kit mutants, transgenic and genetic c-Kit lineage reporters, or dual-recombinase-mediated cell tracking lines are all valuable tools for elucidating cardiac c-Kit biology. However, due to limitations particular to each system, none exclusively capture the full significance and role of c-Kit expressing cells within the developing and adult mammalian heart [38,106,107]. Further refinement and alternative models which incorporate specific markers of stem/progenitor cells are necessary [28,38,42,60,61,108,109]. Various studies demonstrating cardiac c-Kit expression and activity in non-stem cell populations [28,38,42,59] including myocytes, substantiate heterogeneity within the cardiac c-Kit+ cell population and re-focus the discussion on what defines a stem cell beyond surface selection markers [110,111]. Indeed, recent studies demonstrating upregulation of c-Kit expression in “dedifferentiating” adult mammalian cardiomyocytes suggest a somewhat fluid relationship between stemness, post-mitotic commitment and stem cell marker expression [112–115] echoing but not equivalent to lower vertebrate or mammalian neonatal myocardial plasticity.

Similarly, consensus regarding true measures of cardiomyocyte proliferation remains unclear.

Reliable authentication of cell cycle status is essential for understanding the basic biology of and potential for proliferation in adult cardiomyocytes. False positive signals based on traditional measures of cell cycle interpreted as evidence for cell division substantiate the need for new methods and tools that distinguish between true cell division and incomplete progression through the cell cycle. For example, the cardiac specific FUCCI reporter mouse provides a testable real time readout of cardiomyocyte cell cycle status in vivo and in vitro. Together with more conventional measures of cell cycle, the cardiac FUCCI model illustrates that adult cardiomyocytes appear to be poised at the G1/S cell cycle restriction point [116]. Taken further, combining FUCCI with newly described, more precise markers of cell cycle status will help distinguish between actual cell division and other dynamic ways in which cardiomyocytes respond to changes in their environment. These distinctions will be critical to determining where cardiomyocytes get stuck in the cell cycle under various conditions [117], and identifying the cellular and molecular mechanisms that impede cell cycle progression with the goal of revealing potential targets for promoting cell cycle completion in these cells.

Increased recognition of the heterogeneity and plasticity of cardiac myocyte and non-myocyte populations [115] has prompted multiple studies using single cell analysis and individual cell profiling [118]. In the last two years, transcriptomic analysis has progressed from RNA-seq of bulk cardiac insterstitial populations [70], to single cell transcriptional profiling of interstitial and primary cultures of cardiac origin [119,120], and even single nucleus profiling of cardiomyocytes [74]. Single cell transcriptional profiling allows molecular assessment of both heterogeneity and inter-relatedness of cardiac interstitial cell populations as well as the cardiomyocyte pool during development, homeostasis and in response to injury, while transcriptomic analysis of individual isolated cardiomyocyte nuclei overcomes many limitations imposed by cardiomyocyte size and viability following isolation, and facilitates assessment of nuclear content as it pertains to cardiomyocyte biology. More recently, single cell western technology paired with transcriptional profiling aims to capture heterogeneity of protein expression within cardiac populations. Finally, recent findings reveal nonmyocyte ploidy as a previously unrecognized aspect of interstitial cell heterogeneity with possible implications for functional divergence [121].

Taken together, these advances in technology permit deeper investigation into the cellular and molecular biology of cardiac cell populations, and move scientific understanding past fixed perspectives that prevent progress toward cardiac regenerative therapies.

Conclusion

Cardiac disease remains the primary cause of death among humans [122], representing a costly, multifaceted public health dilemma that cannot be resolved with a single minded approach. Reversing these dire statistics requires an integrative program of increased support for basic and clinical research, health education, preventative medicine and regenerative therapies together with

Table 1
Trajectory of cardiac regeneration research as represented by select studies.

Adult mammalian cardiac regeneration			
	Past	Present	Future
Cardiomyocytes (CMs)	Measuring CM cell cycle, overexpressing cell cycle proteins to promote CM proliferation [88,89,123–125]	Retrenching adult CM perspective [86] Renewed interest in CM proliferation for cardiac repair. Identifying new stimuli for CM cell cycle [50,58,90,95,101,126] Developing new tools to assess CM cell cycle progression [98,99,116]	Developing new tools to accurately pinpoint CM cell cycle progression and molecular and cellular targets for promoting CM proliferation [103] Investigating CM heterogeneity using single cell molecular and biochemical analyses [74,127]
Cardiac Stem Cells (CSCs)	Discovery of adult CSCs [2] Development of cardiac cell therapy [10,80,82,83,128]	Retrenching adult CSC perspective [43,86,106,111,129–131] Novel research directions arising from CSC field [44,45,55–57,67,132,133] Advancing combinatorial CSC clinical trials [22,24,84,134]	Potentiating CSCs to improve therapeutic outcomes [135] Investigating CSC heterogeneity using single cell molecular and biochemical analyses [72]

currently available treatments. Reviewing the trajectory of cardiac regeneration research provides perspective and suggests potential areas for innovation to better understand basic cardiac cell biology in an effort to promote myocardial repair (see Table 1). Development of multiple reparative therapies whether based on cardiomyocyte proliferation, stem cell applications, tissue engineering, or some combination therein, is key to moving cardiac regenerative medicine forward. Therefore, research into every aspect of cardiac survival and repair is valid and necessary, and cooperation among researchers crucial if we are ever to succeed in pushing the human heart out of its resolutely postmitotic state. Revisiting old questions with new perspectives and tools such as bioinformatics, innovative animal or cellular models, or cell free systems will provide the fresh insights and interpretations necessary to create effective regenerative therapies for the treatment cardiac disease.

References

[1] Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410(6829):701–5.
[2] Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003;114(6):763–76.
[3] Gage FH. Adult neurogenesis in mammals. Science 2019;364(6443):827–8.
[4] Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 2004;428(6983):668–73.
[5] Chien KR. Stem cells: lost in translation. Nature 2004;428(6983):607–8.
[6] Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004;428(6983):664–8.
[7] Laflamme MA, Murry CE. Regenerating the heart. Nat Biotechnol 2005;23(7):845–56.
[8] Hsieh PC, Segers VF, Davis ME, MacGillivray C, Gannon J, Molkentin JD, et al. Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. Nat Med 2007;13(8):970–4.
[9] Kraitchman DL, Heldman AW, Atalar E, Amado LC, Martin BJ, Pittenger MF, et al. In vivo magnetic resonance imaging of mesenchymal stem cells in myocardial infarction. Circulation 2003;107(18):2290–3.
[10] Amado LC, Saliaris AP, Schuleri KH, St John M, Xie JS, Cattaneo S, et al. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proc Natl Acad Sci USA 2005;102(32):11474–9.
[11] Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001;107(11):1395–402.
[12] Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. Circulation 2007;115(7):896–908.
[13] Barile L, Messina E, Giacomello A, Marban E. Endogenous cardiac stem cells. Prog Cardiovasc Dis 2007;50(1):31–48.
[14] Badorff C, Brandes RP, Popp R, Rupp S, Urbich C, Aicher A, et al. Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. Circulation 2003;107(7):1024–32.
[15] Kawamoto A, Tkebuchava T, Yamaguchi J, Nishimura H, Yoon YS, Milliken C, et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. Circulation 2003;107(3):461–8.
[16] Ii M, Nishimura H, Iwakura A, Wecker A, Eaton E, Asahara T, et al. Endothelial progenitor cells are rapidly recruited to myocardium and mediate protective effect of ischemic preconditioning via "imported" nitric oxide synthase activity. Circulation 2005;111(9):1114–20.
[17] Bearzi C, Leri A, Lo Monaco F, Rota M, Gonzalez A, Hosoda T, et al. Identification of a coronary vascular progenitor cell in the human heart. Proc Natl Acad Sci U S A 2009;106(37):15885–90.
[18] Crisan M, Chen CW, Corselli M, Andriolo G, Lazzari L, Peault B. Perivascular multipotent progenitor cells in human organs. Ann N Y Acad Sci 2009;1176:118–23.
[19] Chen CW, Okada M, Proto JD, Gao X, Sekiya N, Beckman SA, et al. Human pericytes for ischemic heart repair. Stem Cells 2013;31(2):305–16.
[20] Smart N, Bollini S, Dube KN, Vieira JM, Zhou B, Davidson S, et al. De novo cardiomyocytes from within the activated adult heart after injury. Nature 2011;474(7353):640–4.
[21] Trembley MA, Velasquez LS, de Mesy Bentley KL, Small EM. Myocardin-related transcription factors control the motility of epicardium-derived cells and the maturation of coronary vessels. Development 2015;142(1):21–30.
[22] Karantalis V, Suncion-Loescher VY, Bagno L, Golpanian S, Wolf A, Sanina C, et al. Synergistic effects of combined cell therapy for chronic ischemic cardiomyopathy. J Am Coll Cardiol 2015;66(18):1990–9.
[23] Hatzistergos KE, Saur D, Seidler B, Balkan W, Breton M, Valasaki K, et al. Stimulatory effects of mesenchymal stem cells on cKit+ cardiac stem cells are mediated by SDF1/CXCR4 and SCF/cKit signaling pathways. Circ Res 2016;119(8):921–30.
[24] Natsumeda M, Florea V, Rieger AC, Tompkins BA, Banerjee MN, Golpanian S, et al. A combination of allogeneic stem cells promotes cardiac regeneration. J Am Coll Cardiol 2017;70(20):2504–15.
[25] Zwetsloot PP, Vegh AM, Jansen of Lorkeers SJ, van Hout GP, Currie GL, Sena ES, et al. Cardiac stem cell treatment in myocardial infarction: a systematic review and meta- analysis of preclinical studies. Circ Res 2016;118(8):1223–32.
[26] Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabe-Heider F, Walsh S, et al. Evidence for cardiomyocyte renewal in humans. Science 2009;324(5923):98–102.
[27] Bergmann O, Zdunek S, Frisen J, Bernard S, Druid H, Jovinge S. Cardiomyocyte renewal in humans. Circ Res 2012;110(1):e17–18 author reply e19-21.
[28] van Berlo JH, Kanisicak O, Maillet M, Vagnozzi RJ, Karch J, Lin SC, et al. c-kit+ cells minimally contribute cardiomyocytes to the heart. Nature 2014;509(7500):337–41.
[29] Nadal-Ginard B, Ellison GM, Torella D. Absence of evidence is not evidence of absence: pitfalls of cre knock-ins in the c-Kit locus. Circ Res 2014;115(4):415–18.
[30] Molkentin JD. Letter by Molkentin regarding article, "The absence of evidence is not evidence of absence: the pitfalls of Cre Knock-Ins in the c-Kit Locus". Circ Res 2014;115(8):e21–3.
[31] Nadal-Ginard B, Ellison GM, Torella D. Response to Molkentin's letter to the editor regarding article, "the absence of evidence is not evidence of absence: the pitfalls of Cre knock-ins in the c-kit locus. Circ Res 2014;115(12):e38–e39.
[32] Vicinanza C, Aquila I, Cianflone E, Scalise M, Marino F, Mancuso T, et al. Kit(cre) knock- in mice fail to fate-map cardiac stem cells. Nature 2018;555(7697):E1–5.
[33] Vagnozzi RJ, Sargent MA, Lin SJ, Palpant NJ, Murry CE, Molkentin JD. Genetic lineage tracing of sca-1(+) cells reveals endothelial but not myogenic contribution to the murine heart. Circulation 2018;138(25):2931–9.
[34] Vagnozzi RJ, Molkentin JD, Houser SR. New myocyte formation in the adult heart: endogenous sources and therapeutic implications. Circ Res 2018;123(2):159–76.
[35] Aquila I, Cianflone E, Scalise M, Marino F, Mancuso T, Filardo A, et al. c-kit Haploinsufficiency impairs adult cardiac stem cell growth, myogenicity and myocardial regeneration. Cell Death Dis 2019;10(6):436.
[36] Fransioli J, Bailey B, Gude NA, Cottage CT, Muraski JA, Emmanuel G, et al. Evolution of the c-kit-positive cell response to pathological challenge in the myocardium. Stem Cells 2008;26(5):1315–24.
[37] Fischer KM, Cottage CT, Wu W, Din S, Gude NA, Avitabile D, et al. Enhancement of myocardial regeneration through genetic engineering of cardiac progenitor cells expressing Pim-1 kinase. Circulation 2009;120(21):2077–87.
[38] Gude NA, Firouzi F, Broughton KM, Ilves K, Nguyen KP, Payne CR, et al. Cardiac c-Kit biology revealed by inducible transgenesis. Circ Res 2018;123(1):57–72.
[39] Duran JM, Makarewich CA, Sharp TE, Starosta T, Zhu F, Hoffman NE, et al. Bone- derived stem cells repair the heart after myocardial infarction through transdifferentiation and paracrine signaling mechanisms. Circ Res 2013;113(5):539–52.
[40] Mohsin S, Troupes CD, Starosta T, Sharp TE, Agra EJ, Smith S, et al. Unique features of cortical bone stem cells associated with repair of the injured heart. Circ Res 2015;117(12):1024–33.
[41] Ellison GM, Vicinanza C, Smith AJ, Aquila I, Leone A, Waring CD, et al. Adult c-kit(pos) cardiac stem cells are necessary and sufficient for functional cardiac regeneration and repair. Cell 2013;154(4):827–42.
[42] Tallini YN, Greene KS, Craven M, Spealman A, Breitbach M, Smith J, et al. c-kit expression identifies cardiovascular precursors in the neonatal heart. Proc Natl Acad Sci U S A 2009;106(6):1808–13.
[43] Expression of concern. Circulation 2019;139(3):e5–6.
[44] Lee C, Mitsialis SA, Aslam M, Vitali SH, Vergadi E, Konstantinou G, et al. Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. Circulation 2012;126(22):2601–11.
[45] Khan M, Nickoloff E, Abramova T, Johnson J, Verma SK, Krishnamurthy P, et al. Embryonic stem cell-derived exosomes promote endogenous repair mechanisms and enhance cardiac function following myocardial infarction. Circ Res 2015;117(1):52–64.
[46] Davis ME. Exosomes: what do we love so much about them? Circ Res 2016;119(12):1280–2.
[47] Sharma S, Mishra R, Bigham GE, Wehman B, Khan MM, Xu H, et al. A deep proteome analysis identifies the complete secretome as the functional unit of human cardiac progenitor cells. Circ Res 2017;120(5):816–34.
[48] Rosenthal N, Santini MP, Musaro A. Growth factor enhancement of cardiac regeneration. Cell Transplant 2006;15(Suppl 1):S41–5.
[49] Ellison GM, Torella D, Dellegrottaglie S, Perez-Martinez C, Perez de Prado A, Vicinanza C, et al. Endogenous cardiac stem cell activation by insulin–like growth factor-1/hepatocyte growth factor intracoronary injection fosters survival and regeneration of the infarcted pig heart. J Am Coll Cardiol 2011;58(9):977–86.
[50] Nakada Y, Canseco DC, Thet S, Abdisalaam S, Asaithamby A, Santos CX, et al. Hypoxia induces heart regeneration in adult mice. Nature 2017;541(7636):222–7.

[51] Tang YL, Zhu W, Cheng M, Chen L, Zhang J, Sun T, et al. Hypoxic preconditioning enhances the benefit of cardiac progenitor cell therapy for treatment of myocardial infarction by inducing CXCR4 expression. Circ Res 2009;104(10):1209–16.
[52] Mohsin S, Khan M, Toko H, Bailey B, Cottage CT, Wallach K, et al. Human cardiac progenitor cells engineered with Pim-I kinase enhance myocardial repair. J Am Coll Cardiol 2012;60(14):1278–87.
[53] Narsinh KH, Sun N, Sanchez-Freire V, Lee AS, Almeida P, Hu S, et al. Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells. J Clin Invest 2011;121(3):1217–21.
[54] Menard C, Hagege AA, Agbulut O, Barro M, Morichetti MC, Brasselet C, et al. Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study. Lancet 2005;366(9490):1005–12.
[55] Menasche P, Vanneaux V, Hagege A, Bel A, Cholley B, Parouchev A, et al. Transplantation of human embryonic stem cell-derived cardiovascular progenitors for severe ischemic left ventricular dysfunction. J Am Coll Cardiol 2018;71(4):429–38.
[56] Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell 2010;142(3):375–86.
[57] Srivastava D, DeWitt N. In vivo cellular reprogramming: the next generation. Cell 2016;166(6):1386–96.
[58] Mohamed TMA, Ang YS, Radzinsky E, Zhou P, Huang Y, Elfenbein A, et al. Regulation of cell cycle to stimulate adult cardiomyocyte proliferation and cardiac regeneration. Cell 2018;173(1):104–16 e12.
[59] Hatzistergos KE, Takeuchi LM, Saur D, Seidler B, Dymecki SM, Mai JJ, et al. cKit+cardiac progenitors of neural crest origin. Proc Natl Acad Sci USA 2015;112(42):13051–6.
[60] Li Y, He L, Huang X, Bhaloo SI, Zhao H, Zhang S, et al. Genetic lineage tracing of nonmyocyte population by dual recombinases. Circulation 2018;138(8):793–805.
[61] Li Y, Lv Z, He L, Huang X, Zhang S, Zhao H, et al. Genetic tracing identifies early segregation of the cardiomyocyte and non-myocyte lineages. Circ Res 2019.
[62] Long C, Amoasii L, Mireault AA, McAnally JR, Li H, Sanchez-Ortiz E, et al. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science 2016;351(6271):400–3.
[63] Amoasii L, Hildyard JCW, Li H, Sanchez-Ortiz E, Mireault A, Caballero D, et al. Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. Science 2018;362(6410):86–91.
[64] Liu J, Hu Q, Wang Z, Xu C, Wang X, Gong G, et al. Autologous stem cell transplantation for myocardial repair. Am J Physiol Heart Circ Physiol 2004;287(2):H501–11.
[65] Eschenhagen T, Zimmermann WH. Engineering myocardial tissue. Circ Res 2005;97(12):1220–31.
[66] Zimmermann WH. Translating *Myocardial remuscularization*. Circ Res 2017;120(2):278–81.
[67] Gao L, Gregorich ZR, Zhu W, Mattapally S, Oduk Y, Lou X, et al. Large cardiac muscle patches engineered from human induced-pluripotent stem cell-derived cardiac cells improve recovery from myocardial infarction in swine. Circulation 2018;137(16):1712–30.
[68] Madonna R, Van Laake LW, Botker HE, Davidson SM, De Caterina R, Engel FB, et al. ESC working group on cellular biology of the heart: position paper for cardiovascular research: tissue engineering strategies combined with cell therapies for cardiac repair in ischaemic heart disease and heart failure. Cardiovasc Res 2019;115(3):488–500.
[69] Bochmann L, Sarathchandra P, Mori F, Lara-Pezzi E, Lazzaro D, Rosenthal N. Revealing new mouse epicardial cell markers through transcriptomics. PLoS One 2010;5(6):e11429.
[70] Furtado MB, Costa MW, Pranoto EA, Salimova E, Pinto AR, Lam NT, et al. Cardiogenic genes expressed in cardiac fibroblasts contribute to heart development and repair. Circ Res 2014;114(9):1422–34.
[71] Diez-Cunado M, Wei K, Bushway PJ, Maurya MR, Perera R, Subramaniam S, et al. miRNAs that induce human cardiomyocyte proliferation converge on the hippo pathway. Cell Rep 2018;23(7):2168–74.
[72] Jia G, Preussner J, Chen X, Guenther S, Yuan X, Yekelchyk M, et al. Single cell RNA-seq and ATAC-seq analysis of cardiac progenitor cell transition states and lineage settlement. Nat Commun 2018;9(1):4877.
[73] Zhang JZ, Termglinchan V, Shao NY, Itzhaki I, Liu C, Ma N, et al. A human iPSC Double- Reporter System enables purification of cardiac lineage subpopulations with distinct function and drug response profiles. Cell Stem Cell 2019;24(5):802–11 e5.
[74] See K, Tan WLW, Lim EH, Tiang Z, Lee LT, Li PYQ, et al. Single cardiomyocyte nuclear transcriptomes reveal a lincRNA-regulated de-differentiation and cell cycle stress- response in vivo. Nat Commun 2017;8(1):225.
[75] Gude NA, Broughton KM, Firouzi F, Sussman MA. Cardiac ageing: extrinsic and intrinsic factors in cellular renewal and senescence. Nat Rev Cardiol 2018;15(9):523–42.
[76] Lewis-McDougall FC, Ruchaya PJ, Domenjo-Vila E, Shin Teoh T, Prata L, Cottle BJ, et al. Aged-senescent cells contribute to impaired heart regeneration. Aging Cell 2019:e12931.
[77] Broughton KM, Sussman MA. Empowering adult stem cells for myocardial regeneration V2.0: success in small steps. Circ Res 2016;118(5):867–80.
[78] Broughton KM, Sussman MA. Enhancement strategies for cardiac regenerative cell therapy: focus on adult stem cells. Circ Res 2018;123(2):177–87.
[79] Khan AR, Farid TA, Pathan A, Tripathi A, Ghafghazi S, Wysoczynski M, et al. Impact of cell therapy on myocardial perfusion and cardiovascular outcomes in patients with angina refractory to medical therapy: a systematic review and meta-analysis. Circ Res. 2016;118(6):984–93.
[80] Jeevanantham V, Butler M, Saad A, Abdel-Latif A, Zuba-Surma EK, Dawn B. Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis. Circulation 2012;126(5):551–68.
[81] Samanta A, Dawn B. Meta-Analysis of preclinical data reveals efficacy of cardiac stem cell therapy for heart repair. Circ Res 2016;118(8):1186–8.
[82] Chugh AR, Beache GM, Loughran JH, Mewton N, Elmore JB, Kajstura J, et al. 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 2012;126(11 Suppl 1):S54–64.
[83] Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet 2012;379(9819):895–904.
[84] Bolli R, Hare JM, March KL, Pepine CJ, Willerson JT, Perin EC, et al. Rationale and design of the CONCERT-HF Trial (Combination of mesenchymal and c-kit(+) cardiac stem cells as regenerative therapy for heart failure). Circ Res 2018;122(12):1703–15.
[85] Hatzistergos KE, Vedenko A. Cardiac cell therapy 3.0: the beginning of the end or the end of the beginning? Circ Res 2017;121(2):95–7.
[86] Eschenhagen T, Bolli R, Braun T, Field LJ, Fleischmann BK, Frisen J, et al. Cardiomyocyte regeneration: a consensus statement. Circulation 2017;136(7):680–6.
[87] Oh H, Taffet GE, Youker KA, Entman ML, Overbeek PA, Michael LH, et al. Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. Proc Natl Acad Sci USA 2001;98(18):10308–13.
[88] Soonpaa MH, Koh GY, Pajak L, Jing S, Wang H, Franklin MT, et al. Cyclin D1 overexpression promotes cardiomyocyte DNA synthesis and multinucleation in transgenic mice. J Clin Invest 1997;99(11):2644–54.
[89] Pasumarthi KB, Nakajima H, Nakajima HO, Soonpaa MH, Field LJ. Targeted expression of cyclin D2 results in cardiomyocyte DNA synthesis and infarct regression in transgenic mice. Circ Res 2005;96(1):110–18.
[90] Mahmoud AI, Kocabas F, Muralidhar SA, Kimura W, Koura AS, Thet S, et al. Meis1 regulates postnatal cardiomyocyte cell cycle arrest. Nature 2013;497(7448):249–53.
[91] Hirose K, Payumo AY, Cutie S, Hoang A, Zhang H, Guyot R, et al. Evidence for hormonal control of heart regenerative capacity during endothermy acquisition. Science 2019;364(6436):184–8.
[92] Gude N, Muraski J, Rubio M, Kajstura J, Schaefer E, Anversa P, et al. Akt promotes increased cardiomyocyte cycling and expansion of the cardiac progenitor cell population. Circ Res 2006;99(4):381–8.
[93] Muraski JA, Rota M, Misao Y, Fransioli J, Cottage C, Gude N, et al. Pim-1 regulates cardiomyocyte survival downstream of AKT. Nat Med 2007;13(12):1467–75.
[94] Leone M, Magadum A, Engel FB. Cardiomyocyte proliferation in cardiac development and regeneration: a guide to methodologies and interpretations. Am J Physiol Heart Circ Physiol 2015;309(8):H1237–50.
[95] Zebrowski DC, Becker R, Engel FB. Towards regenerating the mammalian heart: challenges in evaluating experimentally induced adult mammalian cardiomyocyte proliferation. Am J Physiol Heart Circ Physiol 2016;310(9):H1045–54.
[96] Walsh S, Ponten A, Fleischmann BK, Jovinge S. Cardiomyocyte cell cycle control and growth estimation in vivo–an analysis based on cardiomyocyte nuclei. Cardiovasc Res 2010;86(3):365–73.
[97] Awad S, Al-Haffar KM, Marashly Q, Quijada P, Kunhi M, Al-Yacoub N, et al. Control of histone H3 phosphorylation by CaMKIIdelta in response to haemodynamic cardiac stress. J Pathol 2015;235(4):606–18.
[98] Hesse M, Doengi M, Becker A, Kimura K, Voeltz N, Stein V, et al. Midbody positioning and distance between daughter nuclei enable unequivocal identification of cardiomyocyte cell division in mice. Circ Res 2018;123(9):1039–52.
[99] Leone M, Musa G, Engel FB. Cardiomyocyte binucleation is associated with aberrant mitotic microtubule distribution, mislocalization of RhoA and IQGAP3, as well as defective actomyosin ring anchorage and cleavage furrow ingression. Cardiovasc Res 2018;114(8):1115–31.
[100] Ali SR, Hippenmeyer S, Saadat LV, Luo L, Weissman IL, Ardehali R. Existing cardiomyocytes generate cardiomyocytes at a low rate after birth in mice. Proc Natl Acad Sci USA 2014;111(24):8850–5.
[101] Kimura W, Xiao F, Canseco DC, Muralidhar S, Thet S, Zhang HM, et al. Hypoxia fate mapping identifies cycling cardiomyocytes in the adult heart. Nature 2015;523(7559):226–30.
[102] Lazar E, Sadek HA, Bergmann O. Cardiomyocyte renewal in the human heart: insights from the fall-out. Eur Heart J 2017;38(30):2333–42.
[103] Leone M, Engel FB. Advances in heart regeneration based on cardiomyocyte proliferation and regenerative potential of binucleated cardiomyocytes and polyploidization. Clin Sci (Lond) 2019;133(11):1229–53.
[104] Vicinanza C, Aquila I, Scalise M, Cristiano F, Marino F, Cianflone E, et al. Adult cardiac stem cells are multipotent and robustly myogenic: c-kit expression is necessary but not sufficient for their identification. Cell Death Differ 2017;24(12):2101–16.

[105] Zaruba MM, Soonpaa M, Reuter S, Field LJ. Cardiomyogenic potential of C-kit(+)- expressing cells derived from neonatal and adult mouse hearts. Circulation 2010;121(18):1992–2000.
[106] Gude NA, Sussman MA. Chasing c-Kit through the heart: taking a broader view. Pharmacol Res 2018;127:110–15.
[107] Marino F, Scalise M, Cianflone E, Mancuso T, Aquila I, Agosti V, et al. Role of c-Kit in myocardial regeneration and aging. Front Endocrinol (Lausanne) 2019;10:371.
[108] Chabot B, Stephenson DA, Chapman VM, Besmer P, Bernstein A. The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. Nature 1988;335(6185):88–9.
[109] Ye L, Zhang EY, Xiong Q, Astle CM, Zhang P, Li Q, et al. Aging kit mutant mice develop cardiomyopathy. PLoS One 2012;7(3):e33407.
[110] Keith MC, Bolli R. "String theory" of c-kit(pos) cardiac cells: a new paradigm regarding the nature of these cells that may reconcile apparently discrepant results. Circ Res 2015;116(7):1216–30.
[111] Maroli G, Braun T. The complex biology of KIT(+) cells in the heart. Nat Rev Cardiol 2018;15(8):443–4.
[112] Kubin T, Poling J, Kostin S, Gajawada P, Hein S, Rees W, et al. Oncostatin M is a major mediator of cardiomyocyte dedifferentiation and remodeling. Cell Stem Cell 2011;9(5):420–32.
[113] Zhang Y, Li TS, Lee ST, Wawrowsky KA, Cheng K, Galang G, et al. Dedifferentiation and proliferation of mammalian cardiomyocytes. PLoS One 2010;5(9):e12559.
[114] Wang WE, Li L, Xia X, Fu W, Liao Q, Lan C, et al. Dedifferentiation, proliferation, and redifferentiation of adult mammalian cardiomyocytes after ischemic injury. Circulation 2017;136(9):834–48.
[115] Kawaguchi N, Takagaki Y, Matsuoka R, Nakanishi T, et al. Molecular analysis of long-term cultured cardiac stem cells for cardiac regeneration. In: Nakanishi T, Markwald RR, Baldwin HS, et al., editors. Etiology and morphogenesis of congenital heart disease: from gene function and cellular interaction to morphology; 2016. p. 345–7. Tokyo.
[116] Alvarez R Jr, Wang BJ, Quijada PJ, Avitabile D, Ho T, Shaitrit M, et al. Cardiomyocyte cell cycle dynamics and proliferation revealed through cardiac-specific transgenesis of fluorescent ubiquitinated cell cycle indicator (FUCCI). J Mol Cell Cardiol 2019;127:154–64.
[117] Broughton KM, Sussman MA. Adult cardiomyocyte cell cycle detour: off-ramp to quiescent destinations. Trends Endocrinol Metab 2019.
[118] Sussman MA. Cardiac nonmyocyte subpopulations: a secular congregation. Regen Med 2019.
[119] Skelly DA, Squiers GT, McLellan MA, Bolisetty MT, Robson P, Rosenthal NA, et al. Single-Cell transcriptional profiling reveals cellular diversity and intercommunication in the mouse heart. Cell Rep 2018;22(3):600–10.
[120] Kim T, Echeagaray OH, Wang BJ, Casillas A, Broughton KM, Kim BH, et al. In situ transcriptome characteristics are lost following culture adaptation of adult cardiac stem cells. Sci Rep 2018;8(1):12060.
[121] Broughton KM, Khieu T, Nguyen N, Rosa M, Mohsin S, Quijada P, et al. Cardiac interstitial tetraploid cells can escape replicative senescence in rodents but not large mammals. Commun Biol 2019;2:205.
[122] Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics-2019 update: a report from the american heart association. Circulation 2019;139(10):e56–e528.
[123] Soonpaa MH, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. Circ Res 1998;83(1):15–26.
[124] Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, et al. Evidence that human cardiac myocytes divide after myocardial infarction. N Engl J Med 2001;344(23):1750–7.
[125] Pasumarthi KB, Field LJ. Cardiomyocyte cell cycle regulation. Circ Res 2002;90(10):1044–54.
[126] Zebrowski DC, Engel FB. The cardiomyocyte cell cycle in hypertrophy, tissue homeostasis, and regeneration. Rev Physiol Biochem Pharmacol 2013;165:67–96.
[127] Hu P, Liu J, Zhao J, Wilkins BJ, Lupino K, Wu H, et al. Single-nucleus transcriptomic survey of cell diversity and functional maturation in postnatal mammalian hearts. Genes Dev 2018;32(19–20):1344–57.
[128] Hatzistergos KE, Quevedo H, Oskouei BN, Hu Q, Feigenbaum GS, Margitich IS, et al. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. Circ Res 2010;107(7):913–22.
[129] Zhou B, Wu SM. Reassessment of c-Kit in cardiac cells: a complex interplay between expression, fate, and function. Circ Res 2018;123(1):9–11.
[130] Braunwald E. Cell-Based therapy in cardiac regeneration: an overview. Circ Res 2018;123(2):132–7.
[131] Nadal-Ginard B, Torella D, De Angelis A, Rossi F. Monographic issue of pharmacological research on adult myocardial repair/regeneration. Pharmacol Res 2018;127:1–3.
[132] Xiao C, Wang K, Xu Y, Hu H, Zhang N, Wang Y, et al. Transplanted mesenchymal stem cells reduce autophagic flux in infarcted hearts via the exosomal transfer of miR-125b. Circ Res 2018;123(5):564–78.
[133] Ye L, Chang YH, Xiong Q, Zhang P, Zhang L, Somasundaram P, et al. Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell- derived cardiovascular cells. Cell Stem Cell 2014;15(6):750–61.
[134] Williams AR, Hatzistergos KE, Addicott B, McCall F, Carvalho D, Suncion V, et al. Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. Circulation 2013;127(2):213–23.
[135] Kulandavelu S, Karantalis V, Fritsch J, Hatzistergos KE, Loescher VY, McCall F, et al. Pim1 kinase overexpression enhances ckit(+) cardiac stem cell cardiac repair following myocardial infarction in Swine. J Am Coll Cardiol 2016;68(22):2454–64.