

Heart regeneration: Past, present and future

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Abstract

The heart has been considered a post-mitotic organ without regenerative capacity for most of the last century. We review the evidence that led to this hypothesis in the early 1900s and how it was progressively modified, culminating with the report that we renew 50% of our cardiomyocytes during our lifetime. The future of cardiac regenerative therapies is discussed, presenting the difficulties to overcome before repair of the diseased heart can come into clinical practice.

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INTRODUCTION

For most of the last century, the heart has classically been viewed as an organ incapable of self-renewal^[1]. The basis for this assumption was laid more than eight decades ago and many still consider it a definitive characteristic^[2,3]. However, the possibility of cardiac self-renewal has been re-examined over the years^[4-6]. Here, we will review heart regeneration research from a historical perspective, presenting the foundations that established the field. Then, we will discuss current knowledge and future possibilities for this exciting and promising area of research.

PAST

From 1850 to the first quarter of the 20th century, the prevailing view among cardiologists was that the heart was capable of regeneration, since organ hypertrophy was attributed to cardiomyocyte hyperplasia^[2,4]. In 1925, Karsner *et al*^[2] examined in detail whether macroscopic cardiac hypertrophy was caused by an increase in the size or in the number of fibers present in adult cardiac muscle. By counting the nuclei stained with hematoxylin and eosin, they concluded that the number of cardiac fibers was unchanged in the hypertrophied human heart when compared to a normal heart, indicating that hypertrophy was caused by enlargement rather than proliferation of cardiomyocytes. Additionally, they also stated that “the most careful search has failed to disclose mitotic figures”. These observations laid the ground for envisaging

the heart as a post-mitotic organ, which is a view that became common knowledge and remained widely accepted for much of the last century.

In spite of this, already in 1937, Macmahon^[4] demonstrated that, even though mitotic figures had not been found in the adult heart, they were present in the hearts of children with hypertrophy and myocarditis. Additionally, Robledo^[7] published work in 1956 demonstrating the presence of mitosis in 4 to 7 d old rats that had been submitted to myocardial injury by burning a small ventricular area.

The first evidence that not only young but also adult hearts could regenerate was presented in 1960. Linzbach^[5] published an article analyzing the anatomic basis of variations in the size of the human heart. First, the average length of sarcomeres, as measured by the distance between Z-bands, was shown to be unchanged in normal, hypertrophied and dilated hearts. Then, it was demonstrated that, when the adult heart was pathologically overloaded and its weight exceeded 500 g (or 200 g for the left ventricle), there was an increase in the number of muscle fibers with little further thickening of these fibers. Hence, even though cardiomyocyte proliferation had not been directly documented, the addition of new fibers suggested that some form of cardiac regeneration had occurred in the adult heart.

In the following years, mitosis started to be documented in uninjured cardiac muscle^[8,9]. In 1968, Sasaki and co-workers described that mitotic figures could be found in normal rats treated with colchicine, both in cardiomyocytes (at the age of 4 wk) and interstitial cells (at 6 mo)^[8]. Moreover, Zak^[9] published a famous review in 1974 discussing the proliferative capacity of cardiac muscle cells. He described, as shown by Sasaki, that cardiomyocytes can undergo mitosis in rats up to 4 wk of age. In addition, he also analyzed the presence of mitosis in other organs; e.g. in the liver no mitotic figures could be found 10 wk after birth, which is, as concluded by the author, an indication that proliferation will stop in any organ that has achieved its adult size. The aspect that really differentiates the liver from the heart is the proliferative capacity in response to injury in adult cells. Zak stated that adult cardiomyocytes were unable to divide in a pressure overload model, although there was proliferation of non-muscle cells, which is a fact that had not been appreciated previously. Therefore, he concluded that cardiac hypertrophy consists of hypertrophy of myocytes and hyperplasia of connective tissue cells, thus reinforcing the notion of the cardiomyocytes as post-mitotic cells.

In 1977, Astorri *et al.*^[10] published an article confirming Linzbach's findings in diseased adult human hearts, demonstrating that cardiomyocyte hyperplasia was evident above the critical left ventricular weight of 250 g. Nonetheless, no direct evidence of mitosis in adult cardiomyocytes had yet been found. Only in the 1990s did the evidence start to appear^[6,11-13]. Quaini *et al.*^[6] demonstrated the presence of proliferating cell nuclear anti-

gen, expressed at the G₁-S boundary of the cell cycle, in adult cardiomyocytes obtained from ischemic and dilated cardiomyopathy patients. However, DNA synthesis and nuclear division do not provide definitive evidence of mitosis in cardiomyocytes since these cells can undergo DNA duplication and karyokinesis, becoming multinucleated without dividing (no cytokinesis). A few years later, the presence of both metaphasic chromosomes and cytokinesis was detected in normal myocardium^[12,13] in ischemic and dilated cardiomyopathy patients^[12], as well as in patients who had suffered a myocardial infarction^[13].

From that point on, it became generally accepted that cardiomyocytes could proliferate in the adult heart. However, there was no agreement on the frequency of this event in normal and diseased myocardium. As reviewed by Soonpaa *et al.*^[14], the frequency of cell division is influenced by the methods used to detect DNA synthesis and identify cardiomyocytes. In normal adult rats and mice, the percentage of cardiomyocytes that were synthesizing DNA is reported to range from 0.005%-3.15% and 0.0004%-0.04%, respectively^[14]. In the injured hearts of adult animals, results were even more variable, ranging from 0.0006%-43.6% in rats and 0.0055%-0.5% in mice^[14].

Thus, even though cardiomyocyte proliferation was accepted by the scientific community, the discrepancies found in the frequency of mitosis led to universal disagreement on the biological significance of this event. Based on clinical observations, several authors argued that cardiomyocyte proliferation had no biological significance since the heart was unable to recover, for instance, from myocardial infarctions and that primary heart tumors were rarely observed in adults^[14,15]. However, as pointed out by Anversa *et al.*^[16], regardless of the proliferative capacity of their parenchymal cells, the outcome of infarction is identical in several organs, including the testis, skin, kidney, brain and intestine. Additionally, using the rarity of primary heart tumors as an argument is also faulty; despite the fact that neurons do not usually proliferate, there are several tumors that arise from the interstitial/supporting cells in the central nervous system. On the other hand, although the heart also has a vast number of interstitial/supporting cells, tumors originating from these cells are as rare as the ones originating from cardiomyocytes. This could possibly indicate that the infrequency of primary heart tumors has more to do with the structural, mechanical and functional characteristics of the organ than with the rate of cardiomyocyte proliferation.

Therefore, no agreement on the importance of cardiac self-renewal was reached and new facts would come to play a role. In the late 1990s, we moved into the present stage with the explosion of stem cell research directed toward regenerative medicine.

CURRENT PERSPECTIVES

Stem cell research was actually implemented in the early

1960s after the observation that lethally irradiated mice could be rescued from death by a bone marrow transplant^[17,18]. Till and McCulloch began to analyze the bone marrow to find out which component was responsible for regenerating blood, leading to the discovery of the hematopoietic stem cell^[18-20]. However, it was only in the late 1990s that scientists started trying to use bone marrow stem cells (BMCs) to regenerate injured organs such as skeletal muscle^[21], brain^[22,23], liver^[24,25] and heart^[26,27]. From this moment on, stem cell research applied to regenerative medicine grew exponentially and a few years later, in 2004, the capacity of BMCs to regenerate the heart started to be challenged^[28-31]. In the mean time, a number of clinical trials using bone marrow-derived cells were started. The majority of these trials used the mononuclear fraction of the patient's own bone marrow. The results have been far more modest than was anticipated, with reported gains of 3%-4% in left ventricular ejection fraction in acute myocardial infarction patients.

From the point of view of cardiac self-renewal, it is not important whether BMCs can or cannot transdifferentiate into cardiomyocytes. In fact, the importance of those disputed initial findings resides on the fact that they triggered the search for resident stem cells in the heart. The first report of such a cell appeared in 2002, indicating the presence of a verapamil-sensitive side population (SP) with stem cell-like activity^[32]. Shortly after, the existence of several other types of cardiac stem cells was reported: c-kit positive^[33], Sca-1 positive^[34,35], cells with persistent expression of Abcg2^[36], cardiosphere-derived cells (CDCs)^[37] and islet-1 positive cells^[38,39]. Since only c-kit positive^[40], CDCs^[41] and islet-1 positive^[38,42] cells were isolated from human tissue, these cell types have received more attention over the years.

Human c-kit positive cells isolated from small samples of myocardium are self-renewing, clonogenic, multipotent and have the ability to generate cardiomyocytes and coronary vessels *in vivo*, improving cardiac function after myocardial infarction in mice^[40]. CDCs isolated from human endomyocardial biopsies form a heterogeneous population that expresses antigens found in other stem cell types, such as c-kit, CD90 and CD105^[41]. When co-cultured with rat neonatal cardiomyocytes, CDCs exhibited calcium transients synchronous with the neighboring myocytes and, when injected *in vivo*, engrafted and improved cardiac function in mice submitted to myocardial infarctions^[41]. Islet-1 is a developmental lineage marker for undifferentiated cardiogenic precursor cells usually found in the fetal human heart^[38]. After birth, few islet-1 positive cells can be found in the myocardium, suggesting they are developmental remnants of the fetal progenitor population. These cells can be isolated and differentiated into fully mature cardiomyocytes that express contractile proteins, generate calcium transients and respond to β -adrenergic stimulation^[38]. However, their presence in the adult human heart and their capacity to engraft, regenerate myocardium and improve cardiac function in animal models remains to be demonstrated.

As pointed out by Laflamme *et al.*^[43], it is unlikely that the heart would harbor multiple non-overlapping sets of cardiomyocyte progenitors. However, some degree of overlapping has been reported; a subset of c-kit positive cells do express Sca-1^[33], CDCs are formed by a heterogeneous population in which c-kit expression has been documented^[37,41] and islet-1 positive cells may not exist in adult myocardium at all. Furthermore, it is possible that these cell types are precursors originating from a more undifferentiated cell that would be the true cardiac stem cell. Regardless of which cell is the right one, the major advance pushed forward by the isolation of cardiac stem cells is the possibility that the heart possesses progenitors that are responsible for the physiological renewal of cardiomyocytes, which involves a slow turnover process, maintaining organ homeostasis. Obviously, these cells cannot fully recover the myocardium in pathological conditions, but even in this scenario some degree of regeneration has already been reported. Hsieh and co-workers, using α -myosin heavy chain *Cre-Lox* transgenic mice, elegantly demonstrated that up to 15% of cardiomyocytes could be regenerated in adult hearts after myocardial infarction^[44].

Finally, definitive evidence that the human heart is capable of self-renewal came in 2009. Bergmann and co-workers^[45] published an important study in which they used the integration of carbon-14, generated by nuclear bomb tests during the Cold War, into DNA to establish the age of cardiomyocytes composing the human heart. They reported that 1% of human cardiomyocytes are renewed annually at the age of 25 and that this rate is reduced to 0.45% at the age of 75. Moreover, total cell renewal over the entire human life span corresponds to approximately 50% of the cardiomyocytes.

Therefore, we can go back to reflect on the end of the 1990s when there was no agreement on the biological significance of cardiac self-renewal. It is now undeniable that heart regeneration does occur and is important to maintain organ homeostasis. This regeneration is probably a result of both stem cell differentiation and cardiomyocyte proliferation. It is now time to move forward and explore all the possibilities that these new advances have opened in the field.

FUTURE POSSIBILITIES

It is impossible to talk about the future of regenerative medicine and cardiac regeneration without mentioning the truly pluripotent cells. Embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) are unquestionably able to generate any cell type in our body and therefore have an insurmountable potential for regeneration. Obvious problems to be overcome are immune rejection (in the case of ESC) and the carcinogenic potential of both cell types. Pre-differentiation of patient specific iPSCs into the desired cell type for transplantation can potentially avoid both immune rejection and carcinogenesis, but differentiation protocols into a

specific cell type are still of very low efficiency. Derivation of iPSCs without true teratoma formation capacity, viewed as a problem for the field^[46], can in fact provide an important advantage for these cell lines in regenerative medicine.

Fast progress seen in the development of defined culture media, free of animal antigens, and stringent purification and expansion of pre-differentiated cells, anticipate the use of pluripotent derived cells in clinical trials in the future.

Use of the cardiac stem/progenitor cells also has great potential for future clinical use. In fact, clinical trials using CDCs and c-kit positive cells are currently underway in California and Louisville, KY, respectively. Advantages include the use of a multipotent cell type, thus unlikely to promote carcinogenesis, and use of autologous cells, since they are derived from biopsies obtained from the heart muscle of the patient. Potential disadvantages are the diminished numbers and regenerative potential of stem cells derived from a diseased organ. Heesch *et al.*^[47] have shown a decrease in colony forming and migration capacity of bone marrow cells obtained from patients with ischemic heart disease.

Introduction or re-introduction of exogenously cultured cells (either genetically or non-genetically manipulated) is the immediate future for cardiac regeneration strategies, but long term goals include use of factors that are capable of enhancing endogenous regeneration and genetic interventions using viral delivery systems. Knowledge gained from pre-clinical and clinical trials using the exogenous cells will allow insights into the relevant factors needed to boost the regenerative capacity of our own stem cells. Another approach would be the genetic manipulation of the cardiomyocyte cell cycle, inducing genes responsible for proliferation after the regulatory mechanisms involved in this event are elucidated^[48].

Finally, a word of caution is offered. Although we may discover the best cell type, administration route and time-window for the regeneration of the diseased heart, some questions remain open. The improvement in cardiac function, our ultimate goal, will depend on the long-term engraftment of the injected cells. In that regard, the results reported by Wu's group have been truly "disheartening"^[49,50]. Using bone marrow derived, cardiac derived or pluripotent derived cells in animal models of ischemic heart diseases, Wu and coworkers have been unable to detect cell survival for more than a few weeks in the heart. This may be the last and most difficult obstacle to conquer: a way to induce permanent engraftment of the injected cells.

REFERENCES

- 1 **Anversa P**, Leri A, Rota M, Hosoda T, Bearzi C, Urbanek K, Kajstura J, Bolli R. Concise review: stem cells, myocardial regeneration, and methodological artifacts. *Stem Cells* 2007; **25**: 589-601
- 2 **Karsner HT**, Saphir O, Todd TW. The State of the Cardiac Muscle in Hypertrophy and Atrophy. *Am J Pathol* 1925; **1**: 351-372.1
- 3 **Tam SK**, Gu W, Mahdavi V, Nadal-Ginard B. Cardiac myocyte terminal differentiation. Potential for cardiac regeneration. *Ann N Y Acad Sci* 1995; **752**: 72-79
- 4 **Macmahon HE**. Hyperplasia and Regeneration of the Myocardium in Infants and in Children. *Am J Pathol* 1937; **13**: 845-854.5
- 5 **Linzbach AJ**. Heart failure from the point of view of quantitative anatomy. *Am J Cardiol* 1960; **5**: 370-382
- 6 **Quaini F**, Cigola E, Lagrasta C, Saccani G, Quaini E, Rossi C, Olivetti G, Anversa P. 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* 1994; **75**: 1050-1063b
- 7 **Robledo M**. Myocardial regeneration in young rats. *Am J Pathol* 1956; **32**: 1215-1239
- 8 **Sasaki R**, Morishita T, Yamagata S. Mitosis of heart muscle cells in normal rats. *Tohoku J Exp Med* 1968; **96**: 405-411
- 9 **Zak R**. Development and proliferative capacity of cardiac muscle cells. *Circ Res* 1974; **35**: suppl II:17-suppl II:26
- 10 **Astorri E**, Bolognesi R, Colla B, Chizzola A, Visioli O. Left ventricular hypertrophy: a cytometric study on 42 human hearts. *J Mol Cell Cardiol* 1977; **9**: 763-775
- 11 **Kajstura J**, Zhang X, Reiss K, Szoke E, Li P, Lagrasta C, Cheng W, Darzynkiewicz Z, Olivetti G, Anversa P. Myocyte cellular hyperplasia and myocyte cellular hypertrophy contribute to chronic ventricular remodeling in coronary artery narrowing-induced cardiomyopathy in rats. *Circ Res* 1994; **74**: 383-400
- 12 **Kajstura J**, Leri A, Finato N, Di Loreto C, Beltrami CA, Anversa P. Myocyte proliferation in end-stage cardiac failure in humans. *Proc Natl Acad Sci USA* 1998; **95**: 8801-8805
- 13 **Beltrami AP**, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med* 2001; **344**: 1750-1757
- 14 **Soonpaa MH**, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Circ Res* 1998; **83**: 15-26
- 15 **Von Harsdorf R**. Can cardiomyocytes divide? *Heart* 2001; **86**: 481-482
- 16 **Anversa P**, Leri A, Kajstura J, Nadal-Ginard B. Myocyte growth and cardiac repair. *J Mol Cell Cardiol* 2002; **34**: 91-105
- 17 **Lorenz E**, Uphoff D, Reid TR, Shelton E. Modification of irradiation injury in mice and guinea pigs by bone marrow injections. *J Natl Cancer Inst* 1951; **12**: 197-201
- 18 **Domen J**, Wagers A, Weissman IL. Bone marrow (hematopoietic) stem cells. In: Regenerative medicine - NIH stem cell report, 2006. Available from: URL: http://stemcells.nih.gov/staticresources/info/scireport/PDFs/Regenerative_Medicine_2006.pdf
- 19 **Becker AJ**, McCulloch EA, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* 1963; **197**: 452-454
- 20 **Till JE**, McCulloch EA, Siminovitch L. A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. *Proc Natl Acad Sci USA* 1964; **51**: 29-36
- 21 **Ferrari G**, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998; **279**: 1528-1530
- 22 **Brazelton TR**, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* 2000; **290**: 1775-1779
- 23 **Mezey E**, Chandross KJ, Harta G, Maki RA, McKecher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science* 2000; **290**: 1779-1782
- 24 **Petersen BE**, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP. Bone marrow as a potential source of hepatic oval cells. *Science*

- 1999; **284**: 1168-1170
- 25 **Lagasse E**, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med* 2000; **6**: 1229-1234
- 26 **Orlic D**, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001; **410**: 701-705
- 27 **Jackson KA**, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 2001; **107**: 1395-1402
- 28 No consensus on stem cells. *Nature* 2004; **428**: 587
- 29 **Chien KR**. Stem cells: lost in translation. *Nature* 2004; **428**: 607-608
- 30 **Murry CE**, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JJ, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 2004; **428**: 664-668
- 31 **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**: 668-673
- 32 **Hierlihy AM**, Seale P, Lobe CG, Rudnicki MA, Megey LA. The post-natal heart contains a myocardial stem cell population. *FEBS Lett* 2002; **530**: 239-243
- 33 **Beltrami AP**, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 2003; **114**: 763-776
- 34 **Oh H**, Bradfute SB, Gallardo TD, Nakamura T, Gaussen V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci USA* 2003; **100**: 12313-12318
- 35 **Matsuura K**, Nagai T, Nishigaki N, Oyama T, Nishi J, Wada H, Sano M, Toko H, Akazawa H, Sato T, Nakaya H, Kasanuki H, Komuro I. Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *J Biol Chem* 2004; **279**: 11384-11391
- 36 **Martin CM**, Meeson AP, Robertson SM, Hawke TJ, Richardson JA, Bates S, Goetsch SC, Gallardo TD, Garry DJ. Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. *Dev Biol* 2004; **265**: 262-275
- 37 **Messina E**, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso F, Salio M, Battaglia M, Latronico MV, Coletta M, Vivarelli E, Frati L, Cossu G, Giacomello A. Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res* 2004; **95**: 911-921
- 38 **Laugwitz KL**, Moretti A, Lam J, Gruber P, Chen Y, Woodward S, Lin LZ, Cai CL, Lu MM, Reth M, Platoshyn O, Yuan JX, Evans S, Chien KR. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature* 2005; **433**: 647-653
- 39 **Barile L**, Messina E, Giacomello A, Marbán E. Endogenous cardiac stem cells. *Prog Cardiovasc Dis* 2007; **50**: 31-48
- 40 **Bearzi C**, Rota M, Hosoda T, Tillmanns J, Nascimbene A, De Angelis A, Yasuzawa-Amano S, Trofimova I, Siggins RW, Lecapitaine N, Cascapera S, Beltrami AP, D'Alessandro DA, Zias E, Quaini F, Urbanek K, Michler RE, Bolli R, Kajstura J, Leri A, Anversa P. Human cardiac stem cells. *Proc Natl Acad Sci USA* 2007; **104**: 14068-14073
- 41 **Smith RR**, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, Giacomello A, Abraham MR, Marbán E. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation* 2007; **115**: 896-908
- 42 **Bu L**, Jiang X, Martin-Puig S, Caron L, Zhu S, Shao Y, Roberts DJ, Huang PL, Domian IJ, Chien KR. Human ISL1 heart progenitors generate diverse multipotent cardiovascular cell lineages. *Nature* 2009; **460**: 113-117
- 43 **Laflamme MA**, Murry CE. Regenerating the heart. *Nat Biotechnol* 2005; **23**: 845-856
- 44 **Hsieh PC**, Segers VF, Davis ME, MacGillivray C, Gannon J, Molkentin JD, Robbins J, Lee RT. Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. *Nat Med* 2007; **13**: 970-974
- 45 **Bergmann O**, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, Jovinge S, Frisén J. Evidence for cardiomyocyte renewal in humans. *Science* 2009; **324**: 98-102
- 46 **Chan EM**, Ratanasirintrao S, Park IH, Manos PD, Loh YH, Huo H, Miller JD, Hartung O, Rho J, Ince TA, Daley GQ, Schlaeger TM. Live cell imaging distinguishes bona fide human iPSC cells from partially reprogrammed cells. *Nat Biotechnol* 2009; **27**: 1033-1037
- 47 **Heeschen C**, Lehmann R, Honold J, Assmus B, Aicher A, Walter DH, Martin H, Zeiher AM, Dimmeler S. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. *Circulation* 2004; **109**: 1615-1622
- 48 **Pasumarthi KB**, Field LJ. Cardiomyocyte cell cycle regulation. *Circ Res* 2002; **90**: 1044-1054
- 49 **Li Z**, Lee A, Huang M, Chun H, Chung J, Chu P, Hoyt G, Yang P, Rosenberg J, Robbins RC, Wu JC. Imaging survival and function of transplanted cardiac resident stem cells. *J Am Coll Cardiol* 2009; **53**: 1229-1240
- 50 **Swijnenburg RJ**, Govaert JA, van der Bogt KE, Pearl JL, Huang M, Stein W, Hoyt G, Vogel H, Contag CH, Robbins RC, Wu JC. Timing of bone marrow cell delivery has minimal effects on cell viability and cardiac recovery after myocardial infarction. *Circ Cardiovasc Imaging* 2010; **3**: 77-85

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