Heart failure and regenerative cardiology

**Annarosa Leri¹,
Toru Hosoda¹,
Jan Kajstura¹
& Piero Anversa^{2†}**

[†]Author for correspondence

¹Cardiovascular Research
Institute, Department of
Medicine, New York Medical
College, Valhalla, New York,
NY 10595, USA

²Cardiovascular Research
Institute, Department of
Medicine, Vossburgh Pavilion,
Room 302, New York
Medical College, Valhalla,
New York, NY 10595, USA
Tel.: +1 914 594 4168;
Fax: +1 914 594 4406;
E-mail: piero_anversa@
nymc.edu

'The bizarre behavior of hematopoietic stem cells has surprised and distressed many of us; they disobey the dogma of embryonic specification and with elegant and plastic arabesques undergo unexpected metamorphoses.'

A scientific revolution

The 17th century was the century of Scientific Revolution. The investigation of natural phenomena acquired a methodology of research that was based on direct observation and experimentation and not on ancient beliefs supported by followers of Classicism [1]. We owe to Galileo the implementation of the scientific experimental method that is currently applied in the daily life of our laboratories. The desire to resolve scientific problems based on factual findings has led to the creation of new instruments – telescope, microscope, barometer – that greatly facilitated the progression of research [2]. As with the sky of Galileo, every aspect of the human life was subjected to the examination of the eye. And painters, scientists and poets investigated areas of reality that were not considered before. However, these new lines of evidence were perceived with mistrust and suspicion. The prevailing reaction was astonishment and disbelief for this novel approach that refused to hand down to posterity observations, which were not validated by the scientific method. This was the climate in which Galileo risked being sentenced to the stake [3]. Providentially, the stake is no longer permitted by law, but scientists who observe old truths proposing new perspectives and interpretations can feel the heat of the flames. At the boundary of the second and third millennia, the discovery that hematopoietic stem cells (HSCs) can acquire cell lineages different from the organ of origin has started a new intriguing scientific revolution [4–8]. The bizarre behavior of HSCs has surprised and distressed many of us; they disobey the dogma of embryonic specification and with elegant and plastic arabesques undergo unexpected metamorphoses [9]. To recompose a safe, comfortable and orderly view, studies reporting negative findings and

affirming the impossibility to reproduce published positive results have suddenly become very popular and have found easy hospitality in high-impact journals [10–15]. This confusion is feeding a fire that may disrupt the stream of scientific consciousness and delay the clinical application of a potentially revolutionary therapeutic tool.

Regenerative cardiology

The epidemic proportions of heart failure together with limitations in its treatment constitute the bases for the recent interest and enthusiasm in regenerative cardiology. Efforts in this field aim at the reconstitution of the myocardium lost upon pathological insults, and involve the utilization of cells, gene products and tissue engineering [16–18]. Soon after the first experimental evidence that bone marrow-derived cells (BMCs) induce cardiac repair in the postinfarcted heart [19], endothelial progenitor cells, mononuclear bone marrow cells and CD34⁺ cells have been administered to patients affected by acute myocardial infarction or chronic ischemic heart failure [20–25]. These interventions had positive outcomes documenting not only the feasibility and safety of this therapeutic approach but also beneficial effects on cardiac function. While patients are currently enrolled in large randomized clinical trials, the documentation of cardiac-specific adult progenitor cells (CPCs) has created great expectation concerning the utilization of this new cell for the management of the human disease [26–34]. Theoretically, the most logical and potentially powerful cell to be employed for cardiac repair is the CPC. Cardiac regeneration would be accomplished by enhancing the normal turnover of myocardial cells [35]. However, difficulties exist in the acquisition of myocardial samples in humans, and the isolation and expansion of CPCs in quantities that can be employed therapeutically. Conversely, BMCs can be easily collected from bone marrow aspirates or the peripheral blood upon their mobilization from the bone marrow with cytokines. Other cell types have been implemented experimentally and attempts with allogeneic cells, including embryonic stem cells, have been made with contradictory results [16,36–38].

The problem at hand is whether these various forms of cell therapy of the damaged heart lead to a passive graft with reduction in myocardial stiffness of the injured ventricular wall or active graft, in which the administered cells home, grow and differentiate, restoring the lost myocardium with newly formed functionally competent tissue. The latter is the ultimate goal, although the possibility of obtaining actual regeneration of myocytes and coronary vessels has been strongly promoted by some and vigorously opposed by others. Thus, the search for the ideal cell continues.

The heart & BMCs

The recognition that BMCs can repair the damaged heart has profoundly changed the future of cardiovascular research and clinical cardiology. BMCs injected in the border zone of a myocardial infarct or mobilized systemically into the circulation with cytokines leads to regeneration of functionally competent myocardium through a process defined as developmental plasticity [39]. The wave of enthusiasm created by these observations and the lack of effective new drugs for the treatment of heart failure, has prompted cardiologists to rapidly implement BMCs in the management of the severely decompensated heart in humans [20,21]. Because of the compelling need to treat severely ill patients, clinicians are leading the field of cellular therapy and cardiac regeneration. Conversely, statements of caution are advanced by the scientific community concerning the necessity to acquire a better understanding of the mechanisms of cardiac repair before these protocols are introduced in a clinical setting.

If regeneration of myocytes and coronary vessels can be promoted by local injection of cells of bone marrow origin, the heart microenvironment represents a permissive milieu that favors BMC transdifferentiation and conditions the growth and maturation of the administered cells. Because of this unexpected characteristic, challenging biological issues have been advanced and the beneficial consequences of BMC treatment have been ascribed to *de novo* formation of myocardium [19,40], paracrine effects on the growth reserve of the heart [41,42] or a combination of both [8]. However, two established models of cellular and cardiac biology were shaken by these astonishing results: the general belief that during embryonic and fetal development cells undergo a hierarchical progressive restriction of developmental options, which was thought to be inviolable in

adulthood; and the perennial view of the heart as an organ incapable of extrinsic or intrinsic regeneration. In this regard, several examples of transition from one cell type to another or from one cell lineage to a different lineage have been reported supporting the notion of stem cell plasticity [9]. Similarly, myocyte replication, karyokinesis and cytokinesis together with foci of spontaneous myocardial regeneration have been documented in the overloaded and infarcted human heart [43,44].

When the twist in fate occurs by transdifferentiation, nuclear reprogramming is required. With transdifferentiation, the reorganization of chromatin is mediated by an alternate turning on of transcription factors to drive the adult stem cell towards the creation of a specific progeny [45]. This process could be slow and limited in efficiency and time is a critical factor in acute heart failure of ischemic origin. The recent documentation that resident progenitor cells exist in the adult myocardium [34] overcomes the need for the complex and time-consuming process of nuclear reprogramming, required by non-resident primitive cells. This is because undifferentiated cells nested in the cardiac microenvironment are predetermined to evolve into cardiac cell lineages, i.e., myocytes and vascular smooth muscle and endothelial cells.

The re-introduction of the notion of cellular fusion, extremely popular in the 1980s, has created uncertainty about stem cell plasticity. After the publication of two *in vitro* reports describing the extremely rare occurrence of fusion in co-culture systems of embryonic stem cells with bone marrow cells or neural progenitors [46,47], the possibility that a phenotype conversion of an adult stem cell could mask cellular fusion *in vivo* has been promoted. Cellular and/or nuclear fusion requires the merging of two distinct cells with formation of a hybrid cell. Although cell fusion referred originally to the joining of two fully differentiated cells, this process is now thought to consist of the merging of an adult stem cell and a differentiated cell resulting in the formation of a binucleated heterokaryon or a mononucleated hyperploid synkaryon [48,49]. In this case, the growth of the binucleated heterokaryon seems to depend on the nucleus of the more undifferentiated cell that dominates the nucleus of the somatic cell by transferring its replication properties while the destiny of the heterokaryon is regulated by the differentiated cell [39,50]. When cellular fusion is accompanied by nuclear fusion, a mononucleated synkaryon

with a hyperploid DNA content is formed. In this condition, the bulky burden of the high nuclear DNA content leads to genetic instability and reduced or null replicative potential [46]. If cell fusion is involved, the replicative potential of the fused cell is at best transient. In organs, such as the brain, cell fusion leads only to a temporary rescue of the old pre-existing terminally differentiated parenchymal cell without additional growth [51].

In the absence of rigorous studies that unequivocally demonstrate the occurrence of cell fusion *in vivo*, cell fusion remains an *in vitro* phenomenon. Cell fusion in organs, such as the skin, lung, brain and the heart, is restricted at most to a few cells, which have little consequence on baseline function or tissue repair in pathologic states [52]. Studies of cell fusion in the liver and skeletal muscle are problematic since cell fusion is an inherent aspect of the growth pathway of hepatocytes and myofibers. In hepatocytes, mitochondria damaged by cumulative oxidative stress can be replaced by intact organelles from BMCs [53]. The possibility that transfer of mitochondria occurs between cells through the formation of nanotubules has been raised but this process was documented only *in vitro* [54]. Similarly, old genetic material could be substituted by the DNA of the progenitor cells [55]. Under normal physiological turnover, fusion of bone marrow progenitor cells (BMPCs) with parenchymal cells is an extremely rare event [16,35,46,52].

The analysis of cell fusion is properly evaluated by the measurement of the number of sex chromosomes in the nuclei of the created cells [16,35]. When this methodology is employed, fusion events in the myocardium are essentially absent [7,40]. This is not the case when the Cre–Lox system is employed [13]. The detection of sex chromosomes is superior to the utilization of the Cre–Lox strategy since this model has severe limitations [46,47]. The Cre–Lox genetic system cannot avoid metabolic cooperation by the potential formation of junctional complexes [56] or creation of nanotubules [57] between the progenitor cells carrying the Cre–recombinase and the recipient cardiac cells. The recipient cells cannot express the β -Gal or enhanced green fluorescent protein (EGFP) since the corresponding gene is repressed by a stop codon flanked by Lox-P sequences [58]. The Lox-P sequences are recognized by the Cre–recombinase that removes this inhibition allowing the appearance of β -Gal or EGFP.

Because of the small molecular weight of the Cre–recombinase, this enzyme can translocate via gap junction channels or nanotubules to the resident cells, mimicking cell fusion [16,46,47].

Multiple lines of evidence support the notion that transdifferentiation is the fundamental mechanism of BMC-mediated cardiac repair (Figure 1A–C) while cell fusion is not the cause of the new cardiac phenotype. Experimentally, the

Figure 1. BMPCs and myocardial regeneration.

The injection of EGFP-tagged male c-kit⁺ BMPCs in the proximity of infarcted myocardium results in the formation of myocytes (A); α -sarcomeric actin (red) within the infarcted myocardium; these cells express EGFP (B; green) and carry the Y-chromosome (C; white dots in nuclei). BMPC: Bone marrow progenitor cell; EGFP: Enhanced green fluorescent protein.

cardiac cells formed in female infarcted hearts have the male phenotype of the injected BMPCs [19,40]. Following permanent coronary occlusion, all cells in the supplied myocardium die in less than 5 h. Essentially, there are no partner cells left for fusion. Adult myocytes have a volume of $\sim 20,000 \mu\text{m}^3$ and if cell fusion would occur, new myocytes should have a volume of at least $20,000 \mu\text{m}^3$. By contrast, the generated myocytes have a maximum size of $\sim 2000 \mu\text{m}^3$ and a minimum size of $\sim 100 \mu\text{m}^3$. Donor-derived cells divide rapidly and extensively while tetraploid cells divide slowly and might not divide at all if one of the partners is a terminally differentiated myocyte [16]. Fusion of a BMPC to a myocyte that has reached irreversible growth arrest cannot stimulate its re-entry into the cell cycle. Cell fusion should generate binucleated myocytes, with one tetraploid and one diploid nucleus, or myocytes with three diploid nuclei. This has never been the case.

The heart & CPCs

According to the old view, the heart survives and exerts its function until death of the organism with the same or lesser number of cells that are present at birth [59]. This notion was challenged by findings documenting activation of the cell cycle machinery and nuclear and cellular division in a subset of myocytes [35,39]. However, the source of cycling myocytes was unclear until CPCs were recognized in small and large mammals, including humans [26–34]. CPCs correspond to undifferentiated lineage negative (Lin⁻) cells that express the stem cell surface antigens c-kit, MDR1 and Sca-1 in variable combinations. Isolated Lin⁻-c-kit⁺ cells from the rat and dog heart [27,60] possess the three properties of stemness: clonogenicity, self-renewal and multipotentiality. An *in vivo* functional assay consisting of the transplantation of clonogenic Lin⁻-c-kit⁺ cells in the infarcted ventricle or their local mobilization by growth factors has confirmed the *in vitro* features of this cell population. Migration, engraftment, proliferation and multi-lineage differentiation of CPCs result in the replacement of dead tissue (Figure 2A–B) with new contracting myocardium [27,60,61]. There is a high degree of co-expression of c-kit, MDR1 and Sca-1 on the same CPCs. Also primitive cells sorted for each of these antigens in mice, rats and dogs show a comparable growth and differentiation behavior.

Stem cells are stored in niches, which are located deep in the tissue for protection from damaging stimuli [62]. The niche constitutes the

microenvironment in which primitive cells divide, differentiate and die. The recognition of stem cells within their natural milieu is of crucial importance; stem cells do not exist in the absence of supporting cells within the niche. The identification of a stem cell niche is a difficult challenge in mammals. This search is more readily accomplished in simpler organisms, such as the *Drosophila* [63]. CPC clusters are present in the adult heart [27,61]. Although CPC pockets are scattered throughout the myocardium, their distribution appears to be conditioned by the distinct levels of wall stress. In fact, the frequency of CPC clusters is inversely related to the hemodynamic load sustained by the anatomical regions of the heart: they accumulate in the atria and apex and are less numerous at the base and mid-portion of the left ventricle [61]. Lin cells expressing a stem cell marker, c-kit, MDR1 or Sca-1, separately or in combination, have been detected clustered together with cardiac progenitors and precursors. In close proximity to Lin cells, myocytes or other cells in an early stage of differentiation are commonly visible.

The notion that the myocardium is not a static organ and cell renewal is not restricted to EC, smooth muscle cells and fibroblasts, but includes parenchymal cells as well, offers a novel view of the biology of the heart and the mechanisms responsible for cardiac aging. Myocytes are generated by activation and commitment of CPCs [35] and the continuous turnover of these cells throughout the lifespan of the organ and organism results in a heterogeneous parenchymal cell population within the heart consisting of young, adult, old and senescent myocytes [43,64,65]. Data in humans suggest that this subdivision corresponds to cells of different sizes raising the possibility that the lifespan of a cell is associated with a progressive increase in the volume of the cell [35]. With time, myocytes age and cellular aging is paralleled by an increase in myocyte volume together with the expression of inhibitors of the cell cycle and markers of cellular senescence [43,64,65]. An increase in old and senescent myocytes has detrimental effects on cardiac performance and may cause the development of an aging myopathy. This negative impact of aging on myocytes and organ function may involve defects at the level of the regulating cell, such as the CPC [64]. Alterations in the stem cell compartment can lead to inadequate generation of committed progeny and inefficient replacement of senescent mechanically depressed myocytes and severe ventricular dysfunction.

Figure 2. CPCs and myocardial regeneration.

The injection of EGFP-tagged CPCs leads to the restoration of infarcted myocardium. The formed myocytes (**B**; α-sarcomeric actin, red) express EGFP (**B**; green) and on their surface connexin 43 (**A, B**; white dots) and N-cadherin (**A, B**; magenta dots).

CPC: Cardiac progenitor cell; EGFP: Enhanced green fluorescent protein.

CPCs express c-Met and insulin-like growth factor (IGF)-1 receptors (IGF-1R). Hepatocyte growth factor (HGF) activates c-Met and through this mechanism promotes cell migration, growth and survival. IGF-1 has no influence on locomotion *in vitro* and *in vivo* but enhances cell proliferation, differentiation, and viability [60,61]. The HGF–cMet and IGF-1–IGF-1R systems oppose apoptotic and necrotic death signals generated by the formation of reactive oxygen species (ROS). ROS, in

turn, can induce DNA damage and, ultimately, cell death [66]. The stem cell antigens and these growth factor receptor systems may operate together, potentiating the commitment of CSCs to distinct cell lineages and their fate.

Although nine independent laboratories have identified CPCs in the adult heart [26–34], the controversy concerning myocyte regeneration has not yet been resolved [37,59,67]. Similarly, cells with the property of stemness have not been characterized consistently. In this regard, a comment may be made concerning a myocyte progenitor documented during cardiac development [68]. The Isl1 transcription factor is associated with the commitment to the myocyte lineage of cardiac cells that have lost their undifferentiated, primitive, stemness state. Isl1, together with GATA-4, is a transcriptional activator of the myocyte transcription factor MEF2C [69]. Nearly 6 years ago, Isl1-positive cells were shown to condition heart morphogenesis in the mouse embryo [70]. The homozygous deletion of Isl1 alters the development of the mouse heart involving the atria, right ventricle and outflow tract [71].

Surprisingly, Isl1-positive cells have been alleged to correspond to the ‘true’ cardiac stem cell with important implications in myocardial regeneration of the failing human heart [68]. Isl1-positive cells are neither self-renewing nor clonogenic or multipotent, lacking the indispensable elements of stem cells. Isl1-positive cells are undetectable in the atrial and ventricular myocardium of the adult mouse and rat heart or in the decompensated human heart. Even during development, Isl1 cells are not implicated in the formation of the left ventricle whose deterioration in the adult is the predominant cause of heart failure and death in the patient population. Whether direct activation, local implantation or systemic mobilization of Isl1-positive cells can repair the infarcted heart remains to be shown. The lack of this information questions the therapeutic import, if any, of this immature myocyte.

Cardiac repair is mediated by several factors including: number of cells to be administered; cell death and survival in the hostile milieu of the infarct and peri-infarcted region; and cell growth and differentiation. An additional critical variable of CPCs is their level of plasticity, which is dictated by their ability to acquire the myocyte, and vascular smooth muscle cell and EC lineages. CPCs have to promote the formation of a proportional number of parenchymal cells and coronary vessels. Moreover, the

injected CPCs can contribute indirectly to cardiac regeneration by releasing a variety of peptides that exert a paracrine action on the myocardium and its resident CPCs. These mechanisms are not mutually exclusive and CPCs may participate directly and indirectly in the repair process. In all cases, progenitor cells

have to engage themselves in homing and engraftment in the myocardium to perform specific functions, ultimately rebuilding the damaged heart.

Acknowledgement

This work was supported by NIH grants.

Bibliography

1. Spielvogel JJ: Revolution and Enlightenment. In: *Glencoe World History*. Glencoe/McGraw Hill, NY, USA, 1550–1800 (2003).
2. Galilei G: *Sidereus Nuntius. Opere di Galileo Galilei*. Riccardo Ricciardi, Florence, Italy (1953).
3. Sobel D: Galileo's Daughter: A Historical Memory of *Science In: Faith and Love*. Walker & Co, NY, USA (1999).
4. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo Cossu G, Mavilio F: Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 279(5356), 528–530 (1998).
5. Brazelton TR, Rossi FM, Keshet GI, Blau HM: From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* 290(5497), 1775–1779 (2000).
6. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR: Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science* 290(5497), 1779–1782 (2000).
7. Eisenberg LM, Burns L, and Eisenberg CA: Hematopoietic cells from bone marrow have the potential to differentiate into cardiomyocytes *in vitro*. *Anat. Rec. A Discov. Mol. Cell Evol. Biol.* 274(1), 870–882 (2003).
8. Yoon YS, Wecker A, Heyd L *et al.*: Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction. *J. Clin. Invest.* 115(2), 326–338 (2005).
9. Tosh D and Slack JM: How cells change their phenotype. *Nat. Rev. Mol. Cell Biol.* 3(3), 187–194 (2002).
10. Wagers AJ, Sherwood RI, Christensen JL, Weissman IL: Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 297(5590), 2256–2259 (2002).
11. Murry CE, Soonpaa MH, Reinecke H *et al.*: Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 428(6983), 664–668 (2004).
12. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC: Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature* 428(6983), 668–673 (2004).
13. Nygren JM, Jovinge S, Breitbach M *et al.*: Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat. Med.* 10(5), 494–501 (2004).
14. Jackson KA, Snyder DS, Goodell MA: Skeletal muscle fiber-specific green autofluorescence: potential for stem cell engraftment artifacts. *Stem Cells* 22(2), 180–187 (2004).
15. Burns TC, Ortiz-Gonzalez XR, Gutierrez-Perez M *et al.*: Thymidine analogs are transferred from pre-labeled donor to host cells in the central nervous system after transplantation: a word of caution. *Stem Cells* (2006) (Epub ahead of print).
16. Leri A, Kajstura J, Anversa P: Cardiac stem cells and mechanisms of myocardial regeneration. *Physiological Rev.* 85, 1373–1416 (2005).
17. Urbich C, Dimmeler S: Endothelial progenitor cells: characterization and role in vascular biology. *Circ. Res.* 95(4), 343–353 (2004).
18. Davis ME, Hsieh PC, Grodzinsky AJ, Lee RT: Custom design of the cardiac microenvironment with biomaterials. *Circ. Res.* 97(1), 8–15 (2005).
19. Orlic D, Kajstura J, Chimenti S *et al.*: Bone marrow cells regenerate infarcted myocardium. *Nature* 410(6829), 701–705 (2001).
20. Strauer BE, Brehm M, Zeus T *et al.*: Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 106(15), 1913–1918 (2002).
21. Assmus B, Schachinger V, Teupe C *et al.*: Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 106(24), 3009–3017 (2002).
22. Perin EC, Dohmann HF, Borojevic R *et al.*: Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 107(18), 2294–2302 (2003).
23. Wollert KC, Meyer GP, Lotz J *et al.*: Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomized controlled clinical trial. *Lancet* 364(3429), 141–148 (2004).
24. Schachinger V, Assmus B, Britten MB *et al.*: Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J. Am. Coll. Cardiol.* 44(8), 1690–1699 (2004).
25. Strauer BE, Brehm M, Zeus T *et al.*: Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease. (IACST-Study). *J. Am. Coll. Cardiol.* 46(9), 1651–1658 (2005).
26. Hierlihy AM, Seale P, Lobe CG, Rudnicki MA, Megeney LA: The post-natal heart contains a myocardial stem cell population. *FEBS Lett.* 530(1–3), 239–243 (2002).
27. Beltrami AP, Barlucchi L, Torella D *et al.*: Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114(6), 763–776 (2003).
28. Oh H, Bradfute SB, Gallardo TD *et al.*: Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc. Natl Acad. Sci. USA* 100(9), 12313–12318 (2003).
29. Matsuura K, Nagai T, Nishigaki N *et al.*: Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *J. Biol. Chem.* 279(12), 11384–11391 (2004).
30. Martin CM, Meeson AP, Robertson SM *et al.*: Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. *Dev. Biol.* 265(1), 262–275 (2004).
31. Messina E, De Angelis L, Frati G *et al.*: Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ. Res.* 95(9), 911–921 (2004).
32. Pfister O, Mouquet F, Jain M *et al.*: CD31- but not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. *Circ. Res.* 97(1), 52–61 (2005).

33. Rosenblatt-Velin N, Lepore MG, Cartoni C, Beermann F, Pedrazzini T: FGF-2 controls the differentiation of resident cardiac precursors into functional cardiomyocytes. *J. Clin. Invest.* 115(7), 1724–1733 (2005).
34. Tomita Y, Matsumura K, Wakamatsu Y *et al.*: Cardiac neural crest cells contribute to the dormant multipotent stem cell in the mammalian heart. *J. Cell Biol.* 170(7), 1135–1146 (2005).
35. Aversa P: Life and death of cardiac stem cells: a paradigm shift in cardiac biology. *Circulation* (2006).
36. Kofidis T, deBruin JL, Yamane T *et al.*: IGF-1 promotes engraftment, differentiation and functional improvement after transfer of embryonic stem cells for myocardial restoration. *Stem Cells* 22(7), 1239–1245 (2004).
37. Laflamme MA, Murry CE: Regenerating the heart. *Nat. Biotechnol.* 23(7), 845–856 (2005).
38. Kofidis T, deBruin JL, Tanaka M *et al.*: They are not stealthy in the heart: embryonic stem cells trigger cell infiltration, humoral and T-lymphocyte-based host immune response. *Eur. J. Cardiothorac Surg.* 28(3), 461–466 (2005).
39. Anversa P, Leri A, Kajstura J: Cardiac regeneration. *J. Am. Coll. Cardiol.* 1305–1378 (2006).
40. Kajstura J, Rota M, Whang B *et al.*: Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell fusion. *Circ. Res.* 96(1), 127–137 (2005).
41. Mangi AA, Noiseux N, Kong D *et al.*: Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat. Med.* 9(9), 1195–1201 (2003).
42. Gneocchi M, He H, Liang OD *et al.*: Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat. Med.* 11(4), 367–368 (2005).
43. Urbanek K, Quaini F, Tasci G *et al.*: Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. *Proc. Natl Acad. Sci. USA* 100(18), 10440–10445 (2003).
44. Urbanek K, Torella D, Sheikh F *et al.*: Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. *Proc. Natl Acad. Sci. USA* 102(24), 8692–8697 (2005).
45. Pomerantz J, Blau HM: Nuclear reprogramming: a key to stem cell function in regenerative medicine. *Nat. Cell Biol.* 6(9), 810–816 (2004).
46. Terada N, Hamazaki T, Oka M *et al.*: Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* 416(6880), 542–545 (2002).
47. Ying QL, Nichols J, Evans EP, Smith AG: Changing potency by spontaneous fusion. *Nature* 416(6880), 545–548, (2002).
48. Leri A, Kajstura J, Anversa P: Identity deception: not a crime for a stem cell. *Physiology* 20, 162–168 (2005).
49. Kajstura J, Leri A, Bolli R, Anversa P: Endothelial progenitor cells: neovascularization or more? *J. Mol. Cell Cardiol.* 40(1), 1–8 (2006).
50. Leri A, Kajstura J, Nadal-Ginard B, Anversa P: Some like it plastic. *Circ. Res.* 94(2), 132–134 (2004).
51. Weimann JM, Johansson CB, Trejo A, Blau HM: Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. *Nat. Cell Biol.* 5(11), 959–966 (2003).
52. Harris RG, Herzog EL, Bruscia EM, Grove JE, Van Arnam JS, Krause DS: Lack of a fusion requirement for development of bone marrow-derived epithelia. *Science* 305(5680), 90–93 (2004).
53. Theise ND, Nimmakayalu M, Gardner R *et al.*: Liver from bone marrow in humans. *Hepatology* 32(1) 11–16 (2000).
54. Koyanagi M, Brandes RP, Haendeler J, Zeiher AM, Dimmeler S: Cell-to-cell connection of endothelial progenitor cells with cardiac myocytes by nanotubes: a novel mechanism for cell fate changes? *Circ. Res.* 96(10), 1039–1041 (2005).
55. Theise ND, d'Inverno M: Understanding cell lineages as complex adaptive systems. *Blood Cells Mol. Dis.* 32(1), 17–20 (2004).
56. Subak-Sharpe H, Burk RR, Pitts JD: Metabolic co-operation between biochemically marked mammalian cells in tissue culture. *Rev. Med. Virol.* 12(2), 69–78 (1969).
57. Onfelt B, Nedvetzki S, Yanagi K, Davis DM: Cutting edge: membrane nanotubes connect immune cells. *J. Immunol.* 173(3), 1511–1513 (2004).
58. Kostetskii I, Li J, Xiong Y *et al.*: Induced deletion of the N-cadherin gene in the heart leads to dissolution of the intercalated disc structure. *Circ. Res.* 96(3), 346–354 (2005).
59. Chien KR: Stem cells: lost in translation. *Nature* 428(6983), 607–608 (2004).
60. Linke A, Müller P, Nurzynska D *et al.*: Cardiac stem cells in the dog heart regenerate infarcted myocardium improving cardiac performance. *Proc. Natl Acad. Sci. USA* 102(25), 8966–8971 (2005).
61. Dawn B, Stein AB, Urbanek K *et al.*: Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proc. Natl Acad. Sci. USA* 102(10), 3766–3771 (2005).
62. Fuchs E, Tumber T, Guasch G: Socializing with the neighbors: stem cells and their niche. *Cell* 116(6), 769–778 (2004).
63. Li L, Xie T: Stem cell niche: structure and function. *Annu. Rev. Cell Dev. Biol.* 21, 605–631 (2005).
64. Chimenti C, Kajstura J, Torella D *et al.*: Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. *Circ. Res.* 93(7), 604–613 (2003).
65. Torella D, Rota M, Nurzynska D *et al.*: Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. *Circ. Res.* 94(4), 514–524 (2004).
66. Fiers W, Beyaert R, Declercq W, Vandenaabee P: More than one way to die: apoptosis, necrosis and reactive oxygen damage. *Oncogene* 18(54), 7719–7730 (1999).
67. Rubart M, Field LJ: Cardiac regeneration: repopulating the heart. *Annu. Rev. Physiol.* 68, 29–49 (2006).
68. Laugwitz KL, Moretti A, Lam J *et al.*: Postnatal Isl1+cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature* 433(7026), 585–587 (2005).
69. Dodou E, Verzi MP, Anderson JP, Xu SM, Black BL: Mef2c is a direct transcriptional target of ISL1 and GATA factors in the anterior heart field during mouse embryonic development. *Development* 131(16), 3931–3942 (2004).
70. Yuan S, Schoenwolf GC: Islet-1 marks the early heart rudiments and is asymmetrically expressed during early rotation of the foregut in the chick embryo. *Anat. Rec.* 260(2), 204–207 (2000).
71. Cai CL, Laing X, Shi Y *et al.*: Isl1 identifies a cardiac progenitor for population that proliferates prior to differentiation and contributes a majority of the cells to the heart. *Dev. Cell* 5(6), 877–889 (2003).