Cardiac-derived stem cell-based therapy for heart failure: progress and clinical applications

Yaoliang L Tang, Yingjie J Wang, Lijuan J Chen, Yaohua H Pan, Lan Zhang and Neal L Weintraub

Division of Cardiovascular Disease, Department of Internal Medicine, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267, USA

Corresponding authors: Dr Yaoliang Tang and Dr Neal L Weintraub, Division of Cardiovascular Diseases, Department of Internal Medicine, College of Medicine, University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45243, USA. Emails: tangyg@ucmail.uc.edu and weintrnl@ucmail.uc.edu

Abstract

Stem cell-based therapy is emerging as a promising strategy to treat end-stage heart failure, a leading cause of morbidity and mortality. Stem cells can be isolated from a variety of sources and exhibit unique characteristics that impact their potential therapeutic utility. The adult heart contains small populations of committed, multipotent cardiac stem cells (CSC), which are adapted to the cardiac microenvironment and participate in postnatal physiological and pathological cardiac renewal or repair. These cells can be isolated, expanded in culture, and administered therapeutically to improve cardiac function in the setting of heart failure. CSC can be differentiated into three distinct cardiovascular lineages and exhibit enhanced paracrine factor production and engraftment as compared with other types of mesenchymal stem cells, which in turn may translate into improved therapeutic efficacy. The cell surface marker expression and phenotype of these CSC, however, depends on the method of isolation, selection and propagation, which likely explains the variable experimental results obtained to date. Moreover, invasive procedures are required to obtain CSC from humans. Early trials using autologous CSC in patients with ischemic cardiomyopathy have demonstrated feasibility and safety, along with variable degrees of therapeutic efficacy in terms of enhancing myocardial viability and cardiac function. Further studies are needed to optimize methods of CSC isolation, manipulation and delivery. If fully realized, the potential of CSC therapy could fundamentally change the approach to the treatment of end-stage heart failure.

Keywords: cardiac stem cells, cardiosphere, heart regeneration, hypoxic preconditioning, microRNA

Experimental Biology and Medicine 2013; **238**: 294–300. DOI: 10.1177/1535370213477982

Introduction

Congestive heart failure is a leading cause of morbidity and mortality worldwide. Approximately 5.7 million Americans have heart failure, and it is responsible for 55,000 deaths and 34 billion dollars in health-care expenditures annually.[1,2] Despite advances in medical therapy, mechanical support and heart transplantation, nearly half of all patients with heart failure die within five years of the initial diagnosis. Consequently, there is a great deal of interest in devising novel approaches, including stem cell therapy, to treat patients with advanced heart failure. Transplantation of bone marrow-derived cells can marginally improve cardiac function in animals and humans with heart failure,[3–6] but the mechanisms for this functional improvement are unclear. Transdifferentiation into cardiomyocytes appears to be limited, and most of the cells disappear from the myocardium within one week of transplantation.[7] Evidence is emerging that the beneficial effects seen in these studies may be linked to stimulation of endogenous cardiogenic stem cells (CSC).[8] Moreover, CSC can be isolated from the heart, expanded in culture, and used as a source of stem cells to elicit cardiac repair. In this article, we will describe the populations of resident CSC, discuss their potential for heart repair, and summarize the limited clinical experience to date using these cells.

Cardiac stem cells

The adult mammalian heart is not a terminally differentiated organ, but harbors stem cells with replicative and regenerative capacity.[9] Many laboratories have reported the existence of an endogenous population of CSC in the

adult heart.[10] Although efforts to compare and identify a true residing CSC population continue, the characteristics that define a CSC include self-renewal in a clonal fashion and multipotent capacity to differentiate into multiple cardiovascular lineages, including cardiomyocytes, smooth muscle cells and endothelial cells, both *in vitro* and *in vivo*.[11] Different biomarkers have been used to isolate and classify CSC. From the literature, it is difficult to effectively compare and determine which stem cell markers best identify CSC for optimal heart regeneration, as discrepancies exist in the methods of isolation and expansion *ex vivo*. A variety of methodologies have been described to isolate CSC, including fluorescence or magnetic activated cell sorting based on the expression of one or more specific cell surface markers,[12,13] isolation of cardiac-derived cells with aggregational properties (i.e. cardiospheres)[14–16] and more sophisticated approaches, such as genetic knock-in of reporter genes, to isolate Islet-1 (Isl-1) + cardiac progenitors.[17–19] In addition, the antigens that are targeted for the isolation of specific CSC populations also vary between laboratories; expression of various surface markers, such as Sca-1, c-kit, ABCG2, have been used to purify CSC from heart tissues. Moreover, the heterogeneity of CSC is underscored by studies that demonstrate variable growth and transdifferentiation potentials of different CSC populations, even individually expanded CSC clones. As a consequence, novel approaches such as microfluidic technology[20] may be useful to enrich a target cell type from a heterogenous cell population in order to select a CSC that is optimally suited for efficient heart repair.

Source of CSC and their potential for heart repair

Although the origins of CSC are yet unclear, they can be isolated from heart tissue and expanded *ex vivo* for use as a cell-based therapy. The fact that they are already adapted to the cardiac microenvironment is an obvious advantage of CSC in the cell-based treatment of heart failure.[12] It is important to acknowledge, however, that CSC constitutes a spectrum of cell types, with distinct features and functional capabilities, dependent in part upon the methods of isolation (summarized in Table 1). Moreover, most of the functional studies conducted to date utilize rodent models of myocardial infarction in young, healthy animals. These studies may not be directly translatable to aged humans with multisystem diseases. Therefore, the superiority of CSC over other stem cells for cardiac repair remains debatable at this point. Here, we will review the basic research conducted to date involving isolation and characterization of the different populations of CSC.

Cardiac Sca-1 + cells

Sca-1 + cells are endogenous CSC which can be isolated from the adult heart and transdifferentiated into cardiomyocytes, endothelial cells and smooth muscle cells *in vitro*.[15] Cardiac Sca-1 cells administered after ischemia/reperfusion can home to injured myocardium and the infarct border zone and differentiate into cardiomyocytes.[13,24] Wang *et al*.[25] reported that transplantation of Sca-1(+)/CD31(−) cells into the acutely infarcted mouse heart attenuated the functional decline and adverse structural re-modeling after experimental myocardial infarction (MI), as evidenced by an increased left ventricular (LV) ejection fraction, decreased LV end-systolic and end-diastolic dimensions, increased myocardial neovascularization and modest cardiomyocyte regeneration. Furthermore, genetic deletion of Sca-1 causes primary cardiac defects in myocardial repair consistent with impairment of resident cardiac progenitor cell proliferative capacity.[26]

Cardiac c-kit + cells

Cardiac c-kit + cells, initially reported by Beltrami *et al*.[12] are self-renewing, clonogenic, multipotent cells, which give rise to myocytes, smooth muscle and endothelial cells. When cardiac c-kit cells are injected into ischemic heart tissue, these cells or their clonal progeny can reconstitute well-differentiated myocardium, formed by blood-carrying vessels and myocytes with the characteristics of young cells.[12] The origin and significance of these cells has remained obscure. Sandstedt *et al*.[27] showed that c-kit + CD45- cells express markers commonly found on endothelial progenitor cells and represent a population of endothelial progenitor cells in human heart. Hattori *et al*.[28] showed that c-kit + cells represent a population of mesothelial epicardial cells, while Zhou *et al*.[29] reported that cardiac c-kit +/CD45 (dim/moderate) progenitor cells may be mast cells. In addition, cardioprotective c-kit + cells have been suggested to originate from the bone marrow and regulate the myocardial balance of angiogenic cytokines, including vascular endothelial growth factor and the angiopoietins.[30] There is also a dispute about the fate of c-kit + precursors; evidence suggests that neonatal c-kit + precursors support postinfarction myogenesis, while adult c-kit precursors only adopt a vascular fate within infarcted tissue, without differentiating into myocytes.[31] Thus, cardiac-resident c-kit progenitor cells in adult heart appear to represent a heterogenous population of cells with diverse origins, functions and differentiation capacity.

Cardiac side population cells

Side population (SP) cells can be isolated via a DNA-binding dye (Hoechst 33342 or rhodamine123) exclusion and dual wavelength flow cytometric analysis method.[32] SP cells have been isolated from multiple adult tissues, including bone marrow, liver, lung, skeletal muscle and adipose tissue.[33–36] Adult hearts contain a subpopulation of SP cells which exhibit stem cell characteristics, and they have been shown to contribute to the development, maintenance and repair of the heart.[23,37] SP cells express ATP-binding cassette transporter (Abcg2) and can differentiate into α-actinin-positive cardiomyocytes. These stem cells are classified as $Sca1^{high}$, $cKit^{low}$, $CD34^{low}$ and $CD45^{low}$ cells.[23] In comparison with bone marrow SP cells,

Table 1 Published methods for cardiac progenitor cell isolation

Cell types	Isolation technology	Source	Markers of identification	Features	Reference
Cardiac c-kit + cells	Heart digestion + MACS/FACS	Rat heart	Lin-c-kit +	Self-renewing, clonogenic and multipotent	12
Cardiac Sca-1 + progenitor cells	Heart digestion + MACS	Mouse heart	Lin-, Sca-1 +, CD31 +, CD38 +, c-kit-, Flk-1-, CD45 −	Expression of cardiogenic transcription factors; cardiac differentiation after 5-aza induction	21
	Two-step procedure: tissue explanting + MACS	Mouse heart	Sca-1 +, c-kit + and CD45 −	Self-renewing, clonogenic and multipotent	15
	MACS	Human heart (pathological specimens)	Sca-1 +	Clonogenic; cardiomyocyte differentiation induced by TGF-β and BMP6 *in vitro*	22
Side-population cells	Capacity to efflux Hoechst dye/FACS	Mouse heart	Abcg2 +, Sca-1 +, c-kit +, CD34 +, and CD45 −	Capable of differentiation and proliferation	23
Cardiosphere-derived cells	Sphere-forming cells from tissue explanting technology	Human atrial, ventricular biopsy; postnatal mouse hearts	KDR + CD31 +, CD34 +, c-kit +, Sca-1 +	Clonogenesis	14
Isl1 + cells	Heart digestion	Isl1-mCrem transgenic mouse heart	Expression of progenitor-specific transcription factors	Renewal in cardiac mesenchymal feeder layer; cardiomyocyte differentiation	17

TGF-β, tissue growth factor-β

cardiac SP cells exhibit high expression of Afp, Ace,Bmp6, Cldn5, Dkk3, Eln, Fst, Foxc2, Tcf21 (capsulin), Sox17, Sox18, Rgs5, Meox1 and Meox2.[23] Using loss- and gain-of-function approaches, Pfister *et al.*[38] showed that Abcg2 tightly regulates cell fate and function. Genetic ablation of Abcg2 resulted in blunted proliferation capacity and augmented cell death, while over-expression of Abcg2 enhanced cell proliferation, although with limited cardiomyogenic differentiation. Cardiac SP cells, however, are not a pure cell population. Yamahara *et al.*[39] reported that cardiac SP cells consist of vascular endothelial cells, smooth muscle cells and mesenchymal stem cells, including potentially cardiomyogenic cells. Thus, SP cells are heterogeneous in nature, and their role in therapeutic heart regeneration remains to be determined.

Epicardium-derived cells

Recent studies reported the existence of progenitors in the pro-epicardium,[40] which express the T-box transcription factor (Tbx18), migrate onto the outer cardiac surface to form the epicardium, and then make a substantial contribution to myocytes in the ventricular septum and the atrial and ventricular walls during cardiac development. Epicardium-derived cells (EPDC) are also important for blood vessel formation. In Zebrafish, a subpopulation of these epicardial cells undergoes epithelial-to-mesenchymal transition (EMT), invades damaged tissues and provides new vasculature to regenerating muscle.[41] EMT might be a major mechanism for generation of cardiovascular progenitor cells that differentiate into cardiovascular lineages, including coronary smooth muscle and endothelial cells, as well as cardiomyocytes. Martinez-Estrada[42] reported that the gene encoding Wilms' tumor-1 (Wt1) is essential for repression of the epithelial phenotype in epicardial cells through direct transcriptional regulation of the genes encoding Snail (Snai1) and E-cadherin (Cdh1), two of the major mediators of EMT. Most recently, Smart *et al.*[43] reported that actin monomer-binding protein, thymosin beta-4 (Tbeta-4), a peptide which has been suggested to be critical for cardiac development and to have cardioprotective properties, is secreted from the ischemic heart and provides a paracrine stimulus to EPDC to promote their inward migration and differentiation into endothelial and smooth muscle cells to form coronary vasculature. Gajzer *et al.*[44] reported that priming EPDC with Tbeta-4 can also facilitate cardiomyocyte differentiation. Tbeta-4 was the subject of a multicenter phase 1 clinical trial for treatment of cardiovascular disease (http://www.regenerx.com), suggesting the potential clinical utility of this peptide.[43] Currently, EPDC do not have well-defined cell surface markers, which makes clinical application of EPDC challenging. Furthermore, the mechanism for cardiomyocyte differentiation of EPDC is poorly understood.

Cardiosphere-derived cardiac cells

Messina *et al.*[14] were first to demonstrate that cardiosphere-derived cardiac cells (CDC) can be clonally expanded from mouse and human myocardial biopsies and form cardiospheres, which express Sca-1, c-kit, Flk and CD31. The primary tissue explant technique facilitates the proliferation of endogenous CSC *in vitro*, which are present in limited numbers in the adult heart. However, cardiospheres contain a mixture of cells, including cardiac fibroblasts, which grow faster than endogenous CSC in the cardiosphere. Contamination of fibroblasts in cardiospheres would limit therapeutic utility because the fibroblasts could facilitate scar formation and hamper efficiency of heart repair. To solve this problem, our laboratory

developed a two-step protocol to purify CDC from fibroblast contamination.[15] Reports from our and other groups show that Sca-1 + CDC have clonogenic ability, form spheroid aggregates in culture and can differentiate into endothelial cells, smooth muscle cells and cardiomyocytes in ischemic heart after cell transplantation.[45] We also showed that c-kit + CDC possess similar properties and can home to myocardium following ischemic injury.[46] Moreover, we showed that intravenously infused c-kit + CDC can stimulate angiogenesis and improve LV function,[46] and that Notch1 activation can efficiently increase smooth muscle cell differentiation of Sca-1 + CDC.[47] Shen *et al.*[48] demonstrated a dose-dependent functional benefit of intramyocardial injection of human cardiospheres into immunodeficient severe combined immunodeficiency mice with acute myocardial infarction. In a head-to-head comparison, transplanted CDC exhibited enhanced functional benefits as compared with other types of stem cells, which may relate to enhanced potency of paracrine factors that suppress apoptosis and induce angiogenesis, as well as increased engraftment and differentiation potential.[49]

Cardiac islet (Isl)-1 + cells

The LIM-homeodomain transcription factor Islet-1 (Isl-1) marks a cell population which makes a substantial contribution to the embryonic heart.[18] After birth, immature Isl-1 + cells localize in the proximal outflow tract, where a pool of these cells persists into adulthood. Islet-1-positive cells, which can represent both neural crest and cardiomyocyte lineages, are relatively few in number in the adult murine heart, and are localized in restricted and rather inaccessible areas.[19,50] Domian *et al.*[51] further identified a committed ventricular progenitor cell in the Isl-1 lineage that is capable of limited *in vitro* expansion, differentiation and assembly into functional ventricular muscle tissue. Itzhaki-Alfia *et al.* compared CSC from atrial and ventricular tissue samples obtained during heart surgery, transplantation or endomyocardial biopsies (113 samples from 94 patients 23–80 years of age). They found that the right atrium is the best source for Isl-1 progenitors, which are multipotent and can differentiate into osteoblasts, adipocytes and myogenic lineages.[52] Cardiospheres may represent an ideal intermediate stem cell niche for isolating any potential CSC, including Isl-1 + cells. Ye *et al.*[45] reported that cloned Sca-1 + cells derived from cardiospheres from infarcted middle aged hearts are enriched in Isl-1 + precursors that give rise to both myocardial and vascular tissues.

In summary, the adult heart contains CSC, the phenotype of which depends on the method of isolation. These CSC are multipotent and can be induced to differentiate into three cardiovascular lineages. In comparison with other tissue-derived stem cells, such as adipose tissue- or bone marrow-derived mesenchymal stem cells, CSC offer several advantages that may lead to enhanced heart repair. The major disadvantages of CSC, however, include (1) an invasive procedure is required to obtain these cells; (2) the number of resident CSC declines in aging; and (3) successfully isolating CSC is technically challenging. Obtaining CSC from an allogeneic source might help to overcome these disadvantages. The CSC present in neonatal hearts exhibit robust regenerative capacity,[53] suggesting an ideal potential source of CSC for allogeneic transplantation.

Strategies to improve the efficiency of CSC transplantation

Although cardiac progenitor cells may seem to be an obvious choice for cell-based cardiac repair, as with other types of stem cells, the success of this approach is determined mainly by the survival of donor cells in diseased myocardium. In particular, the environment in ischemic myocardium is particularly harsh and represents a primary barrier to the effectiveness of cell therapy. Developing techniques that enhance the survival of transplanted CSC is crucial to adequately replenish the resident stem cell pool and to maximize its regenerative potential.[46] Our group has developed a hypoxia preconditioning (HPC) strategy, which induces CXCR4 expression in cardiosphere-derived, Lin-c-kit + cells. We found that HPC markedly augments stem cell survival and recruitment to the ischemic myocardium in a CXCR4-dependent manner, thus enhancing the benefit of CDC therapy post-MI.[46] Recently, Yan *et al.*,[54] testing a similar HPC strategy in CSC, found that HPC blunts apoptosis by regulating phosphorylated Akt and Bcl2.

MicroRNA (miRNAs) are endogenous 21–22-nucleotide (nt) non-coding small RNAs that play a critical role in the coordinated fine-tuning of gene expression.[55] The precursor miRNAs must be processed to mature miRNAs by a series of steps directed by the enzyme Dicer. Mature miRNAs, Dicer and Argonaute proteins combine to form the miRNA-induced silencing complex (RISC). Guided by the miRNA through base-pairing, the RISC can suppress gene translation by binding to the 3′ untranslated regions (3′UTR) of target genes.[56] Hu *et al.*[57] have showed that transducing Sca-1 + CSC with a cocktail of lentiviruses expressing three miRNA precursors (miR-21, miR-24 and miR-221) significantly improved CSC survival both *in vitro* and *in vivo*. In addition, this cocktail of miRNA-expressing lentiviruses boosted the therapeutic efficacy of CSC transplantation in terms of functional cardiac recovery.[57] Therefore, targeting miRNAs offers completely new possibilities for improving the efficiency of CSC therapy.

Clinical trials using CSC

CSC are currently being evaluated in two clinical trials for patients with ischemic cardiomyopathy. The first study, Stem Cell Infusion in Patients with Ischemic cardiomyopathy (SCIPIO), evaluated the safety and feasibility of right atrial appendage-derived c-Kit + CSC obtained at the time of open heart surgery.[58] Initial findings demonstrated that (1) isolation and *ex vivo* expansion of c-Kit + CSC from about 1 g of myocardial tissue is feasible and reproducible

and (2) intracoronary infusion of 1 million *ex vivo* expanded autologous CSC in patients with chronic ischemic cardiomyopathy and heart failure is feasible, safe and efficacious in restoring LV systolic function up to one year after treatment. A recent interim analysis of heart function by magnetic resonance imaging (MRI) in the SCIPIO patients showed that CSC infusion produces a striking improvement in both global and regional LV function, a reduction in infarct size, and an increase in viable tissue that persist at least one year post-treatment.[59] Further studies will be needed to determine the duration of this functional benefit, and to elucidate the underlying mechanisms (i.e. paracrine effects, activation of endogenous CSC and transdifferentiation). Furthermore, it is important to conduct additional studies using cardiac cells isolated with the same approach to replicate these encouraging results.

More recently, results of a prospective, randomized Phase 1 study of Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction (CADUCEUS) were reported.[60] Unlike SCIPIO, CADUCEUS employed CDC isolated from endomyocardial biopsy samples that yielded, on average, 276 mg of tissue per patient. Tissue explanting technology and suspension culture were used to obtain CDC, 95% of which expressed CD105 and 5% of which expressed CD45. In patients with systolic heart failure, CDC treatment resulted in significant reductions in scar mass and increases in viable heart tissue by MRI analysis. Regional contractility and regional systolic wall thickness were improved as well. Interestingly, end-diastolic volume, end-systolic volume and global LV ejection fraction did not differ between groups by six months. Further studies are needed to elucidate the precise mechanisms of actions of CDC in heart repair and, importantly, whether the new heart mass generated by donor cells can form electrical coupling with recipient cardiomyocytes, which might be critical for improvement of global cardiac contractility.

Summary

Multipotent CSC possess a vast therapeutic capacity and have shown potential in the treatment of heart failure in preclinical and some clinical settings. While it is clear that CSC do facilitate heart regeneration, the combination of optimal types of CSC with the appropriate delivery approach remains to be determined. Additional studies are required to resolve many other important issues, including determining the origins of CSC, standardizing isolation and purification protocols, and optimally manipulating the cells to enhance their survival in diseased myocardium. Future studies in small animal models should also be complemented by preclinical studies in large animal models that more closely mimic human heart disease. Resolving these issues should lead to improved efficacy of CSC and more consistent results in clinical trials, thus enabling us to understand the true therapeutic potential of CSC.

Author contributions: YLT and NLW wrote the manuscript; YJW, LJC, YHP and LZ collected and discussed the literatures.

ACKNOWLEDGEMENTS

This work was supported by the American Heart Association Beginning Grant-in-Aid 0765094Y (to YLT); NIH grant HL086555 (to YLT), and NIH grants HL076684 and HL112640 (to NLW).

REFERENCES

1 Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics – 2012 update: a report from the American Heart Association. *Circulation* 2012;**125**:e2–e220

2 Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, Finkelstein EA, Hong Y, Johnston SC, Khera A, Lloyd-Jones DM, Nelson SA, Nichol G, Orenstein D, Wilson PW, Woo YJ. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* 2011;**123**:933–44

3 Heeger CH, Jaquet K, Thiele H, Zulkarnaen Y, Cuneo A, Haller D, Kivelitz D, Schmidt T, Krause K, Metzner A, Schneider C, Kuck KH, Bergmann MW. Percutaneous, transendocardial injection of bone marrow-derived mononuclear cells in heart failure patients following acute ST-elevation myocardial infarction: ALSTER-Stem Cell trial. *EuroIntervention* 2012;**8**:732–42

4 Strauer BE, Yousef M, Schannwell CM. The acute and long-term effects of intracoronary Stem cell Transplantation in 191 patients with chronic heARt failure: the STAR-heart study. *Eur J Heart Fail* 2010; **12**:721–9

5 Perin EC, Willerson JT, Pepine CJ, Henry TD, Ellis SG, Zhao DX, Silva GV, Lai D, Thomas JD, Kronenberg MW, Martin AD, Anderson RD, Traverse JH, Penn MS, Anwaruddin S, Hatzopoulos AK, Gee AP, Taylor DA, Cogle CR, Smith D, Westbrook L, Chen J, Handberg E, Olson RE, Geither C, Bowman S, Francescon J, Baraniuk S, Piller LB, Simpson LM, Loghin C, Aguilar D, Richman S, Zierold C, Bettencourt J, Sayre SL, Vojvodic RW, Skarlatos SI, Gordon DJ, Ebert RF, Kwak M, Moye LA, Simari RD, Cardiovascular Cell Therapy Research. Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. *JAMA* 2012;**307**:1717–26

6 Mathiasen AB, Jorgensen E, Qayyum AA, Haack-Sorensen M, Ekblond A, Kastrup J. Rationale and design of the first randomized, double-blind, placebo-controlled trial of intramyocardial injection of autologous bone-marrow derived Mesenchymal Stromal Cells in chronic ischemic Heart Failure (MSC-HF Trial). *Am Heart J* 2012; **164**:285–91

7 Tang YL, Tang Y, Zhang YC, Qian K, Shen L, Phillips MI. Improved graft mesenchymal stem cell survival in ischemic heart with a hypoxia-regulated heme oxygenase-1 vector. *J Am Coll Cardiol* 2005;**46**:1339–50

8 Loffredo FS, Steinhauser ML, Gannon J, Lee RT. Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. *Cell Stem cell* 2011;**8**:389–98

9 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**:1750–7

10 Barile L, Messina E, Giacomello A, Marban E. Endogenous cardiac stem cells. *Prog Cardiovasc Dis* 2007;**50**:31–48

11 Torella D, Ellison GM, Nadal-Ginard B, Indolfi C. Cardiac stem and progenitor cell biology for regenerative medicine. *Trends Cardiovasc Med* 2005;**15**:229–36

12 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**:763–76
13 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**:11384–91
14 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**:911–21
15 Tang YL, Shen L, Qian K, Phillips MI. A novel two-step procedure to expand cardiac Sca-1 + cells clonally. *Biochem Biophys Res Commun* 2007;**359**:877–83
16 Carr CA, Stuckey DJ, Tan JJ, Tan SC, Gomes RS, Camelliti P, Messina E, Giacomello A, Ellison GM, Clarke K. Cardiosphere-derived cells improve function in the infarcted rat heart for at least 16 weeks – an MRI study. *PloS One* 2011;**6**:e25669
17 Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, Woodard 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**:647–53
18 Moretti A, Lam J, Evans SM, Laugwitz KL. Biology of Isl1 + cardiac progenitor cells in development and disease. *Cell Mol Life Sci* 2007;**64**:674–82
19 Genead R, Danielsson C, Andersson AB, Corbascio M, Franco-Cereceda A, Sylven C, Grinnemo KH. Islet-1 cells are cardiac progenitors present during the entire lifespan: from the embryonic stage to adulthood. *Stem Cells Dev* 2010;**19**:1601–15
20 Green JV, Murthy SK. Microfluidic enrichment of a target cell type from a heterogenous suspension by adhesion-based negative selection. *Lab Chip* 2009;**9**:2245–8
21 Oh H, Chi X, Bradfute SB, Mishina Y, Pocius J, Michael LH, Behringer RR, Schwartz RJ, Entman ML, Schneider MD. Cardiac muscle plasticity in adult and embryo by heart-derived progenitor cells. *Annals of the New York Academy of Sciences* 2004;**1015**:182–9
22 Smits AM, van Vliet P, Metz CH, Korfage T, Sluijter JPG, Doevendans PA, Goumans M-J. Human cardiomyocyte progenitor cells differentiate into functional mature cardiomyocytes: an *in vitro* model for studying human cardiac physiology and pathophysiology. *Nat Prot* 2009;**4**:232–43
23 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**:262–75
24 Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin 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 Ntl Acad Sci USA* 2003;**100**:12313–18
25 Wang X, Hu Q, Nakamura Y, Lee J, Zhang G, From AH, Zhang J. The role of the sca-1 +/CD31 − cardiac progenitor cell population in postinfarction left ventricular remodeling. *Stem Cells* 2006; **24**:1779–88
26 Bailey B, Fransioli J, Gude NA, Alvarez R Jr, Zhan X, Gustafsson AB, Sussman MA. Sca-1 knockout impairs myocardial and cardiac progenitor cell function. *Circ Res* 2012;**111**:750–60
27 Sandstedt J, Jonsson M, Lindahl A, Jeppsson A, Asp J. C-kit + CD45- cells found in the adult human heart represent a population of endothelial progenitor cells. *Basic Res Cardiol* 2010;**105**:545–56
28 Hattori F. CD117, adult cardiac stem cell marker, is transiently expressed in methothelial epicardial cells. *J Mol Cell Cardiol* 2010;**49**:711–2
29 Zhou Y, Pan P, Yao L, Su M, He P, Niu N, McNutt MA, Gu J. CD117-positive cells of the heart: progenitor cells or mast cells? *J Histochem Cytochem* 2010;**58**:309–16
30 Fazel S, Cimini M, Chen L, Li S, Angoulvant D, Fedak P, Verma S, Weisel RD, Keating A, Li RK. Cardioprotective c-kit + cells are from the bone marrow and regulate the myocardial balance of angiogenic cytokines. *J Clin Invest* 2006;**116**:1865–77
31 Jesty SA, Steffey MA, Lee FK, Breitbach M, Hesse M, Reining S, Lee JC, Doran RM, Nikitin AY, Fleischmann BK, Kotlikoff MI. c-kit + precursors support postinfarction myogenesis in the neonatal, but not adult, heart. *Proc Ntl Acad Sci USA* 2012;**109**:13380–85
32 Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. *J Exp Med* 1996;**183**:1797–806
33 Huls M, Russel FG, Masereeuw R. The role of ATP binding cassette transporters in tissue defense and organ regeneration. *J Pharmacol Exp Ther* 2009;**328**:3–9
34 Summer R, Kotton DN, Sun X, Ma B, Fitzsimmons K, Fine A. Side population cells and Bcrp1 expression in lung. *Am J Physiol Lung Cell Mol Physiol* 2003;**285**:L97–104
35 Alvi AJ, Clayton H, Joshi C, Enver T, Ashworth A, Vivanco MM, Dale TC, Smalley MJ. Functional and molecular characterisation of mammary side population cells. *Breast Cancer Res* 2003;**5**:R1–8
36 Sandstedt J, Jonsson M, Kajic K, Sandstedt M, Lindahl A, Dellgren G, Jeppsson A, Asp J. Left atrium of the human adult heart contains a population of side population cells. *Basic Res Cardiol* 2012;**107**:255
37 Meissner K, Heydrich B, Jedlitschky G, Meyer Zu Schwabedissen H, Mosyagin I, Dazert P, Eckel L, Vogelgesang S, Warzok RW, Bohm M, Lehmann C, Wendt M, Cascorbi I, Kroemer HK. The ATP-binding cassette transporter ABCG2 (BCRP), a marker for side population stem cells, is expressed in human heart. *J Histochem Cytochem* 2006;**54**:215–21
38 Pfister O, Oikonomopoulos A, Sereti KI, Sohn RL, Cullen D, Fine GC, Mouquet F, Westerman K, Liao R. Role of the ATP-binding cassette transporter Abcg2 in the phenotype and function of cardiac side population cells. *Circn Res* 2008;**103**:825–35
39 Yamahara K, Fukushima S, Coppen SR, Felkin LE, Varela-Carver A, Barton PJ, Yacoub MH, Suzuki K. Heterogeneic nature of adult cardiac side population cells. *Biochem Biophys Res Commun* 2008;**371**:615–20
40 Cai CL, Martin JC, Sun Y, Cui L, Wang L, Ouyang K, Yang L, Bu L, Liang X, Zhang X, Stallcup WB, Denton CP, McCulloch A, Chen J, Evans SM. A myocardial lineage derives from Tbx18 epicardial cells. *Nature* 2008;**454**:104–8
41 Lepilina A, Coon AN, Kikuchi K, Holdway JE, Roberts RW, Burns CG, Poss KD. A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell* 2006;**127**:607–19
42 Martinez-Estrada OM, Lettice LA, Essafi A, Guadix JA, Slight J, Velecela V, Hall E, Reichmann J, Devenney PS, Hohenstein P, Hosen N, Hill RE, Munoz-Chapuli R, Hastie ND. Wt1 is required for cardiovascular progenitor cell formation through transcriptional control of Snail and E-cadherin. *Nat Genet* 2010;**42**:89–93
43 Smart N, Risebro CA, Melville AA, Moses K, Schwartz RJ, Chien KR, Riley PR. Thymosin beta-4 is essential for coronary vessel development and promotes neovascularization via adult epicardium. *Ann N Y Acad Sci* 2007;**1112**:171–88
44 Gajzer DC, Balbin J, Chaudhry HW. Thymosin beta4 and cardiac regeneration: are we missing a beat? *Stem Cell Rev* 2012; 10.1007/s12015-012-9378-3
45 Ye J, Boyle A, Shih H, Sievers RE, Zhang Y, Prasad M, Su H, Zhou Y, Grossman W, Bernstein HS, Yeghiazarians Y. Sca-1 + cardiosphere-derived cells are enriched for Isl1-expressing cardiac precursors and improve cardiac function after myocardial injury. *PloS One* 2012;**7**:e30329
46 Tang YL, Zhu W, Cheng M, Chen L, Zhang J, Sun T, Kishore R, Phillips MI, Losordo DW, Qin G. Hypoxic preconditioning enhances the benefit of cardiac progenitor cell therapy for treatment of myocardial infarction by inducing CXCR4 expression. *Circ Res* 2009;**104**:1209–16
47 Chen L, Ashraf M, Wang Y, Zhou M, Zhang J, Qin G, Rubinstein J, Weintraub NL, Tang Y. The role of notch 1 activation in cardiosphere derived cell differentiation. *Stem Cells Dev* 2012;**21**:2122–9
48 Shen D, Cheng K, Marban E. Dose-dependent functional benefit of human cardiosphere transplantation in mice with acute myocardial infarction. *J Cell Mol Med* 2012;**16**:2112–6
49 Li TS, Cheng K, Malliaras K, Smith RR, Zhang Y, Sun B, Matsushita N, Blusztajn A, Terrovitis J, Kusuoka H, Marban L, Marban E. Direct comparison of different stem cell types and subpopulations reveals superior paracrine potency and myocardial repair efficacy with cardiosphere-derived cells. *J Am Coll Cardiol* 2012;**59**:942–53
50 Khattar P, Friedrich FW, Bonne G, Carrier L, Eschenhagen T, Evans SM, Schwartz K, Fiszman MY, Vilquin JT. Distinction between two

populations of islet-1-positive cells in hearts of different murine strains. *Stem Cells Dev* 2011;**20**:1043–52
51 Domian IJ, Chiravuri M, van der Meer P, Feinberg AW, Shi X, Shao Y, Wu SM, Parker KK, Chien KR. Generation of functional ventricular heart muscle from mouse ventricular progenitor cells. *Science* 2009;**326**:426–9
52 Itzhaki-Alfia A, Leor J, Raanani E, Sternik L, Spiegelstein D, Netser S, Holbova R, Pevsner-Fischer M, Lavee J, Barbash IM. Patient characteristics and cell source determine the number of isolated human cardiac progenitor cells. *Circulation* 2009;**120**:2559–66
53 Simpson DL, Mishra R, Sharma S, Goh SK, Deshmukh S, Kaushal S. A strong regenerative ability of cardiac stem cells derived from neonatal hearts. *Circulation* 2012;**126**:S46–53
54 Yan F, Yao Y, Chen L, Li Y, Sheng Z, Ma G. Hypoxic preconditioning improves survival of cardiac progenitor cells: role of stromal cell derived factor-1alpha-CXCR4 axis. *PloS One* 2012;**7**:e37948
55 Ying SY, Chang DC, Lin SL. The MicroRNA. *Methods Mol Biol* 2013;**936**:1–19
56 Tang Y, Wang Y, Chen L, Pan Y, Weintraub N. Chapter 8 – Cross Talk Between the Notch Signaling and Noncoding RNA on the Fate of Stem Cells. In: Yaoliang T, ed. *Progress in molecular biology and translational science*. Vol. 111. London: Academic Press, 2012:175–93
57 Hu S, Huang M, Nguyen PK, Gong Y, Li Z, Jia F, Lan F, Liu J, Nag D, Robbins RC, Wu JC. Novel microRNA prosurvival cocktail for improving engraftment and function of cardiac progenitor cell transplantation. *Circulation* 2011;**124**:S27–34
58 Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J, Anversa P. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet* 2011;**378**:1847–57
59 Chugh AR, Beache GM, Loughran JH, Mewton N, Elmore JB, Kajstura J, Pappas P, Tatooles A, Stoddard MF, Lima JA, Slaughter MS, Anversa P, Bolli R. 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**:S54–64
60 Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marban L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marban E. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. *Lancet* 2012;**379**:895–904