

Chapter 2

Resident Cardiac Progenitor Cells

Ayelet Itzhaki-Alfia and Jonathan Leor

Abstract Resident cardiac stem/progenitor cells are implicated in cell replacement, repair and maintenance of the myocardium. These cells are already committed to a specific cardiomyogenic pathway. Stimulation of these cells in injured hearts can open new options in cardiovascular regenerative medicine, and advance our approach to patients with end-stage heart failure. This chapter reviews some of the recent discoveries in the field of resident cardiac stem/progenitor cells, focusing first on human cardiac stem cell characteristics and reparative potential, including the concept of myocardial regeneration. In addition, we will briefly review recent clinical trials, controversies and unresolved issues.

Stem Cells can be Used to Replace Lost and Damaged Cells

Replacement of dead or dysfunctional cardiac myocytes through cell-based therapies represents a novel option for the treatment of cardiovascular diseases. Stem cell-based therapies may achieve true cardiac regeneration by renewing the pool of cardiomyocytes or may lead to cardiac repair by stimulating neovascularization, cytoprotection, modulation of inflammation and by extracellular matrix deposition (Lafamme and Murry 2005, 2011).

There are several approaches for the use of stem cells in cardiovascular therapies. The primary strategy is cell therapy that increases the number of functional cardiomyocytes in the infarcted myocardium by cell transplantation, which entails the delivery of a volume or mass of cells to the damaged tissue. The implanted cells

A. Itzhaki-Alfia (✉)
Tamman Cardiovascular Research Institute, Leviev Heart Center,
Sheba Medical Center, 52621 Tel-Hashomer, Israel
e-mail: itzhaki.ayelet@gmail.com

J. Leor
Tamman Cardiovascular Research Institute,
Leviev Heart Center, Tel-Aviv University, Tel-Hashomer, Israel

Neufeld Cardiac Research Institute, Sackler Faculty of Medicine,
Tel-Aviv University, Tel-Aviv, Israel

J. Hescheler, E. Hofer (eds.), *Adult and Pluripotent Stem Cells*,
DOI 10.1007/978-94-017-8657-7_2, © Springer Science+Business Media Dordrecht 2014

may have the potential for growth, self-repair and remodeling by forming new cardiac tissue (Etzion et al. 2001; Leor et al. 1996). Such an approach can be divided into two treatment strategies: the first uses differentiated cells to replace the scar tissue with living cells, while the second uses undifferentiated stem cells as building blocks to grow specific types of tissue in situ, in an attempt to regenerate the myocardium (Penn et al. 2002; Zimmet and Krum 2008). Stem cells could be used to replace lost or damaged cells, if they possess the capacity to produce a large number of specific cell types in situ or ex vivo for transplantation.

Adult Stem Cells are Involved in Tissue Replacement, Repair and Maintenance

Adult stem cells, which can be found in adult tissue, are involved in tissue replacement, repair and maintenance, and are already committed to a specific developmental pathway. Usually they generate cell types already present in the same tissue from which they are derived. These cells are multi-potent and have the potential to develop into several, but not all cells types (Passier and Mummery 2003). Adult stem cells include bone marrow cells (Orlic et al. 2001), haemopoietic stem cells (HSC) (Muller-Sieburg et al. 2002), endothelial progenitor cells (EPCs) (Asahara et al. 1999), mesenchymal stem cells (MSCs), and resident cardiac stem cells (Smith et al. 2007; Messina et al. 2004; Oh et al. 2003; Urbanek et al. 2003).

Cells for Cardiovascular Regenerative Therapy

The optimal cells for human myocardial regeneration should be autologous, capable of differentiating into all adult heart cell types, without forming tumors. Various cells proposed to be beneficial for myocardial regeneration are shown in Table 2.1.

Compared with adult stem cells, human embryonic (hES) stem cells and induced pluripotent stem cells (iPS) are more flexible and versatile, and can differentiate into almost every type of cell in the human body. They are capable of almost unlimited division and proliferation, can be grown fairly easily and produce large numbers of stem cells *in vitro*. In contrast, most adult stem cells do not multiply readily after isolation, which is a major problem in stem cell replacement therapies. Since adult stem cells can differentiate into only a limited number of cell types, their versatility for therapy is limited. Within this property, however, lies a major advantage: such cells are committed and do not form tumors. Adult stem cells have another advantage over ES cells in cardiovascular regenerative medicine (see Table 2.1). They are autologous and do not carry the risk of rejection or the need for immunosuppressive therapy connected with adverse effects. However, all of these cell sources and their

Table 2.1 Advantages and limitations of various cell sources for myocardial repair

	Autologous	Easily obtainable	Highly expandable	Cardiac myogenesis	Clinical experience	Safety concerns
Human embryonic stem cells	No	No	Yes	Yes	No	Yes
Mesenchymal stem cells	Yes	No	Depends on age	?	Yes	No
Crude bone marrow cells	Yes	Yes	Depends on age	?	Yes	No
Cardiac stem cells	Yes	No	Depends on the method	Yes	Yes	?
iPS	Yes	No	No	Yes	No	Yes

mechanisms need further research to achieve improvements in therapies. Despite significant advances in stem cell biology, the best cell type and delivery strategy remains to be defined. It is possible that different diseases require different cells for optimal therapy.

Human Heart Regeneration

The question of whether human hearts are able to regenerate has been the subject of furious debate for several years. However, data gathered in the last decade have suggested that the human heart has self-renewal potential. For example, in hearts of patients who died after myocardial infarction (MI), 4 and 1 % of myocytes, in the border zone and remote area, are positive for the cell proliferation marker Ki-67 (Beltrami et al. 2001). Some Ki-67-positive cells, however, were also present in control hearts, suggesting that myocyte proliferation not only occurs during life but is further enhanced following injury (Beltrami et al. 2001). It has been estimated that muscle cells of the entire heart are replaced every 4.5 years (Anversa et al. 2006).

Another method to estimate the turnover of human cardiomyocytes utilized ^{14}C levels in the nucleus as a age biomarker (Bergmann et al. 2009). Due to nuclear testing, atmospheric levels of ^{14}C have been increased, and ^{14}C has spread around the world as $^{14}\text{CO}_2$. However, following the nuclear test ban in 1963, atmospheric ^{14}C levels gradually declined. Because ^{14}C is incorporated into the DNA of dividing cells, and DNA remains stable after its last division, ^{14}C concentration in DNA mirrors the atmospheric levels of ^{14}C at the time of its last division, and therefore can be used to determine the age of human cells (Spalding et al. 2005). Mathematical calculations estimated that ~ 1 and $\sim 0.4\%$ of myocytes are renewed every year at the ages of 20 and 80, leading to the estimation that $\sim 45\%$ of myocytes are replaced during a normal human life span (Spalding et al. 2005; Bergmann et al. 2009).

The reason for the difference between the two studies is unclear. Ki-67 labeling may over-estimate cardiomyocyte proliferation because cardiomyocytes sometimes undergo DNA synthesis without nuclear division, or nuclear division without cytokinesis. It is also unclear from these studies whether the newly formed myocytes are derived from pre-existing myocytes or from human cardiac progenitor cells (hCPCs). Genetic fate-mapping studies in mice have suggested that cardiomyocyte renewal following myocardial injury originate from either resident CPCs, (Hsieh et al. 2007) or cardiomyocyte dedifferentiation and proliferation (Porrello et al. 2011; Kubin et al. 2011). Nonetheless, recent human specimen studies provide evidence that the human heart retains self-renewal capacity, but with a low frequency.

Human Resident Cardiac Stem Cells for Myocardial Regeneration and Repair

The discovery that the adult mammalian heart contains a small pool of stem cells has generated a new field in cardiac regenerative medicine. These specific sub-populations of hCPCs are involved in cell replacement, repair and maintenance of the myocardium, cardiac cellular homeostasis during aging and possess stem cell characteristics.

Surprisingly, different researchers have described different markers and phenotypes of hCPCs (Table 2.2). These markers and cell types include c-Kit-positive cells (CD117, the receptor for stem cell factor) (Bearzi et al. 2007), cardiospheres (Messina et al. 2004), cardiosphere-derived cells (Smith et al. 2007), stem cell antigen (Sca)-1-positive cells (Morrison et al. 1997), islet 1-positive cells (Laugwitz et al. 2005), stage-specific embryonic antigen 1-positive cells (Pouly et al. 2008; Ott et al. 2007) and Wt1 epicardial-derived cells (Zhou et al. 2008).

A. P. Beltrami (Beltrami et al. 2003, 2001) from the Anversa group was the first to isolate and grow CPCs from rat heart. C. Bearzi (Bearzi et al. 2007) from the Anversa group was the first to report that human cardiac stem cells (hCSCs), isolated from human biopsies, were positive for c-kit, a stem cell growth factor receptor, also known as CD117. The c-kit receptor was originally detected in murine HSCs with ability to restore bone marrow in irradiated recipients (Orlic et al. 1993). Anversa et al. showed a major contribution of c-kit progenitor cells to cardiomyogenesis during embryonic development (Ferreira-Martins et al. 2012), aging (Tang et al. 2010), and after myocardial infarction (Beltrami et al. 2003). Furthermore, c-kit is re-expressed in dedifferentiated cardiomyocytes during myocardial regeneration (Kubin et al. 2011)

Another proposed marker of CPC population is the insulin gene enhancer protein Islet-1, a marker of the secondary heart field that is involved in embryonic heart formation, gives rise to the outflow tract and ventricles. K.-L. Laugwitz (Laugwitz et al. 2005) was the first to find cardioblasts, Islet-1+ progenitor cells, isolated from

Table 2.2 Human resident cardiac stem cells markers and cell isolation methods

Group	Positive markers	Negative marker	Isolation methods	Source of tissues
(Messina et al. 2004)	c-kit ⁺ , CD34 ⁺ , SCA-1 ⁺ , KDR ⁺		Trypsin and 0.1 % Collagenase IV	Atrial or ventricular biopsy specimens
(Laugwitz et al. 2005)	islet-1 ⁺ , Nkx2.5 ⁺ , GATA4 ⁺	sca1 ⁻ , CD31 ⁻ , c-kit ⁻	Collagenase II	Right atrial tissue pediatric surgery
(Bearzi et al. 2007)	c-kit ⁺	CD45 ⁻ , CD34 ⁻ , CD31 ⁻ , KDR ⁻	Collagenase IV	–
(Smith et al. 2007)	c-kit ⁺ , CD105 ⁺ , CD90 ⁺ , CD34 ⁺ , CD31 ⁺	MDR1 ⁻ , CD133 ⁻ , CD45 ⁻	Tissue fragments were cultured as “explants”	Percutaneous right ventricular biopsy
(Goumans et al. 2008)	CD105 ⁺ , SCA-1 ⁺	CD45 ⁻ , CD34 ⁻ , CD133 ⁻ , CD14 ⁻	Collagenase A	Fetal tissue and atrial biopsies
(Itzhaki-Alfia et al. 2009)	c-kit ⁺ , GATA4 ⁺ , Ki-67 ⁺	CD45 ⁻ , CD31 ⁻ , Collagen ⁻ , CD68 ⁻ , Tryptase ⁻	Trypsin-EDTA 0.25 % and dispase II	Myocardial tissue from right or left atrial appendages, right ventricle or left ventricle

pediatric surgery. Those cells were cultured on cardiac mesenchymal feeder layers and were positive to early cardiac mesoderm markers (Nkx2.5 and GATA-4). Finally, while previous groups described cultured CPCs as adherent cells, Messina et al. described human cardiospheres: suspension of cells obtained from human myocardial biopsy samples (Messina et al. 2004). Thus, the markers that phenotype the hCPC population are varied among different researchers.

The anatomical source of hCPCs in the heart is an important factor in understanding their role in maintaining cardiac homeostasis and therapeutic potential. Pouly et al. conducted a histopathological study analyzing tissue samples from biopsies of a right-sided septum from heart-transplanted patients, and right atrial appendage specimens from patients with ischemic cardiomyopathy (Pouly et al. 2008). They found higher amounts of c-kit⁺ cells in septal biopsies compared with those of the right atrial appendage. However, the septal biopsies were taken from heart transplant patients, a third of whom had some degree of rejection and were on immunosuppressive therapy. When we compared cell cultures from tissue derived from various patients and analyzed the data, multi-variant models were incorporated to eliminate potential differences in baseline characteristics. This showed that the number of cultured c-kit⁺ cells produced from the right atrium was 5 fold higher than from other sources (Itzhaki-Alfia et al. 2009).

An Efficient and Reproducible Enzymatic Protocol to Isolate HCPCs

Each group in the field worked so far with a different isolation method and different tissue sources. The cell yield and cluster formation were different with each of the techniques used for cell isolation. Methods to isolate hCPCs include enzymatic (Goumans et al. 2008; Laugwitz et al. 2005; Bearzi et al. 2007; Argentin et al. 1994; Parker et al. 1990a, b; Zhou et al. 2000; Itzhaki-Alfia et al. 2009) and non-enzymatic approaches (Smith et al. 2007; Messina et al. 2004). Using the enzymatic approach, researchers used various enzymes to dissociate the cells from the myocardial specimens. Then, stem cells were isolated from the dissociated cells with a magnetic cell sorter using antibodies against stem cell markers, such as c-kit (Bearzi et al. 2007). However, the cell yield was low and required several months to grow a sufficient number of cells for potential therapeutic applications (Argentin et al. 1994; Parker et al. 1990a, b; Zhou et al. 2000; Forrester et al. 2009). In the non-enzymatic method, tissue samples were seeded, allowing for the spontaneous outgrowth of cells which could then be harvested as cell aggregates in suspension, e.g. cardiospheres (Messina et al. 2004).

We improved the technology and developed an enzymatic digestion cocktail as well as an efficient and reproducible protocol to isolate c-kit⁺ hCPCs from diverse tissue samples (Itzhaki-Alfia et al. 2009). Compared with other methods (Argentin et al. 1994; Parker et al. 1990a, b; Zhou et al. 2000; Table 2.3), our method has a near 100% success rate in isolating hCPCs (Itzhaki-Alfia et al. 2009). During

Table 2.3 Isolation methods used to dissociate human heart tissue

Technique used	Researchers	Percentage of tissue that yielded progenitor cells	Living/dead cell ratios	The amount of viable cells per gram tissue
RDB proteolytic enzyme (Institute of Biology, Nes-Ziona, Israel)	(Shneyvays et al. 1998)	No data	No data	No data
Collagenase	(Bearzi et al. 2007)	8 of 12	No data	No data
Explanted technique	(Bearzi et al. 2007)	46 of 70	No data	6,000
Explanted technique	(Smith et al. 2007)	69 of 70	No data	No data
0.1 % trypsin, 0.1 % collagenase, 0.025 % DNAase	(Parker et al. 1990b)	No data	No data	No data
Trypsin-EDTA 0.25 % and dispase II	(Itzhaki-Alfia et al. 2009)	249 of 249 samples	95–98 %	$7 \times 10^6 \pm 6.53 \times 10^5/\text{g}$

our study we compared our method with different other methods, enzymatic digestion cocktails and isolation protocols with respect to cell yield. Isolation methods described in Table 2.3 were tested on human tissues, whereas these methods were originally used to obtain cells from rodents, a fact which might explain the low yield compared with our method.

Patient Characteristics and the Number of hCPCs

One of the most important challenges in the field of regenerative medicine is to predict the yield of hCSC production from a specific patient. Previous reports correlated increased numbers of CPCs in humans with aortic stenosis, acute and chronic ischemia, and cardiomyopathies (Beltrami et al. 2001, 2003; Kajstura et al. 1998; Urbanek et al. 2005b; Kubo et al. 2008). Together, these findings, which are consistent with ours, support the notion that an endogenous reparative system is stimulated in the injured heart (Hsieh et al. 2007).

Several studies have attempted to determine the correlation between patient characteristics and hCPC yield. While it has been found that coronary artery disease does not affect CPC yield (Aghila Rani et al. 2009) the use of β -blockers was positively associated with CPC percentage at first passage (Gambini et al. 2012).

We found that patients with hypertension had 2-fold greater percentage of c-kit⁺ cells in culture, compared with normotensive patients. No correlation was found between medical therapy, including aspirin, ACE inhibitors, amiodarone or statins and the number of c-kit⁺ cells (Itzhaki-Alfia et al. 2009). Another intriguing finding was that female gender is associated with a higher number of c-kit⁺ cells (Itzhaki-Alfia et al. 2009). Although the explanation for this finding is unclear, it

is consistent with previous reports suggesting variability in stem cell function and numbers between genders (Olivetti et al. 1995; Anversa et al. 2005; Nelson et al. 2007).

Previous work on stem cell aging in the adult heart has indicated that the cardiac progenitor population undergoes an aging process, which could be attributed to either telomere shortening-mediated cell senescence or cumulative cellular trauma (Rota et al. 2008; Anversa et al. 2005; Sussman and Anversa 2004). Consequently, histological analysis of myocardial tissue from elderly patients with cardiomyopathy showed lower numbers of c-kit⁺ cells compared with younger patients (Chimenti et al. 2003). It has been suggested that in older patients, stem cell isolation from cardiac biopsies may not succeed, and such cells may not be available for cell therapy (Aghila Rani et al. 2009). We found however, that age does not significantly affect the number of hCPCs derived from myocardial specimens (Itzhaki-Alfia et al. 2009), a finding consistent with other researchers who suggest that chronological age is not necessarily the main determinant of hCPC functionality and frequency (Rota et al. 2008). There are probably other background conditions that play an important role in the physiology of hCPCs. An additional explanation is that in the aged heart, hCPCs are probably located in certain regions within the myocardium (e.g. the atrium) that retain significant amounts of telomerase-competent hCPCs with long telomeres, which replicate in response to pathological states and participate in regeneration of damaged areas (Gonzalez et al. 2008).

Cardiac Progenitor Cells for Myocardial Repair

Stimulation of endogenous regenerative activity is an attractive strategy for myocardial regeneration and repair. Regeneration can be achieved by stimulation of self-renewal of cardiomyocytes or hCPCs *in situ*, with a drug or cytokine (Bock-Marquette et al. 2004; Smart et al. 2007; Ruvinov et al. 2011). Alternatively, regeneration can be achieved by *ex vivo* propagation of hCPCs followed by transplantation of the cells into the injured area (Fig. 2.1; Bearzi et al. 2007; Beltrami et al. 2003; Quaini et al. 2004).

Regeneration can be achieved by *ex vivo* proliferation of hCPCs. Small biopsies of the myocardium can be digested by a proteolysis enzyme cocktail, followed by proliferation and expansion in culture. In the final stage, these cells are transplanted into the injured area. Regeneration can be also achieved by stimulation of endogenous cardiomyocytes or hCPCs *in situ*, by delivering to injured myocardium specific growth factors or cytokines.

Animal studies have suggested that transplantation of cells expressing c-kit into the heart could stimulate formation of new blood vessels and myocardium (Beltrami et al. 2003; Tang et al. 2010). Suspension culture of cells obtained from human myocardial biopsy samples yields cardiospheres that contain a mixed population

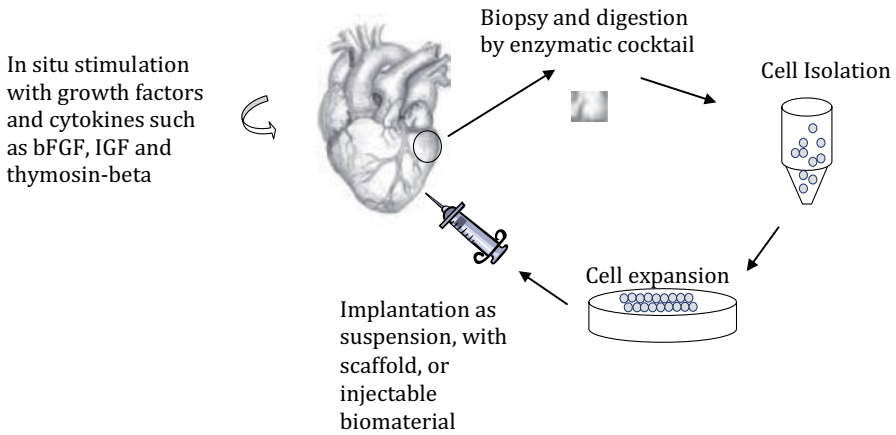


Fig. 2.1 Cardiac progenitor cells for myocardial regeneration and repair

of c-kit⁺ and Sca-1-expressing cells that could stimulate cardiac regeneration (Chimenti et al. 2010).

Whether CSCs are superior to MSCs for myocardial regeneration is unknown. However, a recent study found that hCSCs have a greater ability to engraft and improve cardiac function compared with MSCs (Oskouei et al. 2012). When both hCSCs and MSCs were separately injected into a mouse MI model, both cell types improved ejection fraction, but MSCs required a higher dose (1×10^6) compared with CSCs (3.6×10^4 cells). Moreover, CSCs were superior in reducing scar tissue. These results suggest enhanced engraftment of CSCs compared with MSCs (Oskouei et al. 2012).

To become a viable clinical option, cardiac cell therapy should adopt improved methods of cell engraftment and survival. These improvements can be done by the addition of growth factors (Takehara et al. 2008; Penn and Mangi 2008), genetic engineering (Mohsin et al. 2012), and restoration of the damaged extracellular matrix with scaffolds or injectable biomaterials (Ruvinov et al. 2011; Landa et al. 2008). Alternatively, resident CPCs could be activated in situ by delivering specific growth factors or cytokines to injured myocardium. For example, local delivery of biotinylated IGF-1 complex increased cardiomyocyte growth both *in vitro* and *in vivo* (Davis et al. 2006; D'Amario et al. 2011). Another approach for CSC stimulation was achieved by co-culturing them together with MSCs (Hatzistergos et al. 2010). The number of c-kit CSCs was then 6-fold higher than in CSCs cultured without MSCs (Hatzistergos et al. 2010).

From Bench to Bedside: hCSCs and Cardiospheres in Clinical Trials

The encouraging results of CPCs in animal models led to the initiation of "first in man" clinical trials: ALCADIA (NCT00981006), SCIPIO (NCT00474461), and CADUCEUS (NCT00893360). Preliminary results have recently been published from the SCIPIO and CADUCEUS trials (Makkar et al. 2012; Bolli et al. 2011). Both were designed to assess the feasibility and safety of intracoronary injection of autologous hCPCs (1 million in the SCIPIO, and 12.5–25 million cells in the CADUCEUS) after recent infarction. The SCIPIO study (Bolli et al. 2011) involved c-kit-expressing cells cultured from explanted atrial tissue, whereas the CADUCEUS study (Makkar et al. 2012) involved cardiospheres cultured from biopsies obtained from inter-ventricular septum. Both studies reported on safety and reduction in myocardial scar mass following cell treatment, but only the SCIPIO trial reported improvement in left ventricular ejection fraction. These preliminary results should be viewed with caution, since the number of patients in the treatment group of each study was small (16 in SCIPIO and 17 in CADUCEUS), and neither study included a true placebo group. Many questions are raised following these preliminary trials. Nevertheless, these trials on hCPC treatment for ischemic heart failure established a milestone in the history of cardiac cell therapy.

Unresolved Issues

Substantial gaps remain in our knowledge regarding hCPCs and the mechanisms by which they might promote regeneration. First, the existence and regenerative potential of hCPCs in the adult heart has been questioned and debated. Second, no consensus exists relating to phenotype definition or isolation technique of these populations, many of which have not been compared. Third, genetic fate mapping, a stringent scientific technique used to establish the derivatives of a progenitor cell population, suggests that c-kit-positive cells and cardiospheres do not differentiate into cardiomyocytes (Zaruba et al. 2010). In mice, c-kit-positive cells derived from bone marrow stimulate cardiomyocyte renewal after infarction without direct trans-differentiation (Loffredo et al. 2011). This effect might be attributable to paracrine factors released by the implanted cells on a distinct population of endogenous cardiac cells (Hatzistergos et al. 2010). While advanced age and heart disease did not affect the number of hCPCs (Itzhaki-Alfia et al. 2009), the effect on hCPC function and reparative properties is less clear. Finally, the adoptive transfer of hCSCs results in modest repair due in part to lack of survival, proliferation, and commitment of the transplanted cells after MI.

Summary and Perspective

The area of resident hCPCs is still in its infancy and significant amounts of data regarding the characterization and function of these cells are lacking. However, data from several labs show that the adult human heart, especially the right atrium, retains a unique cell population with stem cell properties. These cells can be isolated, expanded and stored, and could be a viable option to treat patients with heart disease. Augmentation of endogenous regenerative activity in injured hearts will open exciting new options in cardiovascular regenerative medicine, and revolutionize our approach to patients with end-stage heart failure. Larger clinical trials, powered to show clinically meaningful outcomes, are warranted to prove the safety and efficacy of these strategies.

Source of Funding This work was supported by an EC grant (FP7 HEALTH-F2-2009-222995 INELPY) to JL.

References

- Aghila Rani KG, Jayakumar K, Sarma PS, Kartha CC (2009) Clinical determinants of ckit-positive cardiac cell yield in coronary disease. *Asian Cardiovasc Thorac Ann* 17(2):139–142. doi:10.1177/0218492309103292
- Anversa P, Rota M, Urbanek K, Hosoda T, Sonnenblick E, Leri A, Kajstura J, Bolli R (2005) Myocardial aging. *Basic Res Cardio* 100(6):482–493
- Anversa P, Kajstura J, Leri A, Bolli R (2006) Life and death of cardiac stem cells: a paradigm shift in cardiac biology. *Circulation* 113(11):1451–1463. doi:10.1161/CIRCULATIONAHA.105.595181
- Argentin S, Ardati A, Tremblay S, Lihrmann I, Robitaille L, Drouin J, Nemer M (1994) Developmental stage-specific regulation of atrial natriuretic factor gene transcription in cardiac cells. *Mol Cell Biol* 14(1):777–790
- Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM (1999) Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 85(3):221–228. doi:10.1161/01.res.85.3.221
- 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 (2007) Human cardiac stem cells. *Proc Natl Acad Sci U S A* 104(35):14068–14073. doi:10.1073/pnas.0706760104
- Beltrami AP, Urbanek K, Kajstura J, Yan S-M, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P (2001) Evidence that human cardiac Myocytes divide after myocardial infarction. *N Engl J Med* 344(23):1750–1757. doi:10.1056/nejm200106073442303
- 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 (2003) Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114(6):763–776. doi:S0092867403006871 [pii]

- Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabe-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, Jovinge S, Frisen J (2009) Evidence for cardiomyocyte renewal in humans. *Science* 324(5923):98–102. doi:10.1126/science.1164680
- Bock-Marquette I, Saxena A, White MD, Dimaio JM, Srivastava D (2004) Thymosin beta4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair. *Nature* 432(7016):466–472. doi:10.1038/nature03000
- 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 (2011) Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet* 378(9806):1847–1857. doi:10.1016/S0140-6736(11)61590-0
- Chien KR (2005) Alchemy and the new age of cardiac muscle cell biology. *PLoS Biol* 3(4):e131
- Chimenti C, Kajstura J, Torella D, Urbanek K, Hleniak H, Colussi C, Di Meglio F, Nadal-Ginard B, Frustaci A, Leri A, Maseri A, Anversa P (2003) Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. *Circ Res* 93(7):604–613. doi:10.1161/01.res.0000093985.76901.af
- Chimenti I, Smith RR, Li TS, Gerstenblith G, Messina E, Giacomello A, Marban E (2010) Relative roles of direct regeneration versus paracrine effects of human cardiosphere-derived cells transplanted into infarcted mice. *Circ Res* 106(5):971–980. doi:10.1161/CIRCRESA-HA.109.210682
- D'Amario D, Cabral-Da-Silva MC, Zheng H, Fiorini C, Goichberg P, Steadman E, Ferreira-Martins J, Sanada F, Piccoli M, Cappetta D, D'Alessandro DA, Michler RE, Hosoda T, Anastasia L, Rota M, Leri A, Anversa P, Kajstura J (2011) Insulin-like growth factor-1 receptor identifies a pool of human cardiac stem cells with superior therapeutic potential for myocardial regeneration. *Circ Res* 108(12):1467–1481. doi:10.1161/CIRCRESAHA.111.240648
- Davis ME, Hsieh PC, Takahashi T, Song Q, Zhang S, Kamm RD, Grodzinsky AJ, Anversa P, Lee RT (2006) Local myocardial insulin-like growth factor 1 (IGF-1) delivery with biotinylated peptide nanofibers improves cell therapy for myocardial infarction. *Proc Natl Acad Sci U S A* 103(21):8155–8160. doi:0602877103 [pii] 10.1073/pnas.0602877103
- Etzion S, Battler A, Barbash IM, Cagnano E, Zarin P, Granot Y, Kedes LH, Kloner RA, Leor J (2001) Influence of embryonic cardiomyocyte transplantation on the progression of heart failure in a rat model of extensive myocardial infarction. *J Mol Cell Cardiol* 33(7):1321–1330
- Ferreira-Martins J, Ogorek B, Cappetta D, Matsuda A, Signore S, D'Amario D, Kostyla J, Steadman E, Ide-Iwata N, Sanada F, Iaffaldano G, Ottolenghi S, Hosoda T, Leri A, Kajstura J, Anversa P, Rota M (2012) Cardiomyogenesis in the developing heart is regulated by c-kit-positive cardiac stem cells. *Circ Res* 110(5):701–715. doi:10.1161/CIRCRESAHA.111.259507
- Forrester JS, Makkar RR, Marban E (2009) Long-term outcome of stem cell therapy for acute myocardial infarction: right results, wrong reasons. *J Am Coll Cardiol* 53(24):2270–2272. doi:S0735-1097(09)01063-8 [pii] 10.1016/j.jacc.2009.03.023
- Gambini E, Pesce M, Persico L, Bassetti B, Gambini A, Alamanni F, Agrifoglio M, Capogrossi MC, Pompilio G (2012) Patient profile modulates cardiac c-kit⁺ progenitor cell availability and amplification potential. *Transl Res* 160(5):1–11
- Gonzalez A, Rota M, Nuzynska D, Misao Y, Tillmanns J, Ojaimi C, Padin-Iruegas ME, Muller P, Esposito G, Bearzi C, Vitale S, Dawn B, Sanganelmath SK, Baker M, Hintze TH, Bolli R, Urbanek K, Hosoda T, Anversa P, Kajstura J, Leri A (2008) Activation of cardiac progenitor cells reverses the failing heart senescent phenotype and prolongs lifespan. *Circ Res* 102(5):597–606. doi:10.1161/circresaha.107.165464
- Goumans M-J, de Boer TP, Smits AM, van Laake LW, van Vliet P, Metz CHG, Korfage TH, Kats KP, Hochstenbach R, Pasterkamp G, Verhaar MC, van der Heyden MAG, de Kleijn D, Mummery CL, van Veen TAB, Sluijter JPG, Doevendans PA (2008) TGF- β 1 induces efficient differentiation of human cardiomyocyte progenitor cells into functional cardiomyocytes in vitro. *Stem Cell Res* 1(2):138–149
- Hatzistergos KE, Quevedo H, Oskoue BN, Hu Q, Feigenbaum GS, Margitich IS, Mazhari R, Boyle AJ, Zambrano JP, Rodriguez JE, Dulce R, Pattany PM, Valdes D, Revilla C, Heldman

- AW, McNiece I, Hare JM (2010) Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. *Circ Res* 107(7):913–922. doi:10.1161/CIRCRESAHA.110.222703
- Hsieh PCH, Segers VFM, Davis ME, MacGillivray C, Gannon J, Molkentin JD, Robbins J, Lee RT (2007) Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. *Nat Med* 13(8):970–974
- Itzhaki-Alfia A, Leor J, Raanani E, Sternik L, Spiegelstein D, Netser S, Holbova R, Pevsner-Fischer M, Lavee J, Barbash IM (2009) Patient characteristics and cell source determine the number of isolated human cardiac progenitor cells. *Circulation* 120(25):2559–2566. doi:CIRCULATIONAHA.109.849588 [pii] 10.1161/CIRCULATIONAHA.109.849588
- Kajstura J, Lerj A, Finato N, Di Loreto C, Beltrami CA, Anversa P (1998) Myocyte proliferation in end-stage cardiac failure in humans. *Proc Natl Acad Sci U S A* 95(15):8801–8805. doi:10.1073/pnas.95.15.8801
- Kubin T, Poling J, Kostin S, Gajawada P, Hein S, Rees W, Wietelmann A, Tanaka M, Lorchner H, Schimanski S, Szibor M, Warnecke H, Braun T (2011) Oncostatin M is a major mediator of cardiomyocyte dedifferentiation and remodeling. *Cell Stem Cell* 9(5):420–432. doi:S1934-5909(11)00394-8 [pii] 10.1016/j.stem.2011.08.013
- Kubo H, Jaleel N, Kumarapeli A, Berretta RM, Bratinov G, Shan X, Wang H, Houser SR, Margulies KB (2008) Increased cardiac myocyte progenitors in failing human hearts. *Circulation* 118(6):649–657. doi:10.1161/circulationaha.107.761031
- Laflamme MA, Murry CE (2005) Regenerating the heart. *Nat Biotech* 23(7):845–856
- Laflamme MA, Murry CE (2011) Heart regeneration. *Nature* 473(7347):326–335. doi:10.1038/nature10147
- Landa N, Miller L, Feinberg MS, Holbova R, Shachar M, Freeman I, Cohen S, Leor J (2008) Effect of injectable alginate implant on cardiac remodeling and function after recent and old infarcts in rat. *Circulation* 117(11):1388–1396. doi:10.1161/circulationaha.107.727420
- Laugwitz K-L, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, Lin L-Z, Cai C-L, Lu MM, Reth M, Platoshyn O, Yuan JXJ, Evans S, Chien KR (2005) Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature* 433(7026):647–653
- Leor J, Patterson M, Quinones MJ, Kedes LH, Kloner RA (1996) Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? *Circulation* 94(9 Suppl):II332–336
- Loffredo FS, Steinhauser ML, Gannon J, Lee RT (2011) Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. *Cell Stem Cell* 8(4):389–398. doi:10.1016/j.stem.2011.02.002
- 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 (2012) Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. *Lancet* 379(9819):895–904. doi:10.1016/S0140-6736(12)60195-0
- Messina E, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso F, Salio M, Battaglia M, Latronico MVG, Coletta M, Vivarelli E, Frati L, Cossu G, Giacomello A (2004) Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res* 95(9):911–921. doi:10.1161/01.res.0000147315.71699.51
- Mohsin S, Khan M, Toko H, Bailey B, Cottage CT, Wallach K, Nag D, Lee A, Siddiqi S, Lan F, Fischer KM, Gude N, Quijada P, Avitabile D, Truffa S, Collins B, Dembitsky W, Wu JC, Sussman MA (2012) Human cardiac progenitor cells engineered with pim-i kinase enhance myocardial repair. *J Am Coll Cardiol* 60(14):1278–1287. doi:10.1016/j.jacc.2012.04.047
- Morrison SJ, Wandycz AM, Hemmati HD, Wright DE, Weissman IL (1997) Identification of a lineage of multipotent hematopoietic progenitors. *Development* 124(10):1929–1939
- Muller-Sieburg CE, Cho RH, Thoman M, Adkins B, Sieburg HB (2002) Deterministic regulation of hematopoietic stem cell self-renewal and differentiation. *Blood* 100(4):1302–1309

- Nelson WD, Zenovich AG, Ott HC, Stolen C, Caron GJ, Panoskaltsis-Mortari A, Barnes SA III, Xin X, Taylor DA (2007) Sex-dependent attenuation of plaque growth after treatment with bone marrow mononuclear cells. *Circ Res* 101(12):1319–1327. doi:10.1161/circresaha.107.155564
- Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussen V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD (2003) Cardiac progenitor cells from adult myocardium: Homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A* 100(21):12313–12318. doi:10.1073/pnas.2132126100
- Olivetti G, Giordano G, Corradi D, Melissari M, Lagrasta C, Gambert SR, Anversa P (1995) Gender differences and aging: effects on the human heart. *J Am Coll Cardiol* 26(4):1068–1079
- Orlic D, Fischer R, Nishikawa S, Nienhuis AW, Bodine DM (1993) Purification and characterization of heterogeneous pluripotent hematopoietic stem cell populations expressing high levels of c-kit receptor. *Blood* 82(3):762–770
- 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 (2001) Bone marrow cells regenerate infarcted myocardium. *Nature* 410(6829):701–705
- Oskoue BN, Lamirault G, Joseph C, Treuer AV, Landa S, Da Silva J, Hatzistergos K, Dauer M, Balkan W, McNiece I, Hare JM (2012) Increased potency of cardiac stem cells compared with bone marrow mesenchymal stem cells in cardiac repair. *Stem Cells Transl Med* 1(2):116–124. doi:10.5966/sctm.2011-0015
- Ott HC, Matthiesen TS, Brechtken J, Grindle S, Goh SK, Nelson W, Taylor DA (2007) The adult human heart as a source for stem cells: repair strategies with embryonic-like progenitor cells. *Nat Clin Pract Cardiovasc Med* 4(Suppl 1):S27–S39
- Parker TG, Chow KL, Schwartz RJ, Schneider MD (1990a) Differential regulation of skeletal alpha-actin transcription in cardiac muscle by two fibroblast growth factors. *Proc Natl Acad Sci U S A* 87(18):7066–7070
- Parker TG, Packer SE, Schneider MD (1990b) Peptide growth factors can provoke “fetal” contractile protein gene expression in rat cardiac myocytes. *J Clin Invest* 85(2):507–514. doi:10.1172/JCI114466
- Passier R, Mummery C (2003) Origin and use of embryonic and adult stem cells in differentiation and tissue repair. *Cardiovasc Res* 58(2):324–335
- Penn MS, Francis GS, Ellis SG, Young JB, McCarthy PM, Topol EJ (2002) Autologous cell transplantation for the treatment of damaged myocardium. *Prog Cardiovasc Dis* 45(1):21–32
- Penn MS, Mangi AA (2008) Genetic enhancement of stem cell engraftment, survival, and efficacy. *Circ Res* 102(12):1471–1482. doi:10.1161/CIRCRESAHA.108.175174
- Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA (2011) Transient regenerative potential of the neonatal mouse heart. *Science* 331(6020):1078–1080. doi:10.1126/science.1200708
- Pouly J, Bruneval P, Mandet C, Proksch S, Peyrard S, Amrein C, Bousseaux V, Guillemain R, Deloche A, Fabiani J-N, Menasché P (2008) Cardiac stem cells in the real world. *J Thorac Cardiovasc Surg* 135(3):673–678
- Quaini F, Urbanek K, Graiani G, Lagrasta C, Maestri R, Monica M, Boni A, Ferraro F, Delsignore R, Tasca G, Leri A, Kajstura J, Quaini E, Anversa P (2004) The regenerative potential of the human heart. *Int J Cardiol* 95(Suppl 1):S26–S28. doi:10.1016/S0167527304900083 [pii]
- Rota M, Padin-Iruegas ME, Misao Y, De Angelis A, Maestroni S, Ferreira-Martins J, Fiumana E, Rastaldo R, Arcarese ML, Mitchell TS, Boni A, Bolli R, Urbanek K, Hosoda T, Anversa P, Leri A, Kajstura J (2008) Local activation or implantation of cardiac progenitor cells rescues scarred infarcted myocardium improving cardiac function. *Circ Res* 103(1):107–116. doi:10.1161/CIRCRESAHA.108.178525
- Ruvinov E, Leor J, Cohen S (2011) The promotion of myocardial repair by the sequential delivery of IGF-1 and HGF from an injectable alginate biomaterial in a model of acute myocardial infarction. *Biomaterials* 32(2):565–578. doi:10.1016/j.biomaterials.2010.08.097
- Shneyvays V, Nawrath H, Jacobson KA, Shainberg A (1998) Induction of apoptosis in cardiac myocytes by an A3 adenosine receptor agonist. *Exp Cell Res* 243(2):383–397. doi:10.1006/excr.1998.4134

- Smart N, Risebro CA, Melville AA, Moses K, Schwartz RJ, Chien KR, Riley PR (2007) Thymosin beta-4 is essential for coronary vessel development and promotes neovascularization via adult epicardium. *Ann N Y Acad Sci* 1112:171–188. doi:annals.1415.000 [pii] 10.1196/annals.1415.000
- Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, Giacomello A, Abraham MR, Marban E (2007) Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation* 115(7):896–908. doi:10.1161/circulationaha.106.655209
- Spalding KL, Bhardwaj RD, Buchholz BA, Druid H, Frisen J (2005) Retrospective birth dating of cells in humans. *Cell* 122(1):133–143. doi:10.1016/j.cell.2005.04.028
- Sussman MA, Anversa P (2004) Myocardial aging and senescence: where have the stem cells gone? *Ann Rev Physiol* 66(1):29–48. doi:10.1146/annurev.physiol.66.032102.140723
- Takehara N, Tsutsumi Y, Tateishi K, Ogata T, Tanaka H, Ueyama T, Takahashi T, Takamatsu T, Fukushima M, Komeda M, Yamagishi M, Yaku H, Tabata Y, Matsubara H, Oh H (2008) Controlled delivery of basic fibroblast growth factor promotes human cardiosphere-derived cell engraftment to enhance cardiac repair for chronic myocardial infarction. *J Am Coll Cardiol* 52(23):1858–1865
- Tang XL, Rokosh G, Sanganalmath SK, Yuan F, Sato H, Mu J, Dai S, Li C, Chen N, Peng Y, Dawn B, Hunt G, Leri A, Kajstura J, Tiwari S, Shirk G, Anversa P, Bolli R (2010) Intracoronary administration of cardiac progenitor cells alleviates left ventricular dysfunction in rats with a 30-day-old infarction. *Circulation* 121(2):293–305. doi:CIRCULATIONAHA.109.871905 [pii] 10.1161/CIRCULATIONAHA.109.871905
- Urbanek K, Quaini F, Tasca G, Torella D, Castaldo C, Nadal-Ginard B, Leri A, Kajstura J, Quaini E, Anversa P (2003) From the cover: intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. *PNAS* 100(18):10440–10445. doi:10.1073/pnas.1832855100
- Urbanek K, Rota M, Cascapera S, Bearzi C, Nascimbene A, De Angelis A, Hosoda T, Chimenti S, Baker M, Limana F, Nurzynska D, Torella D, Rotatori F, Rastaldo R, Musso E, Quaini F, Leri A, Kajstura J, Anversa P (2005a) Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. *Circ Res* 97(7):663–673. doi:10.1161/01.res.0000183733.53101.11
- Urbanek K, Torella D, Sheikh F, De Angelis A, Nurzynska D, Silvestri F, Beltrami CA, Bussani R, Beltrami AP, Quaini F, Bolli R, Leri A, Kajstura J, Anversa P (2005b) Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. *PNAS* 102(24):8692–8697. doi:10.1073/pnas.0500169102
- Zaruba MM, Soonpaa M, Reuter S, Field LJ (2010) Cardiomyogenic potential of C-kit(+)-expressing cells derived from neonatal and adult mouse hearts. *Circulation* 121(18):1992–2000. doi:10.1161/CIRCULATIONAHA.109.909093
- Zhou YY, Wang SQ, Zhu WZ, Chruscinski A, Kobilka BK, Ziman B, Wang S, Lakatta EG, Cheng H, Xiao RP (2000) Culture and adenoviral infection of adult mouse cardiac myocytes: methods for cellular genetic physiology. *Am J Physiol Heart Circ Physiol* 279(1):H429–H436
- Zhou B, Ma Q, Rajagopal S, Wu SM, Domian I, Rivera-Feliciano J, Jiang D, von Gise A, Ikeda S, Chien KR, Pu WT (2008) Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. *Nature* 454(7200):109–113. doi:nature07060 [pii] 10.1038/nature07060
- Zimmet H, Krum H (2008) Using adult stem cells to treat heart failure-fact or fiction? *Heart Lung Circ* 17(Suppl 4):S48–S54

Adult and Pluripotent Stem Cells
Potential for Regenerative Medicine of the
Cardiovascular System

Hescheler, J.; Hofer, E. (Eds.)

2014, XII, 170 p. 24 illus., 22 illus. in color., Hardcover

ISBN: 978-94-017-8656-0