Cardiac Stem Cells as Biomarkers

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Abstract

This chapter discusses the importance that the discovery of resident cardiac stem cells has had and continues to have on our evolving understanding of myocardial biology. Cardiac stem cells have acquired a progressively critical role in myocardial aging and heart failure suggesting that both these processes may be viewed as stem cell diseases. We have focused on the molecular signature and the telomere-telomerase axis to define novel biomarkers able to characterize the growth reserve of the intact and pathologic heart, major determinant of the adaptive and maladaptive response of the myocardium.

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Keywords
Biomarkers • c-kit • Clonal growth • Cell-based therapy • Heart failure

Abbreviations

Abcg2/Bcrp1/Mdr1	ATP-binding cassette transporters, subfamily G
BMPCs	Bone marrow progenitor cells
CHD	Coronary heart disease
c-kit-CPCs	c-kit-positive cardiac progenitor cells
c-kit-CSCs	c-kit-positive cardiac stem cells
CPCs	Cardiac progenitor cells
ECs	Vascular endothelial cells
EF	Ejection fraction
hCPCs	Human cardiac progenitor cells
hCSCs	Human CSCs
LTL	Leukocyte telomere length
MACE	Major adverse cardiac events
MSCs	Mesenchymal stem cells
PDs	Population doublings
Sca1-CPCs	Sca1-positive cardiac progenitor cells
SMCs	Vascular smooth muscle cells
SP	Side population
SP-CPCs	Side population cardiac progenitor cells
Tbx18	T-box18
TIFs	Telomere dysfunction-induced foci
TL	Mean telomere length
WT1	Wilms tumor 1

Key Facts of Cardiac Stem Cells

- The primary function of CSCs is the control of cell turnover and myocardial regeneration.
- The decline in the growth of CSCs is dictated by defects in the telomere-telomerase system.
- The molecular signature of CPC classes has emphasized the critical role that c-kit expression has in defining the undifferentiated cell phenotype.
- Telomere dysfunction has a critical negative impact of CSC behavior and growth.
- Telomere shortening predicts CSC senescence and is an independent predictor of cardiovascular aging and disease.

Definitions

Clonogenicity Clonogenicity corresponds to the ability of individual stem cells to form a cluster of daughter cells identical to the mother cell.

CPCs CPCs are less undifferentiated than CSCs.

CSCs CSCs are a rare population of resident myocardial cells regulating organ homeostasis and repair following injury.

Holoclones, meroclones, and paraclones Holoclones, meroclones, and paraclones indicate, respectively, cell clusters derived from stem cells, early committed cells, and late-stage transit-amplifying cells.

Multipotency Multipotency reflects the ability of stem cells to generate all specialized cell lineages within the tissue.

Self-renewal Self-renewal reflects the ability of stem cells to undergo long-term proliferation and asymmetric division.

Symmetric and asymmetric division Stem cells can divide symmetrically forming two identical daughter cells and asymmetrically generating two differently fated sibling cells.

Telomerase Telomerase is a reverse transcriptase that synthesizes telomeric repeats utilizing its own RNA as a template.

Telomeres Telomeres are double-stranded highly repetitive DNA sequences located at the end of chromosomes.

Whole genome expression profile The global expression profile of cells can be determined by microarray analysis of transcripts.

Introduction

In a well-known editorial, Morrow and de Lemos defined the three criteria that a clinically valuable novel biomarker should meet: (1) the measurement of the biomarker should rely on analytical methods providing the possibility to perform repeated, accurate, and reliable assessments; (2) the biomarker should offer information that cannot be achieved through clinical evaluation; and (3) the ideal biomarker should help in the diagnosis of the disease, the identification of subjects at risk, the monitoring of the response to therapy, and the prognosis of the disease (Morrow and Lemos 2007). Collectively, these parameters constitute the components of the medical decision-making process (Braunwald 2008, 2009).

The majority of biomarkers used in clinical practice do not satisfy the three criteria, but the aggregate evidence provided by clusters of biomarkers typically reveals important variables concerning the etiology, pathophysiology, and/or the progression of the disease (Morrow and Lemos 2007). The advancements in stem cell biology and the implementation of cell-based therapies pose novel challenges

for the search of relevant biomarkers. Stem cell-related biomarkers can be divided in two classes. The first category comprises the identifiers of the phenotype and functional competence of stem cells. This information has to be utilized in the process of quality control of the product, i.e., progenitor cells, prior to administration to patients. Moreover, the evaluation of the phenotypical and functional state of resident stem cells offers evidence related to the residual growth reserve of the organ and, ultimately, may prove or disprove the concept that pathological conditions correspond to stem cell diseases. The second category includes classic biomarkers for the monitoring of the response to cell-based therapy and the tracking of the evolution of the disease.

Multiple classes of stem and progenitor cells have been recognized in the adult myocardium by using surface and intracellular epitopes, functional assays, and their combination (Leri et al. 2015). *Bona fide* adult tissue-specific stem cells are defined by two biomarkers of "stemness": (1) self-renewal, i.e., the ability to divide and form a daughter stem cell that possesses the same properties of the mother cell, and (2) multipotency, i.e., the ability to differentiate in all specialized lineages within a given organ. Optimal biomarkers for the identification and characterization of cardiac progenitor cells (CPCs) comprise membrane antigens, which allow the isolation of the cells from the tissue where they reside and components of signaling pathways involved in the regulation of stem cell growth and commitment.

c-kit-Positive CPCs

More than a decade ago, cells expressing the surface biomarker c-kit were recognized as a population of stem cells residing in the heart of animals and humans (Beltrami et al. 2003; Bearzi et al. 2007). The receptor tyrosine kinase c-kit identifies a pool of CPCs that are self-renewing and multipotent in vitro and in vivo (Beltrami et al. 2003; Angert et al. 2011; Cottage et al. 2012; Fischer et al. 2009; D'Amario et al. 2011; Goichberg et al. 2013; Hariharan et al. 2015). Myocardial cells that possess the properties of stem cells are characterized not only by the presence of c-kit, but also by the absence of bone marrow and cardiac lineage epitopes. A rare population of c-kit-positive CD45-positive bone marrow-derived cells is present in the adult myocardium (Dey et al. 2013; Sanada et al. 2014). These hematopoietic-committed cells may migrate to the heart acquiring temporary or long-term residence. The combination of c-kit and CD45 is also found on the membrane of mast cells, which express inflammatory mediators and proteases, including tryptase and chymase (Sperr et al. 1994). Freshly isolated c-kit-positive CPCs (c-kit-CPCs) are negative for transcription factors and specialized proteins specific for cardiac and vascular fate. The nuclear localization of Nkx2.5, GATA4, and MEF2C and the cytoplasmic distribution of poorly organized sarcomeres recognize early committed c-kit-CPCs that are destined to become cardiomyocytes (Urbanek et al. 2006; Bearzi et al. 2007; Fischer et al. 2009; Hariharan et al. 2015; Sanada et al. 2014). This process of progressive restriction of developmental options (Fig. 1) is equally

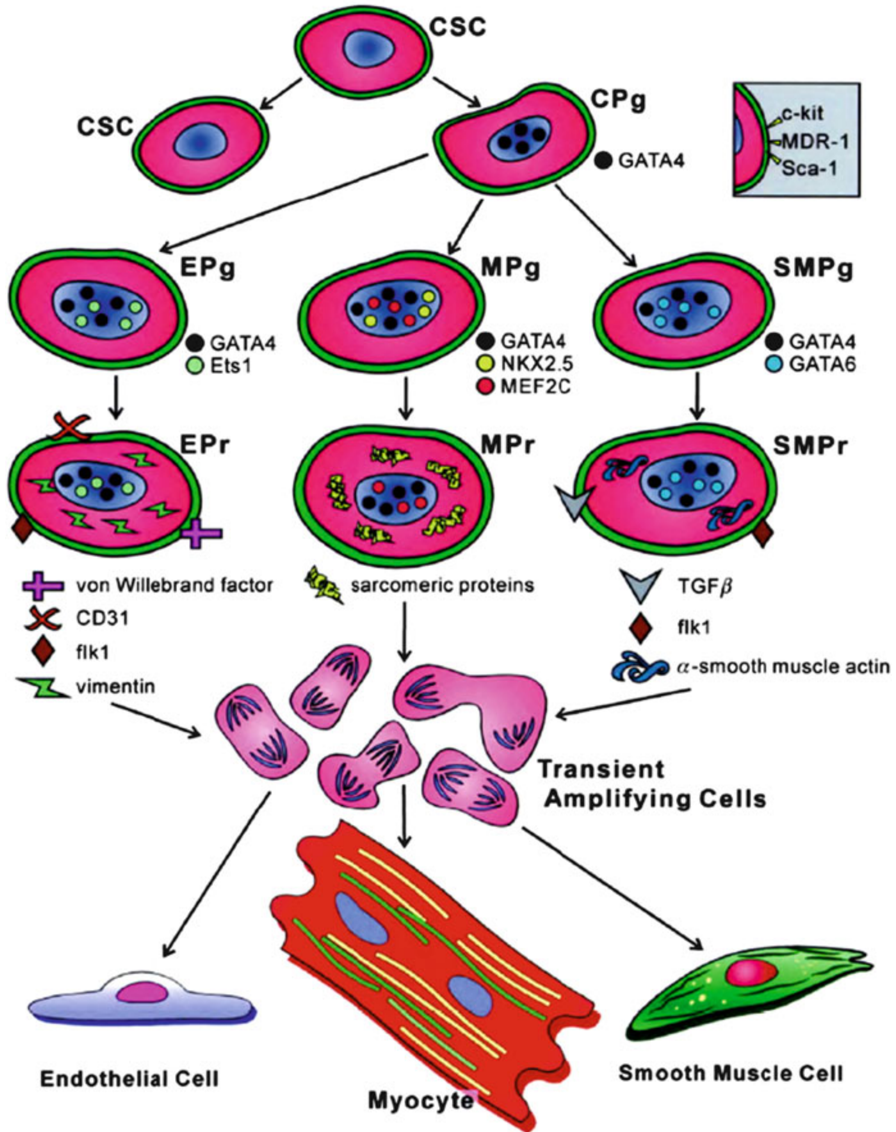


Fig. 1 **Cardiac cells are organized hierarchically**. Asymmetrical division of a CSC into a daughter CSC and a daughter cardiac progenitor (*CPg*). CPg gives rise to myocyte progenitor (*MPg*) and precursor (*MPr*), EC progenitor (*EPg*) and precursor (*EPr*), and SMC progenitor (*SMPg*) and precursor (*SMPr*). Precursors become transient amplifying cells, which divide and differentiate into mature myocytes, ECs, and SMCs. CSCs are lineage-negative cells that express only c-kit, MDR1, or Sca-1. Progenitors express stem cell antigens and transcription factors of cardiac cells but do not exhibit specific cytoplasmic proteins. Precursors possess stem cell antigens, transcription factors, and membrane and cytoplasmic proteins typical of myocytes, ECs, and SMCs. Amplifying cells have nuclear, cytoplasmic, and membrane proteins of cardiac cell lineages but are negative for stem cell antigens (From Anversa et al. 2006)

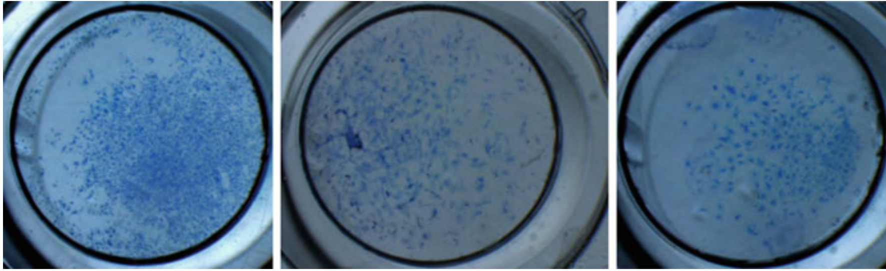


Fig. 2 c-kit-positive cardiac cells form clones with different phenotype. Phase contrast images representative of CSC-derived clones. Clones have different shape and size. Cells are stained with Evans *blue*

operative during the formation of vascular endothelial cells (ECs) and smooth muscle cells (SMCs) and fibroblasts (Anversa et al. 2006).

Lineage-negative c-kit-CPCs undergo serial symmetric divisions in vitro with the formation of daughter stem cells identical to the mother cell (Bearzi et al. 2007; Hariharan et al. 2015). This modality of clonal growth is documented by seeding single c-kit-positive cells in individual wells of Terasaki plates. The characteristics of the clone can be viewed as a biomarker of the functional properties of the parental c-kit-positive cell. Primary cells in culture can form holoclones, meroclones, and paraclones (Jones et al. 2007; Beaver et al. 2014). The fundamental difference among these types of clones is the degree of stemness of the founder cell. Holoclones, which are round in shape and contain a high number of daughter cells, are generated by stem cells capable of undergoing extensive proliferation and self-renewal. Meroclones, which possess an irregular shape, derive from cells with low replicative capacity and self-renewal ability. Paraclones are small in size and are generated by mother cells that have exhausted their growth reserve and rapidly undergo cell cycle withdrawal (Fig. 2). Thus, holoclones, meroclones, and paraclones indicate, respectively, cell clusters derived from stem cells, early committed cells, and late-stage transit-amplifying cells (Jones et al. 2007).

Cardiac c-kit-positive cells form all three types of clones. Large cell clusters that can be expanded to reach a high number of daughter cells are formed by *bona fide* c-kit-positive cardiac stem cells (c-kit-CSCs). In this case, subclones can be obtained upon plating of individual clonal daughter cells (Beltrami et al. 2003). At times, c-kit-positive cardiac cells generate very small colonies, reflecting a stage of early or late commitment; terminal differentiation ensues in a short period of time, indicating that the founder cell has the predicted properties of a precursor (Cesselli et al. 2011). The cells of origin are characterized by low levels of c-kit expression and by the presence of cardiovascular transcription factors and cytoplasmic proteins. These precursors rapidly evolve to the transit-amplifying state giving rise to a small aggregate of cells that are negative for c-kit. These observations underscore the importance of the accurate definition of clones, the thorough characterization of the cell aggregates, and the careful assessment of cloning efficiency. Daughter cells in the stem cell-derived clone preserve their undifferentiated lineage-negative

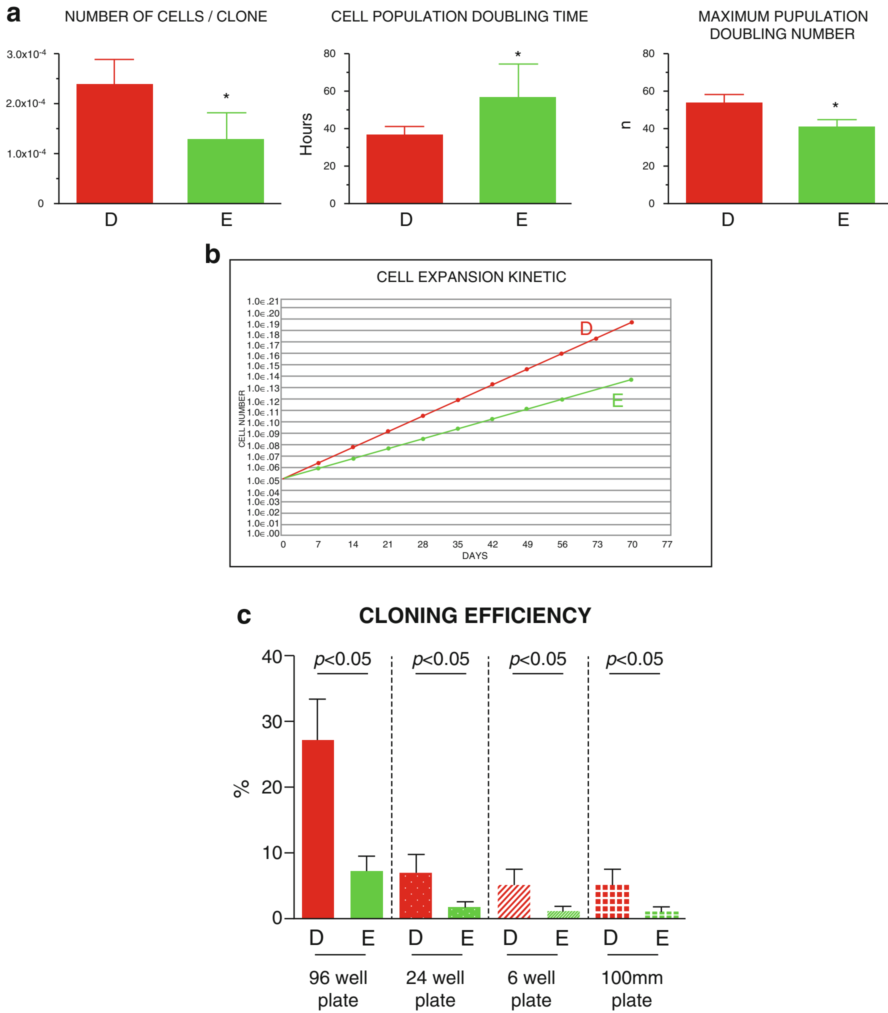


Fig. 3 Human CSCs obtained from control and failing hearts have different functional properties. (**a**) Parameters of cell growth. Results are mean ± SD. *$P < 0.05$ versus donor hearts (*D*). E: Explanted hearts. (**b**) The rate of hCSC expansion is shown in semi-logarithmic scale. (**c**) Cloning efficiency of hCSCs (From Cesselli et al. 2011)

phenotype, continue to express the stem cell antigen, and retain the self-renewal ability and multipotentiality of the founder cell.

To define the effects of disease on CPC clonogenicity, fluorescence activated cell sorting (FACS)-sorted individual c-kit-positive cells were deposited in single wells of 96 multiwell plates (Cesselli et al. 2011). Of 3,682 seeded wells, human CSCs (hCSCs) collected from explanted hearts gave rise to 237 colonies of ~100–200 cells. However, the majority of these small cell clusters underwent growth arrest;

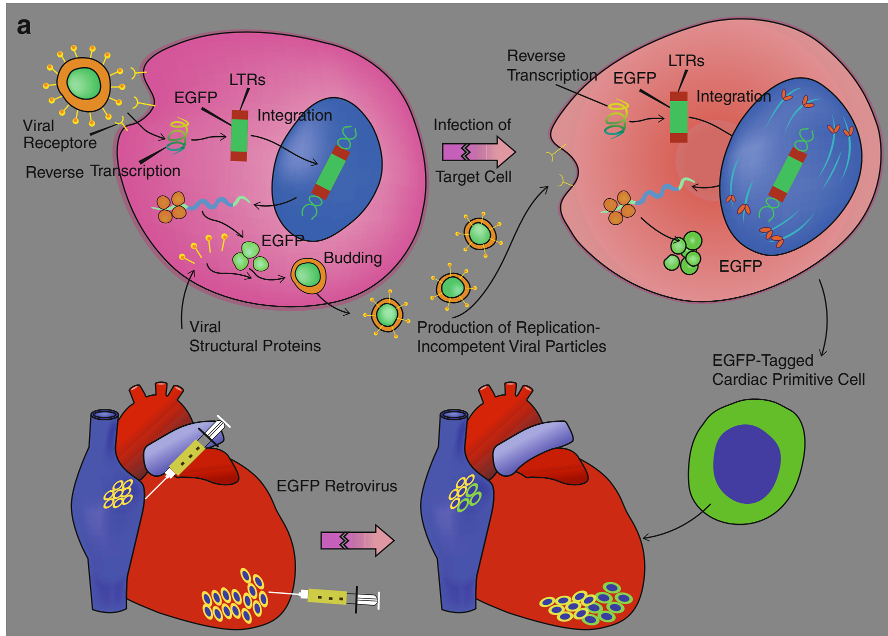
only 39 actively proliferating clones were passaged to 24-well plates where they formed clusters of 40,000–60,000 cells. Nearly 30 % of these colonies stopped growing, while the remaining 70 % were transferred to 6-well plates and continued to replicate undergoing an additional 18 population doublings (PDs) without reaching replicative senescence (Fig. 3a–d). Thus, the actual cloning efficiency of hCSCs from failing hearts was 0.8 % (Fig. 3e). A comparable approach and magnitude of sampling were employed with hCSCs from normal hearts. A cloning efficiency nearly threefold higher was found (Fig. 3e). These clones expanded exponentially for more than 20 PDs (Cesselli et al. 2011).

Evidence of the multipotentiality of c-kit-CSCs can be obtained by exposing clonal cells to differentiation media (Mohsin et al. 2013; Leri et al. 2015). Myocytes, ECs, SMCs, and fibroblasts are formed in various proportions. Typically, CSCs differentiate predominantly into cardiomyocytes and to a lesser extent into ECs and SMCs. Differentiation assays of stem cell clones in vitro have inherent limitations including the possibility that culture conditions result in the preferential acquisition of a selective lineage phenotype, masking the full potential of the founder cell. Similarly, the identification in vivo of multiple cell categories in the progeny of transplanted non-clonal stem cells does not provide direct evidence of the multipotentiality of each delivered cell. This problem can be overcome by the transplantation of clonal populations of CSCs in animal models of the human disease (Beltrami et al. 2003; Bearzi et al. 2007) and by viral gene marking (Hosoda et al. 2009) (Fig. 4).

The adoptive transfer assays have documented that the generation of cardiomyocytes in vivo markedly exceeds the number of cells lost by myocardial infarction. Additionally, a large number of resistance coronary arterioles and a relatively low number of capillary structures develop, resulting in the formation of new myocardial tissue that resembles structurally and functionally the parenchyma of the neonatal heart. The hyperplastic phenotype of the new myocytes and their small size suggest that cardiac repair follows a pattern of growth that reiterates the expected evolutionary changes coupled with the activation of a stem cell (Leri et al. 2015). The reconstitution of myocardium within the necrotic or scarred region reduces proportionally the extent of damage, reversing partly ventricular dilation and thinning of the wall. However, the normal architecture and orientation of myocyte bundles across the wall are not typically acquired.

In the majority of cases, short intervals after c-kit-CSC treatment have been studied, raising the critical question whether young myocytes could become fully mature adult cells with time. This process, which requires a significant increase in cell volume with the insertion of new myofibrils occupying the expanding cytoplasm, may be time dependent. In the acute phases of cardiac repair, in situ activation of resident c-kit-CPCs results in the commitment to the myocyte lineage and formation of small amplifying myocytes with a volume of ~2,000 μm^3 (Urbanek et al. 2005). However, 4 months later, myocyte size averaged ~5,000 μm^3, and nearly 10 % of myocytes reached the adult phenotype, 10,000 μm^3 in volume or larger.

In all organs, stem cells are relatively rare; the frequency of c-kit-CPCs in animals and humans, 1 every 30,000–40,000 myocardial cells (Beltrami et al. 2003; Urbanek et al. 2005; Bearzi et al. 2007), is consistent with that of hematopoietic stem cells in



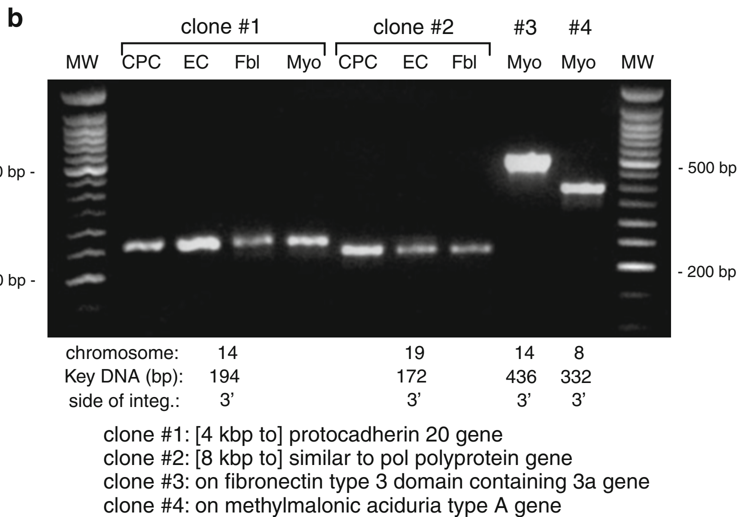


Fig. 4 The self-renewal ability, clonogenicity, and multipotency of CPCs in situ were documented by viral genetic tagging. (**a**) Schematic representation of the protocol employed for clonal marking of mouse CPCs in situ. Infection of CPCs in situ with EGFP retroviruses or EGFP lentiviruses results in the semi-random insertion of the proviral integrant in the genome of the recipient cell. Transcription and translation of the viral DNA result in expression of EGFP and fluorescent labeling of the infected CPC. The unique insertion site of the viral genome is inherited by the entire population derived from the parental cell and can be amplified by PCR. CPCs nested in

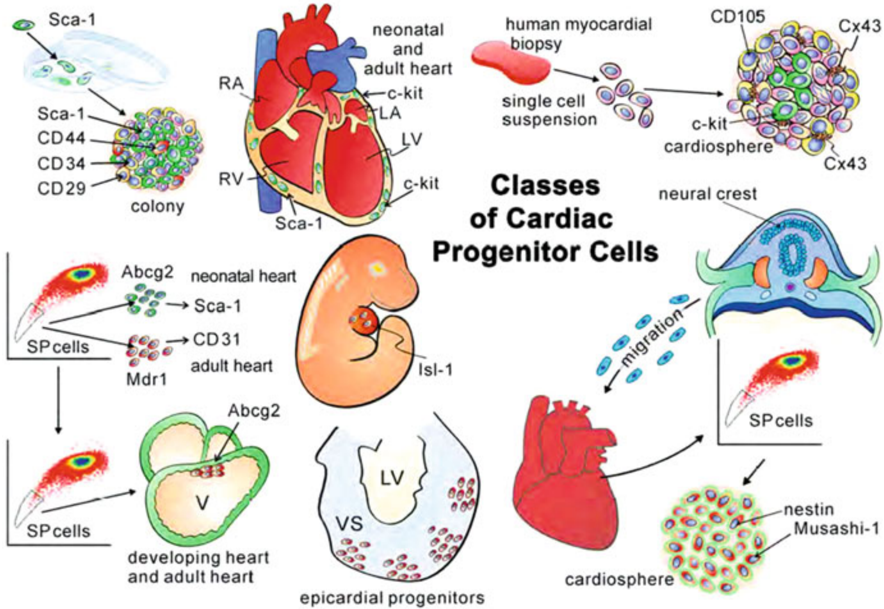


Fig. 5 **The developing and adult heart contains multiple classes of CPCs**. Schematic representation of CPC populations

the bone marrow, 1 every 10,000–100,000 (Shepherd et al. 2007). c-kit-CPCs are present in the entire ventricular myocardium, but are preferentially distributed to the atria and apex (Urbanek et al. 2006). The adult heart typically shows interstitial structures with the architectural organization of stem cell niches. c-kit-CPCs are functionally coupled to myocytes and fibroblasts by adherens junctions expressing N- and E-cadherins and by gap junctions expressing connexin 43 and 45 (Leri et al. 2014). Because of this anatomical configuration, myocytes and fibroblasts operate as supporting cells within the cardiac niches, which provide the necessary permissive milieu for the long-term residence, survival, and growth of CPCs. The function of cardiomyocytes as modulators of c-kit-CPC growth and differentiation has been documented in vitro and in vivo.

Fig. 4 (continued) atrial and apical niches were labeled in situ to identify their progeny in vivo. One to 6 months later. The heart was enzymatically dissociated to obtain cardiomyocytes, c-kit-CPCs, ECs, and fibroblasts. (**b**) Four distinct clones were identified in EGFP-tagged CPCs, ECs, fibroblasts (*Fbl*), and cardiomyocytes (*Myo*) isolated from the ventricle of one mouse heart. Multiple PCR products (bands in agarose gel) were identified. Bands of the same molecular weight correspond to identical sites of integration of the proviral sequence in the host genome of CPCs, myocytes, ECs, and fibroblasts, documenting the multipotency of CSCs in vivo

Myocardial Progenitors

Different classes of progenitor cells have been characterized in the adult heart, but whether they represent distinct categories of undifferentiated cells with diverse functional properties is currently unknown (Fig. 5). The first identification of myocardial progenitors was based on the ability of stem cells to expel toxic compounds and dyes through an ATP-binding cassette transporter (Hierlihy et al. 2002). This property, used initially to isolate side population (SP) hematopoietic cells (Challen and Little 2006), defines a pool of putative cardiac progenitors that form colonies in semisolid media and differentiate into cardiomyocytes. Depletion of cardiac SP cells occurs after infarction in mice overexpressing a dominant-negative MEF2C, documenting that SP cells are committed to the myocyte lineage. This work introduced the concept of a myocardial stem cell that participates in the response of the heart to ischemic injury (Hierlihy et al. 2002).

Evidence of ABC-transporter activity can be obtained by exposing cardiac cells to the DNA-binding dye Hoechst 33342; functionally competent cells clear the fluorochrome, become Hoechst-low, and occupy a side position in the FACS profile (Pfister et al. 2010; Lin and Goodell 2011). SP cells, which are Sca1^{high}, c-kit^{low}, CD34^{low}, and CD45^{low}, comprise 2 % of cardiac cells in the mouse heart. The ability to extrude dyes is attributed to the expression of the multidrug resistance protein Abcg2/Bcrp1 (Martin et al. 2004, 2008). However, most Bcrp1-positive cells express CD31 and are located within the intima of the vessel wall. After injury, SP cells generate predominantly vimentin-positive fibroblasts and calponin-positive SMCs; only few cells acquire the myocyte and EC lineage. A subset of SP cells expresses markers of neural precursors, including nestin and Musashi-1; they form in vitro neuron-like dendrites and peripheral nerve cells (Tomita et al. 2005). These SP cells correspond to embryonic fetal remnants of neural crest-derived cells.

By introducing a novel protocol, an interesting class of SP cardiac progenitor cells (SP-CPCs) was identified (Sereti et al. 2013). Only the Sca1-positive CD31-negative subset of SP-CPCs retained a high cardiomyogenic potential (Pfister et al. 2005). Abcg2 promotes proliferation and survival of SP cells inhibiting differentiation (Sereti et al. 2013). Dysregulation of Abcg2 may alter the fate of these progenitors, resulting in uncontrolled cell growth or death. Bone marrow SP cells do not contribute to the maintenance of the SP-CPCs in physiological conditions, but can repopulate the resident pool after injury (Mouquet et al. 2005).

State-of-the-art imaging protocols have been developed to track in vivo the destiny of Sca1-positive cardiac progenitor cells (Sca1-CPCs). These cells were isolated from transgenic mice that constitutively express luciferase and enhanced green fluorescent protein (EGFP), enabling in vivo tracking by noninvasive imaging and postmortem identification by immunolabeling (Li et al. 2009; Swijnenburg et al. 2010). A strong bioluminescence signal was detected at 2 days after cell delivery; however, this signal decayed rapidly with time. The nonviable portion of the ventricular wall, which was studied by (18F)-FDG positron emission tomography scan, was not reduced in cell-treated mice. Consistently, echocardiographic and MRI analyses did not show functional improvement, and a very small number of

EGFP-positive cardiomyocytes and vascular structures were found. Poor survival and massive apoptosis of the delivered cells are commonly observed when neonatal cardiomyocytes, mesenchymal stem cells (MSCs), bone marrow mononuclear cells, and human embryonic stem cell-derived cardiomyocytes are adoptively transferred (Dowell et al. 2003; Leri et al. 2005; Laflamme et al. 2007; Robey et al. 2008; Wiliams and Hare 2011; Quijada et al. 2012; Siddiqi and Sussman 2013). This phenomenon has prompted the development of novel strategies involving preactivation with growth factors, application of bioengineering methods, and genetic modifications to achieve long-term homing to the injured myocardium (Laflamme et al. 2007; Tillmanns et al. 2008; Siddiqi and Sussman 2013). Collectively, the results with SP cells and Sca1-CPCs suggest that a small pool of primitive cells, distinct from c-kit-positive CPCs, is present in the myocardium. Genetic deletion of Sca1 alters the function of resident c-kit-CPCs, leading to premature alterations in cardiac performance and poor tolerance of the heart to stress (Bailey et al. 2012).

An alternative source of progenitor cells has been found in the epicardium, which represents an epithelial sheet on the cardiac surface. The epicardial marker Wt1 regulates the epithelial-mesenchymal transition (von Gise et al. 2011). Wt1-positive progenitors travel from the proepicardium to the myocardium where they form the epicardium and electrically coupled cardiomyocytes (Zhou et al. 2008). Moreover, a population of proepicardial Tbx18-positive progenitors may give rise to a substantial fraction of cardiomyocytes (Cai et al. 2008). A recent study has challenged the initial findings obtained with Wt1-based Cre/LoxP strategy (Rudat and Kispert 2012). Limitations have been identified in the lineage-tracing mouse employed. They include the poor recombination efficiency of Wt1-positive cells, the lack of specificity of the promoter due to the endogenous extra-epicardial localization of Wt1 in ECs, and the possibility of ectopic recombination (Rudat and Kispert 2012; Zhou and Pu 2012).

A pool of c-kit-positive epicardial cells has been identified in the human infarcted heart (Castaldo et al. 2008). Experimentally, c-kit-positive epicardial cells migrate from the epicardium to the infarcted myocardium, where they proliferate and differentiate into myocyte precursors and vascular cells (Limana et al. 2007). This process is coupled with upregulation of fetal epicardial markers. The recognition of growth factors modulating the behavior of epicardial progenitors may allow their in situ activation, possibly influencing the treatment of the human disease.

Expansion of cardiac cells from human endomyocardial biopsies leads to the formation of floating spheres. Cardiospheres contain a small core of c-kit-positive primitive cells, several layers of differentiating cells expressing myocyte proteins and connexin 43, and an outer sheet composed of MSCs (Smith et al. 2007; Davis et al. 2010; Lee et al. 2011). c-kit-positive cells within the aggregates do not correspond to a uniform class of progenitors because of their heterogeneity dictated by the uncommitted or early committed state, their quiescent or cycling condition, and their migratory properties. This may explain the observed differences in the regenerative potential of single-cell-derived clonal c-kit-CSCs (Beltrami et al. 2003) and c-kit-positive cells sorted from cardiospheres (Cheng et al. 2014).

In summary, different progenitor cell categories may participate in myocardial homeostasis and repair after damage. The surface antigens, transcription factors, and functional assays discussed above constitute novel biomarkers that allow the characterization of resident myocardial progenitors. This information may provide important insights on the etiology, pathophysiology, and evolution of cardiac diseases. Currently, two distinct classes of cardiac-derived cells have been tested clinically, c-kit-CPCs and cardiosphere-derived cells (Bolli et al. 2011; Makkar et al. 2012). The phase 1 trial SCIPIO (Stem Cell Infusion in Patients with Ischemic cardiOmyopathy) involved the delivery of autologous c-kit-positive human CPCs (hCPCs) for the treatment of severe chronic heart failure of ischemic origin (Bolli et al. 2011). Patients with ejection fraction (EF) lower than 40 % at 4 months after coronary artery bypass grafting were enrolled in the treatment and control groups. Treated patients received a single intracoronary infusion of one million autologous hCPCs. Importantly, no adverse effects were reported in the 14 patients treated with hCPCs. EF increased from 30 % to 38 % at 4 months after infusion. The beneficial effects of CPCs were even more pronounced at 1 year and remained stable thereafter. In treated patients, infarct size decreased 24 % and 30 % at 4 and 12 months, respectively.

The prospective, randomized CADUCEUS (CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction) trial included patients with subacute myocardial infarction and 25–45 % EF (Makkar et al. 2012). Autologous cells grown from endomyocardial biopsy specimens were infused into the infarct-related artery 1.5–3 months after infarction. The primary endpoint consisted of the proportion of patients at 6 months who died due to arrhythmic events or had myocardial infarction after cell infusion, new cardiac tumor, or a major adverse cardiac event (MACE). Additionally, preliminary data concerning the efficacy of the treatment were collected by MRI at 6 months. At baseline, mean EF was 39 % and the scar occupied 24 % of the left ventricular mass. At 6 months, no patients had died, developed cardiac tumors, or MACE in either group. However, four patients in the cell-treated group had serious adverse events compared with one control. Cell therapy resulted in a reduction in scar mass, increase in viable heart mass, and enhanced regional contractility and regional systolic wall thickening. End-diastolic volume, end-systolic volume, and EF did not differ between treated and untreated groups. The initial results of SCIPIO and CADUCEUS trials are highly encouraging and warrant further, larger, phase 2 studies.

Molecular Signature of CPCs

The discovery of multiple CPC classes in the adult myocardium is intriguing, but it may not be surprising if we consider other stem cell-regulated tissues. The implementation of complementary strategies has led to the identification of distinct pools of progenitor cells in several organs, including the small intestine, the skin, and the brain. Side-by-side comparisons of the characteristics of different CPC types have rarely been performed, leaving unanswered the question whether the several cell

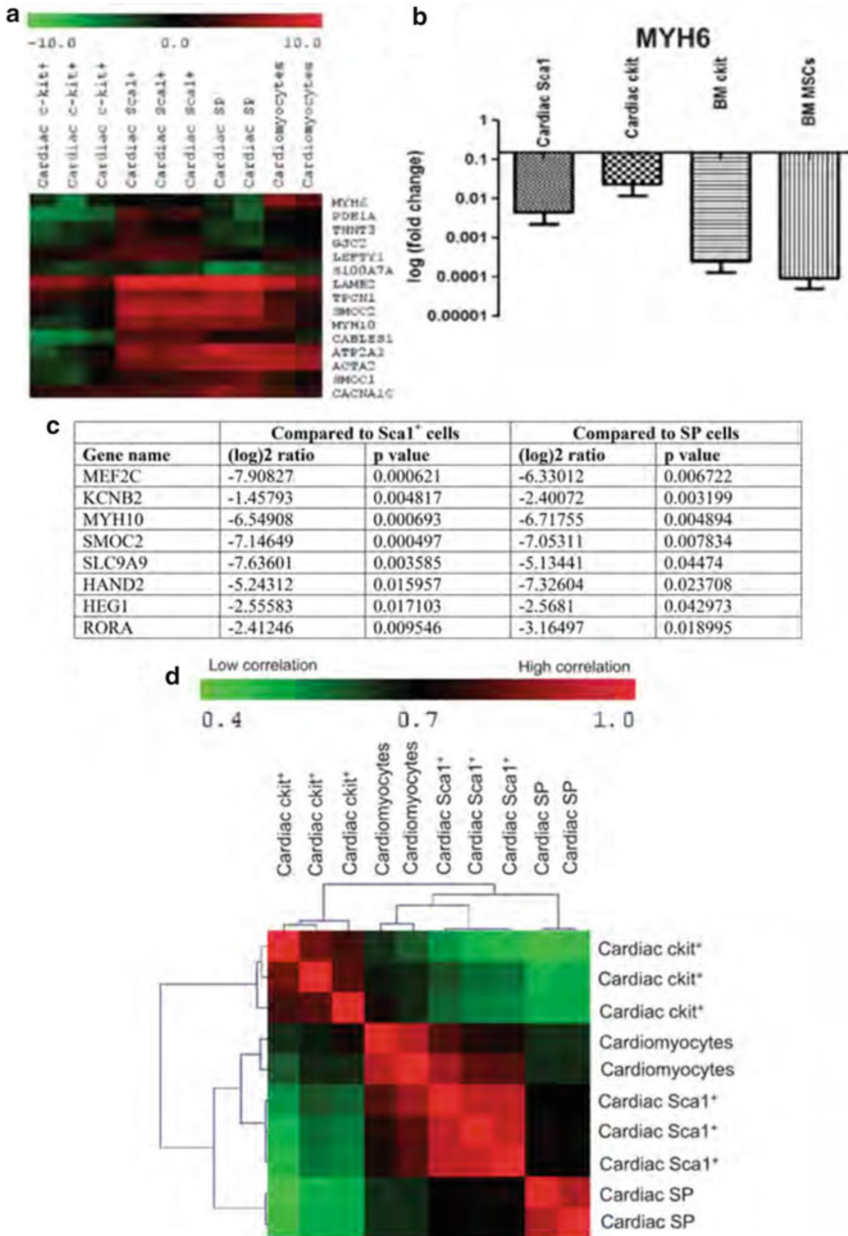
populations identified in the adult heart correspond to different stages of maturation of the same parental cell. In the search for novel biomarkers specific for distinct CPC populations, the molecular signature of c-kit-CPCs, Sca1-CPCs, and SP-CPCs was studied using whole genome transcriptional profiling. The molecular footprint of CPCs was compared with that of cardiomyocytes, bone marrow c-kit-positive progenitor cells (BMPCs), and MSCs.

A significant expression difference of 1,438 genes was found among the three classes of CPCs and between CPCs and cardiomyocytes. The global expression of genes characteristic of the terminally differentiated state of cardiomyocytes was significantly downregulated in the three CPC populations. However, the extent of commitment of CPCs to the myocyte fate differed in the three cell pools: Sca1-CPCs showed the highest correlation with cardiomyocytes, SP-CPCs an intermediate value, and c-kit-CPCs the lowest (Fig. 6a–c). Myocyte-restricted transcription factors, sarcomeric proteins, ion transporters, and calcium-binding proteins were upregulated in Sca1-CPCs and SP-CPCs (Dey et al. 2013).

These findings indicate that early myocyte-lineage genes are poised for expression in Sca1-CPCs and SP-CPCs. Mef2c, Nkx2.5, Tbx5, and Gata4 constitute the critical core of transcription factors that regulate cardiomyogenesis in the embryonic and fetal heart. Similarly, Kv2.2 isoform mRNA is highly enriched in Sca1-CPCs and SP-CPCs. This potassium channel subunit is typically present in embryonic myocytes at very early stages of development and may correspond to the immature equivalent of the protein in the adult organ. Expression of the skeletal and smooth muscle genes Tnnt3 and Acta2 in Sca1-CPCs and SP-CPCs is also indicative of an early state of myocyte lineage determination of the two cell subsets. Collectively, the expression of genes of early myocyte commitment in Sca1-CPCs and SP-CPCs is consistent with the view that myocyte renewal in the adult heart recapitulates cardiac morphogenesis in prenatal life. By comparative analysis and hierarchical clustering, the transcriptional profile of c-kit-CPCs reflects a highly undifferentiated phenotype (Fig. 6d).

The chromatin structure predictive of the multipotent state of c-kit-CPCs may carry a bivalent conformation of histones characterized by activating and inactivating modifications in the same or adjacent nucleosomes. In multipotent progenitor cells, genes that are required in the differentiated progeny are transiently held in a repressed state by histone modifications, while genes that are associated with stemness are stably maintained in an active state. With differentiation, genes that are crucial for multipotency are silenced through histone modifications and DNA methylation. The acquisition of a specific lineage imposes the upregulation of a selected network of genes and the silencing of all other differentiation programs within the cells.

Early mesoderm genes and genes involved in the Notch and canonical Wnt signaling pathways were upregulated in c-kit-CPCs (Fig. 7a). Enzymes controlling cell signaling and metabolism, ATP-binding cassette transporters, anionic transporters, chemokines, and interleukin receptors showed enriched expression in c-kit-CPCs (Fig. 7b, c). The main downregulated core of genes in c-kit-CPCs comprised genes encoding for extracellular matrix proteins, integrins, matrix metalloproteases, and gap junctions (Fig. 7d). Additionally, transcripts for



	Compared to Sca1⁺ cells		Compared to SP cells	
Gene name	(log)2 ratio	p value	(log)2 ratio	p value
MEF2C	-7.90827	0.000621	-6.33012	0.006722
KCNB2	-1.45793	0.004817	-2.40072	0.003199
MYH10	-6.54908	0.000693	-6.71755	0.004894
SMOC2	-7.14649	0.000497	-7.05311	0.007834
SLC9A9	-7.63601	0.003585	-5.13441	0.04474
HAND2	-5.24312	0.015957	-7.32604	0.023708
HEG1	-2.55583	0.017103	-2.5681	0.042973
RORA	-2.41246	0.009546	-3.16497	0.018995

Fig. 6 Transcriptional profile of cardiac-derived cells. (**a**) Heat map representing hierarchical clustering of cardiac and/or muscle-specific genes. (**b**) Transcripts for α-myosin heavy chain (*MYH6*). Results are shown as fold changes in mRNA level with respect to cardiomyocytes. (**c**) Myocardial-restricted genes upregulated in Sca1-CPCs and SP-CPCs with respect to c-kit-CPCs.

biomarkers of vascular ECs and fibroblasts and genes involved in connective tissue remodeling were downregulated in c-kit-CPCs. These findings confirm that the transcriptional profiling of c-kit-CPCs differs significantly from that of Sca1-CPCs and SP-CPCs (Dey et al. 2013).

Despite the shared expression of the c-kit receptor tyrosine kinase, c-kit-BMPCs and c-kit-CPCs showed a highly distinct molecular signature (Fig. 8a). The upregulation of a large gene network involved in DNA replication, repair, and cell cycle regulation in c-kit-BMPCs is consistent with the rapid turnover of the hematopoietic system. Conversely, 40–60 % of c-kit-CPCs are quiescent in the young and old myocardium. Thus, in healthy organisms, the level of expression of genes involved in DNA damage response and cell division appears to represent a cluster of biomarkers able to distinguish c-kit-positive progenitor cells with long-term residence in the myocardium and c-kit-positive cells, which migrate from the bone marrow to the heart (Fig. 8b).

These data document that c-kit-CPCs, Sca1-CPCs, and SP-CPCs represent three distinct cell populations at different stages of commitment. However, the demonstration whether Sca1-CPCs and SP-CPCs originate from c-kit-CPC requires carefully designed lineage-tracing studies in vivo. The findings discussed thus far do not provide information concerning the effects of age and disease on human c-kit-CPCs. The transcriptional profile of human c-kit-CPCs obtained from the atria of donor and explanted hearts was conducted by microarray analysis (Cesselli et al. 2011). Transcripts of genes involved in wound healing and response to stress were upregulated in hCSCs from explanted hearts. Similarly, a group of chemokines and inflammatory factors were more represented in these stem cells, possibly reflecting their proficiency to engraft within the microenvironment of the failing heart. The expression of a subset of genes implicated in lipid metabolism was decreased in hCPCs from failing hearts, suggesting a metabolic shift from fatty acid to carbohydrate metabolism, together with a reduction in mitochondrial-encoded gene expression and protein synthesis. An attenuated response of genes involved in the adaptation to oxidative stress was also observed (Fig. 9).

Highly differentially expressed genes in hCPCs from donor and explanted hearts were then analyzed using Ingenuity Pathway Analysis Software. This independent approach highlighted the presence of two functional gene cores differentially represented in the two classes of hCPCs. The first core included the components of the molecular systems that regulate cell growth, proliferation, motility, lipid and carbohydrate metabolism, and cell-to-cell signaling. The second core involved genes implicated in cardiac development, homeostasis, and repair. Molecular targets of known therapeutic agents used in the treatment of cardiac diseases were upregulated

Fig. 6 (continued) (**d**) Correlation among the three cardiac-derived CPCs and cardiomyocytes represented as a hierarchical cluster matrix, based on Pearson's correlation of significant ($p < 0.05$) differentially expressed genes (≥2-fold) among all samples. *Red* represents high correlation; *green* represents low correlation (From Dey et al. 2013)

Early developmental genes, mesodermal-specific markers and stem cell signaling molecules upregulated in cardiac ckit$^+$ cells with respect to cardiac Sca1$^+$ and SP cells

	Compared to Sca1$^+$ cells		Compared to SP cells	
Gene name	**(log)2 ratio**	**p value**	**(log)2 ratio**	**p value**
NKX2-3	2.947868	0.006842	2.315157	0.022146
TBX1	3.258979	0.002865	1.880969	0.044091
MEST	4.596251	0.030854	6.397683	0.040677
EDNRB	5.880017	0.009592	5.555687	0.033576
Fzd10	1.506756	0.035476	2.121195	0.030591
DLL4	3.793231	0.021916	4.900770	0.014582
LRP8	5.047116	0.002544	3.812955	0.004395
ERN1	3.293769	0.030332	4.990999	0.043222
SOX18	5.022194	0.043175	6.245570	0.005383
ASPSCR1	1.655397	0.004993	1.650760	0.024680
IGHMBP2	1.515657	0.031217	1.255008	0.047935

Genes encoding kinases and metabolic pathway molecules upregulated in cardiac ckit$^+$ cells with respect to cardiac Sca1$^+$ and SP cells

	Compared to Sca1$^+$ cells		Compared to SP cells	
Gene name	**(log)2 ratio**	**p value**	**(log)2 ratio**	**p value**
MYLK2	2.449544	0.033229	2.459230	0.018207
GCLM	4.914408	0.001751	4.731067	0.018651
GSR	3.404869	0.015610	3.704986	0.035554
CHST4	2.989557	0.004743	2.096534	0.042825
HPGDS	3.826584	0.037901	2.244285	0.026049
INPP4A	4.294689	0.002351	3.124071	0.026109
PIK3CG	2.281688	0.034717	3.170903	0.034520
NQO1	6.992044	0.004179	6.124394	0.033839
ATP7B	1.275695	0.001582	7.361509	0.000859
ADH7	3.046368	0.025319	2.518860	0.022582
PHLDA2	5.328909	0.023177	7.249410	0.029028

Genes encoding transporters and ion channels which are upregulated in cardiac ckit$^+$ cells with respect to cardiac Sca1$^+$ and SP cells

	Compared to Sca1$^+$ cells		Compared to SP cells	
Gene name	**(log)2 ratio**	**p value**	**(log)2 ratio**	**p value**
ABCG5	1.489033	0.040843	1.412719	0.031027
SLC7A11	5.855062	0.002628	6.779846	0.011295
SLC25A13	2.666909	0.007270	2.044830	0.035511
KCNJ2	5.562056	0.046619	6.318745	0.022832
OTOP1	2.597527	0.025956	3.157605	0.021380

Chemokines, interleukin receptors, and hematopoietic cell-specific genes upregulated in cardiac ckit$^+$ cells with respect to cardiac Sca1$^+$ and SP cells

	Compared to Sca1$^+$ cells		Compared to SP cells	
Gene name	**(log)2 ratio**	**p value**	**(log)2 ratio**	**p value**
CMA1	5.451747	0.006161	4.940153	0.009952
CSF2RB	4.919763	0.026923	6.292858	0.027126
HHEX	5.982009	0.046852	6.403108	0.009518
SRGN	2.927116	0.040110	6.508746	0.008196
SLPI	7.372099	0.010182	9.403814	0.007264
IL7R	6.913959	0.006677	4.000399	0.033862
CCL22	3.912088	4.46E-05	2.949126	0.001592
TNFRSF18	3.050739	0.005701	2.696391	0.041761
ST8SIA4	5.269708	0.024284	9.390443	0.011375
AIRE	1.692580	0.030879	2.930811	0.007024
TPSB2	3.911608	0.002290	3.933493	0.007794
ILIF10	2.634135	0.021475	3.133199	0.015748

Fig. 7 Gene enrichment in c-kit-CPCs. (**a**–**d**) Genes pertaining to four functional categories were upregulated in c-kit-CPCs versus Sca1-CPCs and SP-CPCs (From Dey et al. 2013)

in hCPCs from decompensated hearts. Thus, the molecular identity of hCPCs from failing hearts differs substantially from that of hCPCs from control myocardium, providing potential biomarkers of their growth and regeneration capacity.

Telomere Length and Cardiovascular Diseases

Telomeres are composed of double-stranded TTAGGG repeats that encompass 9–15 kb in humans. Telomere length is partly maintained by telomerase, a specialized ribonucleoprotein that adds telomeric DNA at the end of chromosomes. Epidemiologic evidence suggests that shortening of mean telomere length (TL) in white blood cells is correlated with cardiac and vascular pathologies (Spyridopoulos and Dimmeler 2007; De Meyer et al. 2011). However, not all studies are in agreement, and the relevance of telomere length as biomarker of cardiac disease and aging has been questioned (Hoffmann and Spyridopoulos 2011). Telomere dysfunction has been implicated in aging and senescence, and shorter TL in peripheral blood cells predicts cardiovascular disease and mortality. Numerous factors have prevented its broad use as a surrogate endpoint; they include the type and stage of the disease, the

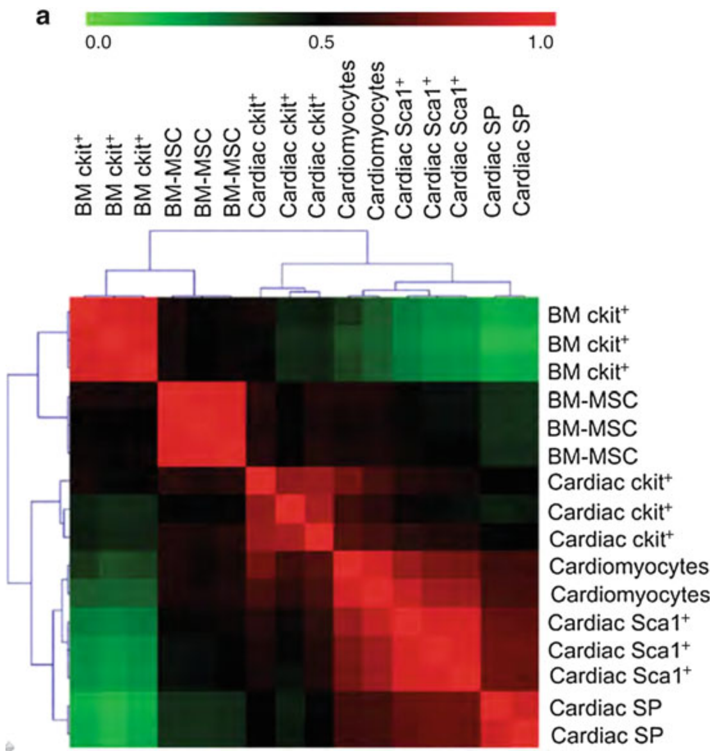


Fig. 8 (continued)

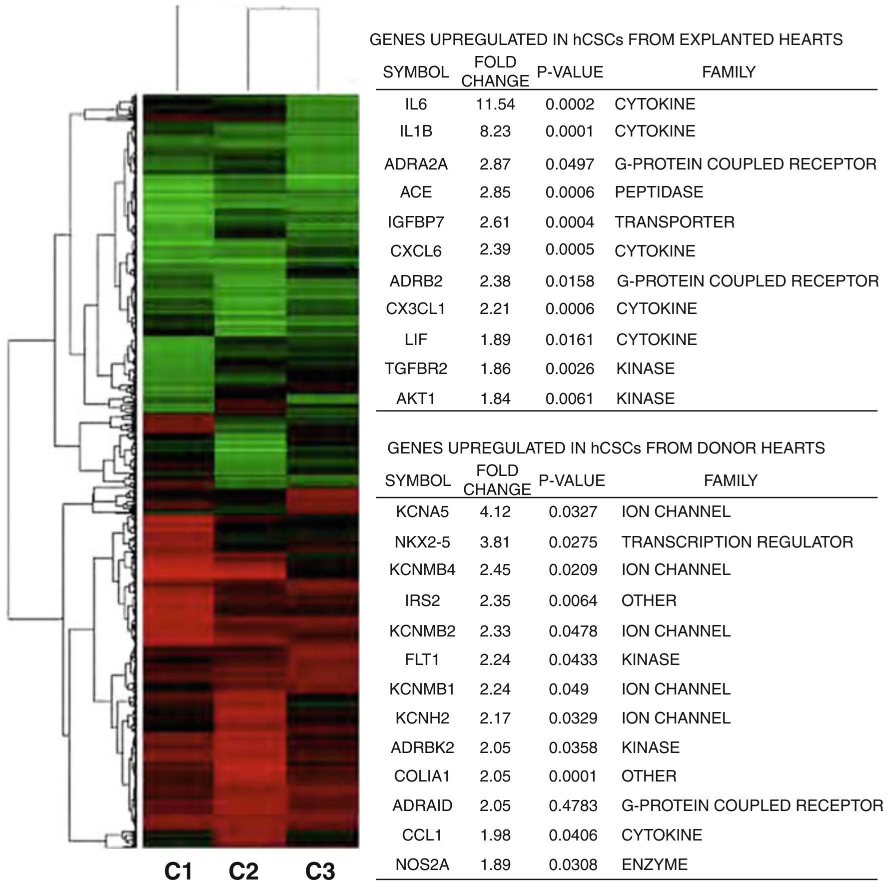
b

Genes downregulated in cardiac cells in comparison to BM cells: Cell cycle and DNA repair

	Compared to BM cells			Compared to BM cells	
Gene name	**(log)2 ratio**	**p value**	**Gene name**	**(log)2 ratio**	**p value**
RAD51	-6.65702	3.81E-05	CCNA2	-8.64993	2.58E-06
RAD51AP1	-2.93829	0.004609	NEK2	-6.43639	1.93E-05
RAD54L	-1.61247	0.017775	NUSAP1	-3.17454	0.005471
DCLRE1B	-3.51313	0.001809	PSRC1	-3.20433	5.57E-05
MCM10	-6.1459	1.13E-05	PSMC3IP	-4.99759	0.001038
MCM7	-1.6482	0.024817	FOXM1	-6.27482	2.69E-05
ZWILCH	-4.14524	3.7E-05	LSM11	-2.26321	5.66E-06
CDKN3	-5.91979	1.01E-05	CKAP2	-4.51619	6.97E-06
CKS2	-1.99491	0.022946	CLSPN	-3.6339	1.89E-05
TOP2A	-5.89237	7.81E-05	MKI67	-6.82292	3.69E-09
PLK1	-5.30944	1.96E-08	CENPA	-7.73735	4.31E-05
NCAPG2	-4.20411	0.001383	CENPH	-7.51336	2.06E-08
NCAPD2	-3.38608	1.77E-05	PMS2	-2.28623	0.034174
BUB1	-8.51468	2.31E-08	SMC2	-3.1528	0.000551
KIF22	-4.98415	4.73E-08	RRM1	-2.30607	0.004292
KIF15	-6.66958	1.4E-06	CENPE	-4.5074	1.85E-05
KIF20B	-3.38301	0.001008	CDCA8	-4.9866	0.000151
KIF23	-5.3509	2.06E-05	CDCA5	-3.95208	3.59E-05
TPX2	-5.39631	0.001477	CDC25C	-5.3415	1.45E-05
POLA1	-4.11884	0.001609	GMNN	-6.20988	6.41E-06
MAD2L1	-6.13612	4.39E-05	CIT	-5.19111	0.000299
MASTL	-2.61873	0.00051	UHRF1	-6.17743	0.000559
HAUS4	-2.8222	0.017249	POLA1	-4.11884	0.001609
BUB1	-8.51468	2.31E-08	PNKP	-5.61481	0.000371
SEPT9	-2.61778	0.000299	TTK	-5.60226	1.62E-05
PMF1	-2.16669	0.002	EXO1	-5.38878	1.43E-05
E2F8	-6.59104	4.49E-06	HELLS	-3.17076	0.000423
E2F2	-6.01447	9.7E-07	FBXO5	-3.78804	3.34E-07
DBF4	-4.43398	0.003481	POLE	-4.13327	0.035291
DTL	-4.17687	1.79E-05	XPC	-2.58405	0.035842
GSG2	-5.47065	4.05E-06	RECQL5	-2.40987	0.011819
SKA1	-1.62069	0.007228	DNA2	-5.85404	0.000402
BRIP1	-1.65905	0.000102	NEIL3	-8.59313	1.06E-08
NDC80	-6.07966	0.000156	CCN5	-8.08324	1.73E-05
CCDC88B	-7.37681	1.42E-07	POLQ	-7.21789	4.2E-08
CASC5	-7.35892	2.42E-06	GAS2L3	-7.04776	1.98E-07
MKI67	-6.82292	3.69E-09			

Fig. 8 Hierarchical clustering among cardiac-derived and bone marrow-derived cells. (**a**) Correlation among the three cardiac-derived CPCs, cardiomyocytes, and BM-derived cells, represented as a hierarchical cluster matrix, based on Pearson's correlation test of significant ($p < 0.05$) differentially expressed genes ($\geq$2-fold) among all samples. *Red* represents high correlation; *green* represents poor correlation. (**b**) A subset of genes involved in DNA repair response and cell cycle is enriched in c-kit-BMPCs with respect to CPCs (From Dey et al. 2013)

age and gender of healthy individuals and patients, the methodology used for the measurement of TL, and the peripheral blood cell population in which TL is assessed.



GENES UPREGULATED IN hCSCs FROM EXPLANTED HEARTS

SYMBOL	FOLD CHANGE	P-VALUE	FAMILY
IL6	11.54	0.0002	CYTOKINE
IL1B	8.23	0.0001	CYTOKINE
ADRA2A	2.87	0.0497	G-PROTEIN COUPLED RECEPTOR
ACE	2.85	0.0006	PEPTIDASE
IGFBP7	2.61	0.0004	TRANSPORTER
CXCL6	2.39	0.0005	CYTOKINE
ADRB2	2.38	0.0158	G-PROTEIN COUPLED RECEPTOR
CX3CL1	2.21	0.0006	CYTOKINE
LIF	1.89	0.0161	CYTOKINE
TGFBR2	1.86	0.0026	KINASE
AKT1	1.84	0.0061	KINASE

GENES UPREGULATED IN hCSCs FROM DONOR HEARTS

SYMBOL	FOLD CHANGE	P-VALUE	FAMILY
KCNA5	4.12	0.0327	ION CHANNEL
NKX2-5	3.81	0.0275	TRANSCRIPTION REGULATOR
KCNMB4	2.45	0.0209	ION CHANNEL
IRS2	2.35	0.0064	OTHER
KCNMB2	2.33	0.0478	ION CHANNEL
FLT1	2.24	0.0433	KINASE
KCNMB1	2.24	0.049	ION CHANNEL
KCNH2	2.17	0.0329	ION CHANNEL
ADRBK2	2.05	0.0358	KINASE
COLIA1	2.05	0.0001	OTHER
ADRAID	2.05	0.4783	G-PROTEIN COUPLED RECEPTOR
CCL1	1.98	0.0406	CYTOKINE
NOS2A	1.89	0.0308	ENZYME

Fig. 9 **Transcriptional profile of hCPCs**. Heat map showing the differentially expressed genes in hCPCs from donor and explanted hearts. Each column (*C1*, *C2*, and *C3*) represents the direct comparison of hCSCs collected from age- and sex-matched donor and failing hearts. C1, C2, and C3 reflect three different sex- and age-matched pairs. Genes downregulated and upregulated in hCSCs from donor hearts are shown in *green* and *red*, respectively. Fold changes are shown for a selected list of genes (From Cesselli et al. 2011)

Although leukocyte telomere length (LTL) has been associated with a variety of aging-related cardiovascular diseases, recent studies have emphasized the relevance of specific peripheral blood lymphocyte and myeloid cell subpopulations to the aging and pathology of heart and vessels. In a comprehensive report, TL was measured by flow-FISH in 12 leukocyte subsets obtained from age-matched healthy individuals and patients with coronary heart disease (CHD) (Spyridopoulos et al. 2008, 2009; Hoffmann et al. 2009). In both groups, TL in granulocytes and monocytes was comparable to that of their cell of origin, CD34-positive progenitor cells. LTL in CD34-positive progenitors, granulocytes, monocytes, and B and T lymphocytes was approximately 0.5 kb shorter in patients with CHD than in

controls. However, a twofold higher degree of telomere erosion was detected in cytotoxic CD8-positive T lymphocytes of CHD patients (Spyridopoulos et al. 2009). Moreover, TL shortening of this T lymphocyte subset in CHD patients was coupled with a decrease in left ventricular function.

In the majority of studies, patients with early-onset CHD and patients with severe complicated diabetes mellitus have shorter LTL than healthy subjects. A recent meta-analysis of 24 published reports was conducted to determine whether LTL is significantly associated with CHD and cerebrovascular disease (Haycock et al. 2014). This analysis involved 43,725 participants and 8,400 patients with cardiovascular disease. An inverse association between LTL and risk of CHD, independently from conventional vascular risk factors, was found. In contrast, shorter telomeres were not significantly associated with cerebrovascular disease risk. Moreover, the Halcyon study was designed to establish whether women have longer telomeres than men (Gardner et al. 2014). In 36 cohorts for a total of 36,230 participants, LTL was found to be longer in women, and this difference did not vary with chronological age. Conflicting data were obtained in patients with hypertension, in which elongated and shortened telomeres were identified; TL was preserved in patients with left ventricular hypertrophy (Nilsson et al. 2013).

Peripheral blood LTL has been considered as a systemic marker for biological aging, but the importance of telomere shortening as independent biomarker of organ and organism senescence has been challenged. Most studies agree that LTL adds predictive power to chronological age and can be considered a marker of cardiovascular aging (Fyhrquist et al. 2013).

The Telomerase-Telomere System in CPCs

The fraction of CPCs with critically shortened telomeres is the major determinant of the growth reserve of the heart (Leri et al. 2015). This is because hCPCs with shortened telomeres give rise to progenitors, precursors, and amplifying cells which inherit the characteristics of the mother cell and generate myocytes that rapidly acquire the senescent cell phenotype and express $p16^{INK4A}$. The link between the past history of CPCs, their telomere length, and the age of the formed progeny has been documented in a rigorous manner not only in humans (Cesselli et al. 2011) but also in small animal models of physiological aging (Sanada et al. 2014), strengthening the notion that myocardial biology and function are determined by the state of the controlling cell, i.e., the CPC. Aging supervenes when pro-senescence stimuli negatively affect cellular longevity, contributing to the physiological decline of the organ and organism.

Telomerase activity delays but cannot prevent telomere erosion in hCPCs, which lose ~130 bp at each round of division (Bearzi et al. 2007). With serial passaging, telomerase undergoes a 50 % decrease, but the catalytic activity remains at a considerably high level. A comprehensive evaluation of the biomarkers of cellular senescence was conducted in hCPCs isolated from the atrial myocardium of donor and explanted hearts (Cesselli et al. 2011). hCPCs obtained from explanted tissue

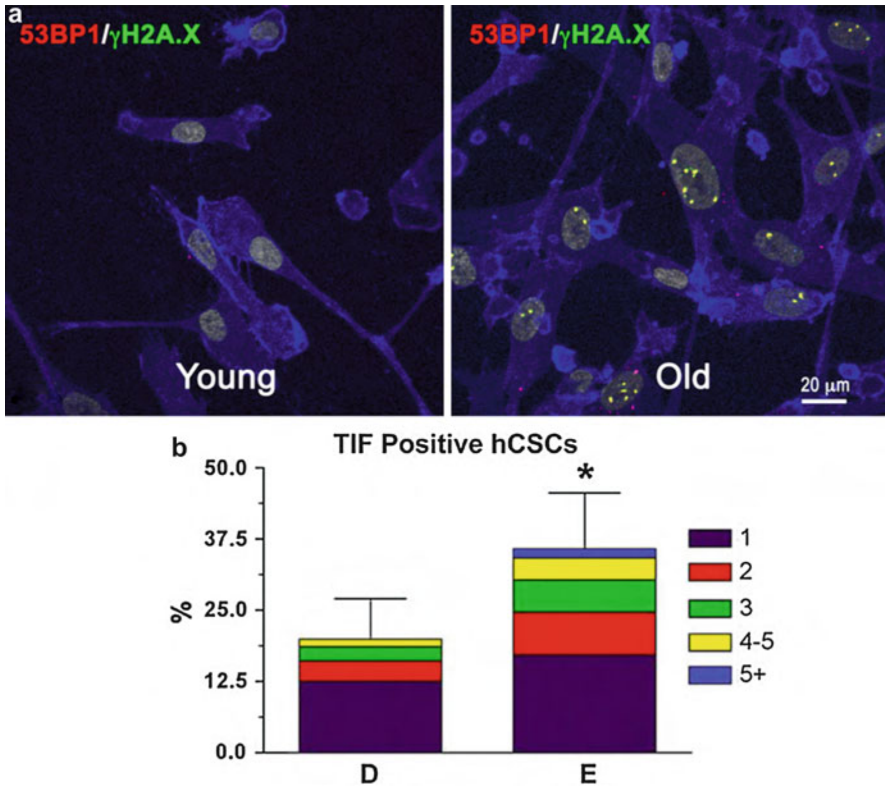


Fig. 10 **DNA damage response (DDR) foci in c-kit-CPCs**. (**a**) DDR foci labeled by γH2A.X (*green*) and 53BP1 (*red*) are more frequent in old CPCs. (**b**) The co-localization of telomere and DDR proteins was measured in hCSCs. The fraction of hCSCs with one to five TIFs is shown as mean ± SD. $^*P < 0.05$ versus donor hearts (*D*). E: Explanted hearts (Panel **b** from Cesselli et al. 2011)

display a fivefold lower level of telomerase activity and a 25 % shorter TL. Although telomerase activity and average telomere length are valid indicators of the age of a cell compartment, the fraction of cells with critically shortened telomeres is the major determinant of the growth reserve of a cell population. Telomere erosion beyond a critical value and/or loss of telomere integrity elicits a DNA damage response that enables cells to block cell cycle progression and initiate DNA repair. The DNA damage response involves interaction of the adaptor protein p53-binding protein 1 (53BP1) and the chromatin modifier phosphorylated histone H2AX (γ-H2AX). The localization of these proteins within telomeric sequences reflects dysfunctional telomere-induced foci (TIFs) (Fig. 10a). TIFs activate the ataxia telangiectasia mutated (ATM) kinase which phosphorylates p53 at serine 15.

With respect to hCPCs from donor hearts, a 75 % larger fraction of hCPCs from explanted hearts showed TIFs in their telomeres (Fig. 10b). Consistent with these

observations, the quantity of phospho-p53^{Ser15} and the expression of the p53 target gene p21^{Cip1} were nearly twofold higher in explanted hCPCs. Importantly, a twofold larger fraction of hCPCs from explanted hearts was positive for the senescence-associated protein p16^{INK4a}. Thus, prolonged pathology and aging have comparable effects on hCPCs that display dysfunctional telomeres and express markers of cellular senescence.

Based on the assumption that telomere length, telomerase activity, TIFs, p16^{INK4a}, and p21^{Cip1} are biomarkers of hCPC function, their interrelation was established. Telomere length was directly related to telomerase activity and inversely correlated with TIFs, p16^{INK4a}, and p21^{Cip1}. Additionally, the level of catalytic activity of telomerase decreased with increased number of hCPCs showing TIFs, p16^{INK4a}, and p21^{Cip1}. Positive relationships were also found among TIFs, p16^{INK4a}, and p21^{Cip1}, indicating that these parameters of stem cell behavior were not independent; each of them could be used as a biomarker of the growth reserve of hCPCs.

Chronological age was recognized as a major predictor of telomere shortening, attenuation in telomerase activity, and increased incidence of TIFs, p16^{INK4a}, and p21^{Cip1} in hCPCs. To establish whether the presence of cardiac disease negatively affected hCPC function, hCPCs from subjects of comparable age were studied. In comparison with hCPCs from donor hearts, hCPCs from age-matched explanted hearts had shorter telomere length, lower telomerase activity, higher frequency of TIFs, and enhanced expression of p16^{INK4a} and p21^{Cip1}. These results suggest that both aging and pathological insults trigger DNA lesions at the level of the telomeric repeats with initiation of the DNA repair response. Activation of telomerase was apparent in hCPCs from control hearts, and this molecular mechanism may preserve partly telomere integrity.

The young heart is characterized by asymmetric growth kinetics of CPCs, a process that has been defined “invariant” and is operative in organs in a steady state. Changes in this pattern of growth have been observed in the old and diseased heart, suggesting that quantitative and qualitative alterations occur in hCPCs. Thus, the human heart is a self-renewing organ regulated by a CSC pool; CSCs condition the destiny of the organ throughout its life span and in the presence of various cardiac pathologies.

Potential Applications to Prognosis, Other Diseases, or Conditions

The discovery that the adult heart contains a compartment of resident CSCs has changed our understanding of myocardial biology and has projected a rather unexpected view of the growth reserve mechanisms of the myocardium. The discussion above has analyzed the variables that can affect the turnover of cardiac cells physiologically and the possibility of cardiac repair following myocardial damage. More importantly, the critical determinants of CSC replication, senescence, and death can be prospected as novel biomarkers able to define the age, pathology, and function of the myocardium with aging and heart failure. The molecular signature

and the telomere-telomerase system are proposed as relevant biomarkers describing the fate of the organ.

Summary Points

- This chapter focuses on the identifiers of the phenotypic and functional properties of cardiac progenitor cells (CPCs).
- CPCs are a rare population of cells that reside in the myocardium where they are clustered in niches.
- Stem cells are self-renewing, clonogenic, and multipotent, which are the fundamental properties of tissue-specific adult stem cell.
- CPCs regulate myocardial homeostasis and tissue repair following injury by generating cardiomyocytes and coronary resistance arterioles and coronary capillaries.
- c-kit-CPCs constitute a pool of undifferentiated cells while SP-CPCs and Sca1-CPCs show markers of early myocyte commitment.
- Transcriptional profile of c-kit-CPCs differs in normal and failing human hearts.

References

Angert D, Berretta RM, Kubo H, et al. Repair of the injured adult heart involves new myocytes potentially derived from resident cardiac stem cells. Circ Res. 2011;108:1226–37.
Anversa P, Kajstura J, Leri A, et al. Life and death of cardiac stem cells: a paradigm shift in cardiac biology. Circulation. 2006;113:1451–63.
Bailey B, Fransioli J, Gude NA, et al. Sca-1 knockout impairs myocardial and cardiac progenitor cell function. Circ Res. 2012;111:750–60.
Bearzi C, Rota M, Hosoda T, et al. Human cardiac stem cells. Proc Natl Acad Sci U S A. 2007;104:14068–73.
Beaver CM, Ahmed A, Masters JR. Clonogenicity: holoclones and meroclones contain stem cells. PLoS ONE. 2014;9:e89834.
Beltrami AP, Barlucchi L, Torella D, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell. 2003;114:763–76.
Bolli R, Chugh AR, D'Amario D, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet. 2011;378:1847–57.
Braunwald E. Biomarkers in heart failure. N Engl J Med. 2008;358:2148–59.
Braunwald E. Biomarkers in heart failure. Preface. Heart Fail Clin. 2009;5:xii–v.
Cai CL, Martin JC, Sun Y, et al. A myocardial lineage derives from Tbx18 epicardial cells. Nature. 2008;454:104–8.
Castaldo C, Di Meglio F, Nurzynska D, et al. CD117-positive cells in adult human heart are localized in the subepicardium, and their activation is associated with laminin-1 and alpha6 integrin expression. Stem Cells. 2008;26:1723–31.
Cesselli D, Beltrami AP, D'Aurizio F, et al. Effects of age and heart failure on human cardiac stem cell function. Am J Pathol. 2011;179:349–66.
Challen GA, Little MH. A side order of stem cells: the SP phenotype. Stem Cells. 2006;24:3–12.
Cheng K, Ibrahim A, Hensley MT, et al. Relative roles of CD90 and c-kit to the regenerative efficacy of cardiosphere-derived cells in humans and in a mouse model of myocardial infarction. J Am Heart Assoc. 2014;3:e001260.

Cottage CT, Neidig L, Sundararaman B, et al. Increased mitotic rate coincident with transient telomere lengthening resulting from pim-1 overexpression in cardiac progenitor cells. Stem Cells. 2012;30:2512–22.

D'Amario D, Fiorini C, Campbell PM, Goichberg, et al. Functionally competent cardiac stem cells can be isolated from endomyocardial biopsies of patients with advanced cardiomyopathies. Circ Res. 2011;108:857–61.

Davis DR, Ruckdeschel Smith R, Marbán E. Human cardiospheres are a source of stem cells with cardiomyogenic potential. Stem Cells. 2010;28:903–4.

De Meyer T, Rietzschel ER, De Buyzere ML, et al. Telomere length and cardiovascular aging: the means to the ends? Ageing Res Rev. 2011;10:297–303.

Dey D, Han L, Bauer M, et al. Dissecting the molecular relationship among various cardiogenic progenitor cells. Circ Res. 2013;112:1253–62.

Dowell JD, Rubart M, Pasumarthi KB, et al. Myocyte and myogenic stem cell transplantation in the heart. Cardiovasc Res. 2003;58:336–50.

Fischer KM, Cottage CT, Wu W, et al. Enhancement of myocardial regeneration through genetic engineering of cardiac progenitor cells expressing Pim-1 kinase. Circulation. 2009;120:2077–87.

Fyhrquist F, Saijonmaa O, Strandberg T. The roles of senescence and telomere shortening in cardiovascular disease. Nat Rev Cardiol. 2013;10:274–83.

Gardner M, Bann D, Wiley L, et al. Gender and telomere length: systematic review and meta-analysis. Exp Gerontol. 2014;51:15–27.

Goichberg P, Kannappan R, Cimini M, et al. Age-associated defects in EphA2 signaling impair the migration of human cardiac progenitor cells. Circulation. 2013;128:2211–23.

Hariharan N, Quijada P, Mohsin S, et al. Nucleostemin rejuvenates cardiac progenitor cells and antagonizes myocardial aging. J Am Coll Cardiol. 2015;65:133–47.

Haycock PC, Heydon EE, Kaptoge S, Butterworth, et al. Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. BMJ. 2014;349:g4227.

Hierlihy AM, Seale P, Lobe CG, et al. The post-natal heart contains a myocardial stem cell population. FEBS Lett. 2002;530:239–43.

Hoffmann J, Spyridopoulos I. Telomere length in cardiovascular disease: new challenges in measuring this marker of cardiovascular aging. Futur Cardiol. 2011;7:789–803.

Hoffmann J, Erben Y, Zeiher AM, et al. Telomere length-heterogeneity among myeloid cells is a predictor for chronological ageing. Exp Gerontol. 2009;44:363–6.

Hosoda T, D'Amario D, Cabral-Da-Silva MC, et al. Clonality of mouse and human cardiomyogenesis in vivo. Proc Natl Acad Sci U S A. 2009;106:17169–74.

Jones PH, Simons BD, Watt FM. Sic transit gloria: farewell to the epidermal transit amplifying cell? Cell Stem Cell. 2007;1:371–81.

Laflamme MA, Chen KY, Naumova AV, et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol. 2007;25:1015–24.

Lee ST, White AJ, Matsushita S, et al. Intramyocardial injection of autologous cardiospheres or cardiosphere-derived cells preserves function and minimizes adverse ventricular remodeling in pigs with heart failure post-myocardial infarction. J Am Coll Cardiol. 2011;57:455–65.

Leri A, Kajstura J, Anversa P. Cardiac stem cells and mechanisms of myocardial regeneration. Physiol Rev. 2005;85:1373–416.

Leri A, Rota M, Hosoda T, et al. Cardiac stem cell niches. Stem Cell Res. 2014;13:631–46.

Leri A, Rota M, Pasqualini FS, et al. Origin of cardiomyocytes in the adult heart. Circ Res. 2015;116:150–66.

Li Z, Lee A, Huang M, et al. Imaging survival and function of transplanted cardiac resident stem cells. J Am Coll Cardiol. 2009;53:1229–40.

Limana F, Zacheo A, Mocini D, et al. Identification of myocardial and vascular precursor cells in human and mouse epicardium. Circ Res. 2007;101:1255–65.

Lin KK, Goodell MA. Detection of hematopoietic stem cells by flow cytometry. Methods Cell Biol. 2011;103:21–30.

Makkar RR, Smith RR, Cheng K, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet. 2012;379:895–904.

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. 2004;265:262–75.

Martin CM, Ferdous A, Gallardo T, et al. Hypoxia-inducible factor-2alpha transactivates Abcg2 and promotes cytoprotection in cardiac side population cells. Circ Res. 2008;102:1075–81.

Mohsin S, Khan M, Nguyen J, et al. Rejuvenation of human cardiac progenitor cells with Pim-1 kinase. Circ Res. 2013;113:1169–79.

Morrow DA, de Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. Circulation. 2007;115:949–52.

Mouquet F, Pfister O, Jain M, et al. Restoration of cardiac progenitor cells after myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. Circ Res. 2005;97:1090–2.

Nilsson PM, Tufvesson H, Leosdottir M, et al. Telomeres and cardiovascular disease risk: an update 2013. Transl Res. 2013;162:371–80.

Pfister O, Mouquet F, Jain M, et al. CD31- but Not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. Circ Res. 2005;97:52–61.

Pfister O, Oikonomopoulos A, Sereti KI, et al. Isolation of resident cardiac progenitor cells by Hoechst 33342 staining. Methods Mol Biol. 2010;660:53–63.

Quijada P, Toko H, Fischer KM, et al. Preservation of myocardial structure is enhanced by pim-1 engineering of bone marrow cells. Circ Res. 2012;111:77–86.

Robey TE, Saiget MK, Reinecke H, et al. Systems approaches to preventing transplanted cell death in cardiac repair. J Mol Cell Cardiol. 2008;45:567–81.

Rudat C, Kispert A. Wt1 and epicardial fate mapping. Circ Res. 2012;111:165–9.

Sanada F, Kim J, Czarna A, et al. c-Kit-positive cardiac stem cells nested in hypoxic niches are activated by stem cell factor reversing the aging myopathy. Circ Res. 2014;114:41–55.

Sereti KI, Oikonomopoulos A, Unno K, et al. ATP-binding cassette G-subfamily transporter 2 regulates cell cycle progression and asymmetric division in mouse cardiac side population progenitor cells. Circ Res. 2013;112:27–34.

Shepherd BE, Kiem H, Lansdorp PM, et al. Hematopoietic stem-cell behavior in nonhuman primates. Blood. 2007;110:1806–13.

Siddiqi S, Sussman MA. Cell and gene therapy for severe heart failure patients: the time and place for Pim-1 kinase. Expert Rev Cardiovasc Ther. 2013;11:949–57.

Smith RR, Barile L, Cho HC, et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. Circulation. 2007;115:896–908.

Sperr WR, Bankl HC, Mundigler G, et al. The human cardiac mast cell: localization, isolation, phenotype, and functional characterization. Blood. 1994;84:3876–84.

Spyridopoulos I, Dimmeler S. Can telomere length predict cardiovascular risk? Lancet. 2007;369:81–2.

Spyridopoulos I, Erben Y, Brummendorf TH, et al. Telomere gap between granulocytes and lymphocytes is a determinant for hematopoietic progenitor cell impairment in patients with previous myocardial infarction. Arterioscler Thromb Vasc Biol. 2008;28:968–74.

Spyridopoulos I, Hoffmann J, Aicher A, et al. Accelerated telomere shortening in leukocyte subpopulations of patients with coronary heart disease: role of cytomegalovirus seropositivity. Circulation. 2009;120:1364–72.

Swijnenburg RJ, Govaert JA, van der Bogt KE, et al. Timing of bone marrow cell delivery has minimal effects on cell viability and cardiac recovery after myocardial infarction. Circ Cardiovasc Imaging. 2010;3:77–85.

Tillmanns J, Rota M, Hosoda T, Misao Y, et al. Formation of large coronary arteries by cardiac progenitor cells. Proc Natl Acad Sci U S A. 2008;105:1668–73.
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. 2005;170:1135–46.
Urbanek K, Rota M, Cascapera S, et al. Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. Circ Res. 2005;97:663–73.
Urbanek K, Cesselli D, Rota M, et al. Stem cell niches in the adult mouse heart. Proc Natl Acad Sci U S A. 2006;103:9226–31.
von Gise A, Zhou B, Honor LB, et al. WT1 regulates epicardial epithelial to mesenchymal transition through β-catenin and retinoic acid signaling pathways. Dev Biol. 2011;356:421–31.
Williams AR, Hare JM. Mesenchymal stem cells: biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. Circ Res. 2011;109:923–40.
Zhou B, Pu WT. Genetic Cre-loxP assessment of epicardial cell fate using Wt1-driven Cre alleles. Circ Res. 2012;111:e276–80.
Zhou B, Ma Q, Rajagopal S, et al. Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. Nature. 2008;454:109–13.