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Cardiac Stem Cells: Biology and Clinical Applications

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Abstract

Significance: Heart disease is the primary cause of death in the industrialized world. Cardiac failure is dictated by an uncompensated reduction in the number of viable and fully functional cardiomyocytes. While current pharmacological therapies alleviate the symptoms associated with cardiac deterioration, heart transplantation remains the only therapy for advanced heart failure. Therefore, there is a pressing need for novel therapeutic modalities. Cell-based therapies involving cardiac stem cells (CSCs) constitute a promising emerging approach for the replenishment of the lost tissue and the restoration of cardiac contractility. **Recent Advances:** CSCs reside in the adult heart and govern myocardial homeostasis and repair after injury by producing new cardiomyocytes and vascular structures. In the last decade, different classes of immature cells expressing distinct stem cell markers have been identified and characterized in terms of their growth properties, differentiation potential, and regenerative ability. Phase I clinical trials, employing autologous CSCs in patients with ischemic cardiomyopathy, are being completed with encouraging results. **Critical Issues:** Accumulating evidence concerning the role of CSCs in heart regeneration imposes a reconsideration of the mechanisms of cardiac aging and the etiology of heart failure. Deciphering the molecular pathways that prevent activation of CSCs in their environment and understanding the processes that affect CSC survival and regenerative function with cardiac pathologies, commonly accompanied by alterations in redox conditions, are of great clinical importance. **Future Directions:** Further investigations of CSC biology may be translated into highly effective and novel therapeutic strategies aiming at the enhancement of the endogenous healing capacity of the diseased heart. *Antioxid. Redox Signal.* 21, 2002–2017.

Introduction

EXPERIMENTAL AND CLINICAL evidence of the regenerative potential of the adult human heart has accumulated over the years (11, 40, 65, 68, 79, 87, 123, 137, 159). The discovery of resident cardiac stem cells (CSCs) in the myocardium (9, 10, 56, 95, 99, 105, 107, 114, 135, 157, 159) elicited extensive investigations by multiple groups in an attempt to understand CSC biology, the mechanisms of cardiac repair, and the therapeutic implications of this process.

Stem cells are distinguished from more mature cells by their ability to differentiate into multiple specialized cell types and concurrently generate an identical stem cell following asymmetric division. This process may occur after long periods of inactivity, since stem cells replicate rarely. Stem cells are postulated to serve as an internal tissue repair system for the replenishment of damaged parenchymal cells.

Adult tissue-resident CSCs with the capacity to differentiate into cardiomyocytes, and vascular endothelial cells and smooth muscle cells have been identified in early 2000 (9, 10, 137, 157). When activated, CSCs proliferate and form lineage-committed progenitors, which will progressively acquire a fully mature phenotype, forming new contractile muscle and blood vessels for tissue oxygenation (1, 52, 79, 80). Preclinical studies have established that transplanted or resident CSCs have the potential to reduce the infarct size and improve cardiac function in a variety of animal models of myocardial injury (1, 40, 71, 90, 153). Thus, there is growing interest in the implementation of CSCs for the management of the diseased heart. Several laboratories have reported the identification of CSCs expressing distinct stem cell antigens. In this review, we will discuss the current understanding of the mechanisms controlling the activity of tissue-resident CSCs, the impact of changes in redox biology on CSC

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biology, and the possibility of using CSCs for the treatment of human heart failure.

Adult Resident CSC Populations

C-kit-positive CSCs

Beltrami *et al.* were the first to identify a unique population of cells in the myocardium that have the phenotypic appearance of primitive cells and express the stem cell marker, c-kit (10). Overall, these cells occur at a frequency of 1:30,000 myocardial cells. The expression of VEGF-receptor 2 (KDR) in the pool of c-kit-positive CSCs distinguishes myocyte progenitor cells (KDR negative) and vasculogenic progenitor cells (KDR positive), both of which can differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells; however, myocyte progenitor cells have a greater propensity to produce cardiomyocytes, whereas vascular progenitors preferentially acquire vascular smooth muscle cell and endothelial cell fates (Fig. 1) (28). In the heart, a small fraction of c-kit-positive CSCs expresses the transcription factors Nkx2.5 and GATA-4, indicating their commitment to the myogenic lineage. Isolated c-kit-positive CSCs form *in vitro* endothelial cells, fibroblasts, smooth muscle cells, and cardio-

myocytes, although spontaneously beating myocytes have not been demonstrated as yet (9, 10, 28). Upon transplantation into the damaged heart, c-kit-positive CSCs generate a large pool of functionally competent cardiomyocytes, resistance arterioles, and capillary profiles repairing, in part, the infarcted myocardium, reducing the infarct size, and attenuating ventricular remodeling (10, 27–29, 44, 46, 59, 120, 158).

Cardiac side population cells

Pfister and Liao selectively isolated cardiac side population (SP) cells, which are characterized by the efflux of Hoechst 33342 DNA-binding fluorescent dye due to the presence of ATP-binding cassette transporters, Abcg2 and MDR1 (114). SP cells account for 1.2% to 2% of adult myocardial cells. Abcg2 expression enhances proliferation and survival of SP cells, although inhibiting their differentiation (115). SP cells generally express the stem cell antigen-1 (Sca-1) and PECAM-1, also termed CD31. The minority of cardiac SP cells that are CD31-negative have been shown to be endowed with cardiomyogenic potential (114). Following culture, the expression of the cardiogenic transcription factors GATA-4 and MEF2c, and the sarcomeric proteins α -actinin and troponin I, is increased in SP cells lacking

FIG. 1. Differentiation of vascular progenitor cells (VPCs) and myocyte progenitor cells (MPCs). (A) Human VPCs differentiated predominantly into endothelial cells (ECs) (von Willebrand factor [vWf], yellow) and smooth muscle cells (SMCs) (α -smooth muscle actin [α -SMA], green), while MPCs formed mostly cardiomyocytes (α -sarcomeric actin [α -SA], red). (B) Percentage of VPCs and MPCs differentiating into ECs, SMCs, and myocytes. The EC phenotype was recognized based on the expression of the transcription factor Ets1 and the surface markers CD31 and vWf; the SMC phenotype was defined based on the presence of the transcription factor GATA-6, the membrane antigen TGF- β 1 receptor (TGF- β 1R), and the contractile protein α -SMA. The myocyte phenotype was identified based on the expression of the transcription factors Nkx2.5 and MEF2c, and the sarcomeric proteins α -cardiac actinin (α -CA) and α -SA. * $p < 0.05$ versus ECs and SMCs.

CD31. In coculture with adult cardiomyocytes, cardiac SP cells differentiate, develop organized sarcomeres, and efficiently contract (95, 109, 114, 153).

Sca-1-positive CSCs

Oh *et al.* demonstrated that Sca-1-positive cells with a phenotype distinct from hematopoietic lineages reside in the adult heart and are colocalized with small capillary vessels (105). In resting state, Sca-1-positive cells are uncommitted and do not express markers of cardiac or endothelial lineages; however, these cells are capable of adapting cardiomyogenic fate during embryogenesis and differentiate into myocytes after transplantation (47, 96, 97, 105, 161). In the myocardial interstitium, the population of Sca-1-expressing cells accounts for ~2% of myocardial cells. Sca-1-positive cells express also CD29 (β 1-integrin) and CD44 (hyaluronic acid receptor), but are negative for CD31, CD45 (pan-leukocyte marker), and c-kit. Of interest, cardiac SP cells are highly enriched for Sca-1 (102, 114), suggesting a relationship between Sca-1-positive cells and SP cells in the heart (32).

Cardiosphere-derived cells

Smith *et al.* introduced a methodology for the isolation and expansion of cells able to acquire the cardiomyocyte phenotype; they are derived from nonadherent multicellular clusters termed cardiospheres, which are obtained from endomyocardial biopsies (135). The cardiosphere-derived cells (CDCs) represent a heterogeneous cell population consisting of undifferentiated and committed cells, expressing c-kit and Sca-1; they are also positive for CD105 (endogelein), CD90 (Thy-1), and CD29 (β 1-integrin), typical epitopes of mesenchymal stromal cells (MSCs). CDCs can differentiate into beating cardiomyocytes when cocultured with mature myocytes; vascular smooth muscle cells and endothelial cells can also derive from differentiation of CDCs (99, 146). Other

putative stem cell classes have been described in the heart, including bone marrow-derived hematopoietic cells (87, 108), MSCs (57, 147), and epicardial cells (16). The cardiomyogenic potential and clinical significance of these cells are discussed in (34, 58, 71, 98, 123, 124, 142, 162).

The Role of CSCs in Heart Homeostasis and Injury

The maintenance of cardiac function requires the continuous replacement of lost cardiomyocytes (1, 80). The magnitude of myocyte renewal in the adult heart and the decline in the endogenous regenerative capacity of the aging myocardium are currently the subject of intense debate (11, 65, 68, 140). Additionally, there is no agreement concerning the origin of newly formed myocytes: dedifferentiation and proliferation of pre-existing myocytes (126) have been proposed, and transdifferentiation of cells derived from the epicardium (134) or from extracardiac origins (67, 118) has been described. In this review, we will focus on the critical role of resident CSCs in the control of the development, postnatal growth, and aging of the heart. Accumulating evidence indicates that a direct lineage relationship exists between CSCs and cardiomyogenesis. Similarly, the generation of the various classes of the coronary circulation *via* activation and lineage specification of CSCs has repeatedly been shown.

Embryonic and fetal hearts contain a defined population of uncommitted c-kit-positive CSCs, which regulate the growth and maturation of the prenatal organ and generate myocytes upon transplantation (38). In addition, CDCs from a neonatal heart include a high number of undifferentiated cells that express c-kit or Sca-1, and have strong regenerative ability in a model of myocardial infarction (132). Mathematical modeling predicts that the size and growth properties of the c-kit-positive population in the course of embryogenesis account for the number of myocytes present in the ventricle of a fully formed neonatal heart (38).

FIG. 2. Cellular and extracellular components of cardiac niches. Apical niche containing 23 c-kit-positive (A, D: green) cardiac stem cells (CSCs). The distribution of fibronectin (B–D: yellow) within the niche is shown. Fibronectin is present throughout the niche and accumulates in close proximity of 7 c-kit-positive GATA-4-negative CSCs (arrows). GATA-4 is illustrated in white in (A, C), and (D). Sixteen c-kit-positive cells express GATA-4 in their nuclei.

CSCs and early lineage-committed cells are nested in specialized microdomains of the myocardial interstitium defined as stem cell niches (156) (Fig. 2). Myocyte progenitors within the niches express c-kit and the myocyte-specific transcription factors GATA-4, Nkx2.5, or MEF2c. The more differentiated myocyte precursors retain the stem cell antigen and contain the contractile proteins sarcomeric α -actinin and troponin. With further maturation, amplifying myocytes are formed; they lack c-kit and have the ability to divide and concurrently differentiate until the adult cell phenotype is acquired.

The activation of endogenous CSCs, accompanied by proliferation and differentiation, occurs in humans in response to injury, including myocardial infarction and chronic aortic stenosis (10, 157, 159). Myocardial regeneration mediated by growth and commitment of multipotent CSCs has been shown in human heart failure of ischemic and non-ischemic origin (64, 158). Experimentally, genetic tagging of c-kit-positive CSCs has established that, following delivery

into the damaged myocardium, a single human CSC is capable of creating multilineage progeny consisting of myocytes, vascular smooth muscle cells, and endothelial cells (51). In the settings of xenotransplantation when human CSCs are administered to an infarcted rodent heart, the donor cells generate a band of new human myocardium that replaces the acutely infarcted portion of the left ventricular wall. As a result of this intervention, myocardial recovery is dramatically improved (10, 27, 29, 44, 53, 62, 84, 96, 101, 120). The human origin of the regenerated tissue has been documented by the presence of human-specific *Alu* sequences in the DNA of the newly formed myocytes (Fig. 3). Independent studies by numerous groups have confirmed that with acute ischemia, the intramyocardial administration or intracoronary delivery of CSCs leads to tissue regeneration due to successful homing of the donor cells to the site of damage, their differentiation into cardiac lineages, and functional coupling with pre-existing myocytes and coronary vessels [for recent review, see Refs. (1, 30, 75, 79, 80, 124)].

FIG. 3. Human CSCs (hCSCs) regenerate infarcted myocardium. Myocardial infarction (MI) was produced in anesthetized female immunodeficient Scid mice and Fischer 344 rats treated with a standard immunosuppressive regimen. Shortly after coronary occlusion, two injections of $\sim 40,000$ hCSCs were made at the opposite sites of the border zone. Animals were killed 5–21 days after surgery. (A) Mouse heart 21 days after infarction and injection of hCSCs. Human myocardium (arrowheads) is present within the infarct (MI). BZ, border zone. Areas in rectangles are shown at higher magnification below. Human myocytes are α -SA (red) positive and *Alu* (green) positive. Asterisks indicate spared myocytes. (B) Ventricular hemodynamics was measured in sham-operated (SO), untreated infarcted (MI), and hCSC-treated infarcted animals (MI-hCSCs). Results are mean $\pm$ SD. * and † $p < 0.05$, versus SO and MI, respectively. (C) Calcium transient in EGFP-positive human myocytes and EGFP-negative mouse myocytes recorded by two-photon microscopy and laser line-scan imaging (calcium indicator Rhod-2, red). (D) Myocardial regeneration at 3 weeks. Connexin 43 (yellow; arrowheads in the inset) is present between human myocytes (α -SA, red; *Alu*, green) and spared rat myocytes (α -SA, red; *Alu* negative).

In analogy with other tissues, physiological aging and diseases negatively impact on the stem cell compartment of the heart (2, 5, 33, 63, 68, 80, 104, 129, 131, 138, 148). The population of CSCs in the failing myocardium displays characteristics of cellular senescence (18, 21, 31, 116), an impaired capacity to migrate to the areas of need (45, 46), and reduced differentiation ability (P.G., unpublished observations). The myocyte progeny derived from old CSCs rapidly acquires a senescent phenotype characterized by depressed contractile ability and a shorter lifespan (21, 46, 63, 68, 148, 159). The decline in CSC function with age may condition the deterioration in the structural integrity of the myocardium, leading to heart failure. Indeed, in rodent models of cardiac aging or chemotherapy-induced cardiomyopathy, transplantation of the CSCs from young hearts results in enhanced cardiomyogenesis and ameliorates the performance of the senescent hearts (31, 46).

Molecular Mechanisms That Regulate CSC Growth and Activation

Despite their high potential for myocardial regeneration, the activity of CSCs remains inadequate in the setting of extensive damage (1). Importantly, a subset of CSCs with preserved growth reserve persists in very old and sick hearts, and can be isolated for further propagation and clinical applications (13, 28, 63, 68). Significant effort has been applied to the understanding of the factors that limit the endogenous regenerative capacity of CSCs and the pathways that potentiate CSC growth and lineage specification.

CSCs reside in myocardial niches, in which parenchymal cells, for example, myocytes, serve to sustain their function. CSCs are structurally connected to the supporting cells *via* cadherin-mediated adherence junctions and connexin-mediated gap junctions (156) (Fig. 4). This intercellular communication affects multiple aspects of CSC behavior, including proliferation and self-renewal, survival in stressful conditions, migration from the niches, and differentiation. For example, activation of the Notch receptor by the Jagged1 ligand, which is expressed on cardiomyocytes, stimulates CSC division and drives myocyte lineage commitment. This signaling mechanism sustains the formation of a pool of transiently amplifying immature myocytes (14). If the Notch pathway is inhibited, myocardial repair is perturbed (Fig. 5). Pharmacological ablation of the Notch activity in the first week of postnatal life causes a dilated cardiomyopathy, underscoring the importance of Notch signaling for heart growth and myocyte formation (155).

An additional example of the direct role of myocytes in the control of CSC fate has been described in a recent study, which suggests that microRNAs, specifically miR-499, are actively transported *via* gap junctions from mature myocytes into CSCs, promoting their commitment and differentiation (53).

The potential contribution of MSCs to cardiac repair has also been postulated [reviewed in Refs. (15, 162)]. Although it has not been established whether cardiac-resident MSCs act as supporting cells within myocardial niches, transplanted bone marrow-derived MSCs successfully engraft in the heart after infarction, connect with the resident progenitors, and

FIG. 4. Cardiac niches and supporting cells. Within the niches, CSCs are structurally and functionally connected to the supporting cells, cardiomyocytes, and fibroblasts, by gap and adherens junctions. Connexin 43 and 45 and N- and E-cadherin in atrial niches containing c-kit-positive CSCs (green) are represented by yellow dots located between two CSCs (arrowheads), a CSC and a fibroblast (procollagen, white; arrows), and a CSC and a myocyte (α -SA, red; double arrowheads). Areas in the rectangles are shown at higher magnification in the insets.

FIG. 5. Myocardial regeneration and Notch1 function *in vivo*. Regenerative response of infarcted mice injected with a γ -secretase inhibitor (treated) or vehicle (untreated). (A) Border zone of an untreated infarct in which the area included in rectangles is shown at higher magnification in the insets. CSCs (c-kit, green) express activated Notch1 intracellular domain (N1ICD; magenta, arrows) and Nkx2.5 (yellow). Cardiomyocytes: tropomyosin, red. (B) Number of CSCs and percentage of CSCs positive for N1ICD and Nkx2.5 in the border zone of untreated and treated infarcts. * $p < 0.05$ versus untreated. (C–F) Area of regeneration (new) located within the infarct (dead). New myocytes are BrdU positive (C), N1ICD positive (D), and Nkx2.5 positive (E). (F) Merge of (C–E). (G) γ -secretase inhibition decreases the fraction of BrdU-labeled myocytes. * $p < 0.05$ versus untreated.

stimulate proliferation and myogenic commitment of endogenous c-kit-positive CSCs (49).

Cell-to-cell contacts are favorable for CSCs; cardiac SP cells in three-dimensional cell aggregates are protected from cell death induced by oxidative stress and anoxia/reoxygenation. This approach improves stem cell survival after transplantation in the hostile environment of the infarcted myocardium (6).

The components of the extracellular matrix (ECM) regulate multiple cellular processes by direct binding to the cells *via* specific receptors, by providing mechanical stimuli, or by modulating the signaling pathways triggered by several growth factors (8). In the myocardial niches, undifferentiated CSCs express the ECM receptor α 4-integrin (CD49d, VLA-4) (156), which mediates retention and mobilization of stem cells in the hematopoietic system (96, 111). Recently, it was

demonstrated that after myocardial infarction, an accumulation of the ECM component fibronectin is necessary for CSC expansion and tissue regeneration (74). Moreover, CSCs produce the ECM proteins in an autocrine fashion and the profile of the secreted ECM changes in the course of myogenic differentiation (7, 156). The *ex vivo* expansion of CSCs enhances the expression of the ECM and adhesion molecules, which have a beneficial effect on the function of CSCs after delivery to the injured heart (83).

One of the major regulators of CSC growth is Wnt signaling. The canonical Wnt pathway, through a downstream effector IGFBP3, limits the renewal of adult cardiac SP cells, blocks endogenous regeneration, and impairs cardiac performance (107). In contrast, Wnt/ β -catenin is upregulated in c-kit-positive CSCs that are induced to proliferate *in vivo* by stem cell factor (SCF) secreted by cardiomyocytes (166).

The SCF activates c-kit receptor. Gene transfer of SCF into myocytes promotes the expansion of the c-kit CSC compartment in the mouse heart after infarction and increases the recruitment of these cells to the injured area (166).

In addition to SCF, insulin-like growth factors (IGF) -1 and -2 have been shown to critically affect CSC behavior. IGF-1 signaling activates nuclear phospho-Akt and telomerase activity, diminishes oxidative stress, and delays CSC aging and death (148). Moreover, the expression of the IGF-1 receptor distinguishes a pool of phenotypically young CSCs, characterized by longer telomeres, a higher telomerase activity, enhanced proliferation, and higher resistance to apoptosis (27, 89). In an autocrine and a paracrine fashion, IGF-2 potentiates the myogenic differentiation of CSCs (27). Conversely, CSCs that present the IGF-2 receptor, rather than the IGF-1 receptor, and coexpress the angiotensin II receptor type 1, exhibit impaired growth and a high rate of cell death. The activation of IGF signaling in CSCs by pretreatment with the growth factor before transplantation or by *in vivo* administration using self-assembling peptide nanofibers facilitates cardiomyogenesis and vasculogenesis, ameliorating cardiac recovery after infarction (27, 110). Notably, IGF-1 can induce the expression of a downstream target of Akt kinase, named Pim-1 [for review Ref. (43)], which is an important regulator of CSC proliferation, differentiation, and survival in the infarcted myocardium (25, 26, 101, 151).

Abcg2 and MDR1 are known to contribute to multidrug resistance and play a role in protecting the CSCs from toxins. Cardiac SP cells from Abcg2 knockout mice show augmented cell death and reduced proliferation ability, whereas Abcg2 overexpression enhances CSC growth, but limits their myogenic differentiation (113). Additionally, Abcg2 controls the fate of CSCs, favoring symmetric cell division and stem cell renewal (127). Of interest, oscillations in Ca^{2+} levels in c-kit-positive CSCs similarly affect the cell cycle progression and dictate the pattern of stem cell proliferation. The spontaneous rises in intracellular Ca^{2+} , which are generated by IP3 receptor activation, condition the asymmetrical stem cell division potentiating myocyte lineage specification (38, 39).

The mobilization of CSCs to the site of injury is of paramount importance for their regenerative potential. Multiple factors affect CSC trafficking in the myocardium. For instance, the cytokine SDF-1 (CXCL12) is upregulated in the ischemic tissue (3, 17). SDF-1 activates the CXCR4 receptor, and this axis stimulates the migration of cardiac SP cells, Sca-1-positive cells, and CDCs (86, 146). Hepatocyte growth factor/scatter factor (HGF/SF) operates by binding to a high-affinity receptor c-Met. HGF has been shown to accumulate in the myocardium after infarction, and the expression of c-Met is controlled by hypoxia (17, 152). C-kit-positive CSCs contain functional c-Met and are mobilized to the infarcted region by the intramyocardial administration of HGF (89, 158), facilitating cardiac regeneration (46, 120). In addition to cytokines, the interaction of CSCs with cardiomyocytes controls cell translocation. The cell-guidance receptor EphA2 is preferentially expressed in c-kit-positive CSCs, whereas one of its ligands, ephrin A1, is presented in a complimentary fashion on cardiomyocytes (44). Intramyocardial administration of ephrin A1 promotes the mobilization of resident CSCs (44) and ameliorates heart recovery after myocardial infarction (36). EphA2 activation

by ephrin A1 increases the chemotaxis of CSCs to HGF, improving stem cell recruitment and cardiac repair after infarction (44).

Redox Control of CSC Function

Reactive oxygen and nitrogen species (ROS) such as superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), are highly chemically reactive by-products of aerobic metabolism. The major sources of ROS in cells are mitochondria and NADPH oxidase (4). Balanced ROS production and sequestration are necessary for proper cell responses to environmental stimuli, including activation of the signaling by growth factor receptors, regulation of cell adhesion, and differentiation [recently reviewed in Refs. (41, 73, 122)]. On the other hand, it is generally accepted that an excessive ROS level is harmful to cells, since it produces DNA damage, lipid and carbohydrate peroxidation, and protein oxidation and inactivation, leading to cellular senescence or death (42, 73, 128, 136). In pathological conditions, for example, atherosclerosis, heart failure, hypertension, diabetes, and aging, ROS create an inflammatory and oxidative microenvironment (167). Mitochondrial ROS emission is a major contributor to the overall oxidizing environment in cardiovascular diseases and heart failure. It is now emerging that the monoamine oxidase activity augments mitochondrial ROS generation and exacerbates heart dysfunction in the state of chronic stress (69–71).

In CSCs, oxidative stress has been shown to induce DNA lesions, upregulation of p53 and p16^{INK4a}, telomeric shortening, and death of stem and progenitor cells (Fig. 6). For example, diabetic hearts are characterized by a marked reduction in CSC abundance. High glucose promotes ROS accumulation, which causes CSC dysfunction in a p66^{shc}-dependent manner (119), as well as inhibition of the pentose phosphate and prosurvival signaling pathways, leading to cell apoptosis (72). Similarly, doxorubicin-induced cardiotoxicity is related to oxidative stress and is accompanied by accelerated apoptosis and premature senescence of CSCs (31, 45, 54, 116). This impairs the regenerative capacity of the heart following antineoplastic treatments.

Of interest, myocardial aging is associated with an increase in CSC numbers (68); however, CSCs in old hearts display signs of cellular senescence and an inferior growth and differentiation ability (18, 21, 46, 63, 66, 148). This is in accordance with numerous studies demonstrating that stem cell exhaustion due to extensive replication conditions organ aging [for review, see Refs. (2, 60, 130, 131)]. Surprisingly, a residual compartment of CSCs with preserved growth reserve is detectable in the senescent heart (18, 46, 65); yet, this stem cell population is idle and does not adequately contribute to myocardial repair. One of the most intriguing aspects of ROS function is the control of stem cell quiescence. Recent findings in the aging heart document an accumulation of quiescent CSCs, which persist in a hypoxic state due to alterations in their microenvironment and changes in metabolism (121). Although these CSCs possess longer telomeres and a significant proliferative ability, the low level of cellular ROS precludes re-entry into the cell cycle and new myocyte formation. Similarly, quiescent hematopoietic stem cells with the highest potential to reconstitute the circulating blood cells are found in the hypoxic

FIG. 6. Heart failure and CSC senescence. Shown are control myocardium (A), acute infarcts (C, E), and chronic infarcts (B, D, and F). c-kit-positive CSCs (green; arrows, A–F) express p16^{INK4a} (yellow, A and B) and p53 (magenta, C and D). The detection of telomeres in c-kit-positive CSCs is shown by small white fluorescence dots (E and F).

microenvironment of the bone marrow (106, 112). The low endogenous amount of ROS is correlated with hematopoietic stem cell quiescence and the preservation of the undifferentiated phenotype (61, 73, 103). The increase in cellular ROS, specifically, H₂O₂, has been shown to trigger hematopoietic stem cell proliferation, differentiation, and migration (20, 81, 94, 117). For instance, after ischemic injury, accelerated ROS production is required to modulate the activity of HIF-1 α , a key regulator of the intracellular response to hypoxia that induces hematopoietic stem cell expansion in the bone marrow and their mobilization into the circulation (17, 154). In agreement, experimental hyperoxia produces changes in the redox state of CSCs residing in the old myocardium, promoting their activation and ameliorating the aging heart phenotype (121).

In this regard, if the rise in cellular ROS is not well adjusted, as in the case of the genetic ablation of the ATM or FoxO proteins, the self-renewal capacity and differentiation pattern of hematopoietic stem cells are severely compromised (55, 100, 149, 164, 165). In a similar manner, the lack of expression of a transcription factor Prdm16 causes oxidative stress, interfering with the maintenance and function

of the neural stem cell compartment (23). Importantly, as discussed above, uncontrolled generation of ROS by mitochondria and a depressed antioxidant defense are among the major contributors to the overall oxidizing environment in cardiac pathologies and heart failure (19, 139, 150).

In contrast to these findings, an increased oxidative status promotes activity of neuroepithelial stem cells in the central nervous system (76). Likewise, oxidation favors self-renewal and division of cells that generate neurons and hippocampal cells (88).

Changes in cellular redox state, due to a decrease in ROS generation by mitochondria, are implicated in cardiomyogenic differentiation during development (50). Conversely, models of *ex vivo* differentiation suggest that mitochondrial oxidative metabolism and high ROS formation facilitate the acquisition of the cardiac phenotype by stem cells (24, 35, 77, 82). Recently, it has been shown that HIF-1 α links redox pathways and cellular metabolism (133, 144, 145). Thus, physiologic induction of ROS in CSCs may be regulated by cell-intrinsic mechanisms, including cell energetics, the metabolic state, and the microenvironment (73, 141, 160). In cardiac and embryonic stem cells, the concept

of oxidative optimum for the control of genetic stability has been proposed (85), implying that ROS production is tightly regulated to promote cell proliferation, differentiation, and survival; the adequate ROS level for these processes may depend on the cellular context.

It is important to indicate that nitrogen oxide (NO) plays a central role in cardiovascular physiology, and the interplay between NO and ROS, that is, the nitroso/redox balance, is a key regulator of cardiovascular homeostasis [for comprehensive review, see Refs. (48, 125)]. Future research may help to identify the impact of NO and ROS on CSC behavior and the potential therapeutic targets for the restoration of stem cell function in the pathologic heart.

Current CSC Therapies for Heart Diseases

The recognition that CSCs govern cardiac homeostasis and are endowed with regenerative capacity led to the development of stem cell-based therapies for myocardial repair. Numerous cell types have been introduced clinically in an attempt to restore cardiac structure and ameliorate heart function in patients. The results of the clinical trials with stem cells from extracardiac origins, such as circulating CD34-positive cells and bone marrow-derived MSCs, are extensively discussed elsewhere (58, 123, 124, 142, 162).

The therapeutic efficacy of resident c-kit-positive CSCs was recently assessed in the phase I trial Cardiac Stem Cells in Patients with Ischemic Cardiomyopathy (SCIPIO) (12). In this trial, c-kit-positive cells were obtained from the right atrial appendage of patients for autologous transplantation. The isolated CSCs were successfully expanded *ex vivo* and lacked the markers of lineage commitment, which reflects the features of primitive stem cells. The infusion of CSCs in the SCIPIO trial produced no adverse effects and exerted a lasting beneficial impact on the cardiac performance and quality of life in the patient population (22). Another therapeutic approach, which was employed in the Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction (CADECEUS) proof-of-concept clinical trial (92), involved the injection of CDCs that were expanded from endomyocardial biopsies. Although this trial failed to detect an improvement in cardiac function, a reduction in the size of scar tissue and an increase in the viable myocardium were observed in treated patients. Additionally, in a small-scale preliminary trial Autologous Human Cardiac-Derived Stem Cell To Treat Ischemic Cardiomyopathy (ALCADIA), the administration of undifferentiated cardiac cells together with a controlled-release formulation of basic fibroblast growth factor was evaluated in patients with ischemic cardiomyopathy (143). The findings from these clinical trials confirm the feasibility and safety of autologous CSC transplantation, and open an avenue for further efforts to harness the regenerative capacity of stem cells for cardiovascular therapies.

Innovation

There are significant clinical and experimental evidences demonstrating that the heart can heal itself, although to a limited extent. The repair process requires the activity of cardiac stem cells (CSCs) residing in the adult myocardium. Different classes of CSCs have been described and recently employed in clinical trials for the treatment of heart diseases,

producing encouraging results. Advances in the understanding of CSC biology indicate that cell-autonomous and environmental changes in the redox status also control CSC growth, survival, differentiation and, ultimately, regenerative potential. Thus, establishing approaches to modulate the redox signaling in the heart have important therapeutic implications.

Future Directions

Although considerable strides have been made in the course of the past decade to gain insight on the biology of CSCs, there are important aspects of CSC function that remain to be investigated. The molecular mechanisms leading to a decline in CSC regenerative potential with age constitute a fundamental question pertaining to the etiology of the aging myopathy and heart failure. Conceivably, an increasingly pro-oxidizing environment of the old myocardium impedes the CSC activity. Furthermore, in view of the potential implementation of CSCs for autologous transplantation in the elderly, the age-associated decrease in stem cell potency may significantly limit their therapeutic efficacy. Similarly, it is unclear why the endogenous regenerative reserve is inadequate to repair an extensive damage, which leads to myocardial scarring and progressive deterioration in the structural integrity of the heart. Thus, therapeutic strategies able to enhance the function of resident CSCs may prove to be required to interfere with the aging myopathy and heart failure in the old patient population.

It is equally important to study the factors that affect the efficacy of CSC transplantation. Approaches are to be developed to optimize cell preparation and routes of delivery, which will ensure a better survival of donor CSCs, adequate engraftment, and successful differentiation into mechanically efficient myocytes and integrated coronary vessels. Tissue engineering and combination therapies consisting of co-transplantation of multiple cell types, or CSC preconditioning with various factors are discussed in the literature and examined in preclinical settings (58, 71, 78, 93, 98, 163). To this end, treatments with antioxidants to prevent CSC senescence and death (37, 91), or establishing the tools that modulate the redox status of CSCs and thereby their activation and differentiation have significant therapeutic potential.

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Abbreviations Used

α -CA = α -cardiac actinin
 α -SA = α -sarcomeric actin
 α -SMA = α -smooth muscle actin
 CDCs = cardiosphere-derived cells
 CSCs = cardiac stem cells
 ECs = endothelial cells
 ECM = extracellular matrix
 H_2O_2 = hydrogen peroxide
 hCSCs = human cardiac stem cells
 HGF/SF = hepatocyte growth factor/scatter factor
 IGF = insulin-like growth factor
 MI = myocardial infarction
 MPCs = myocyte progenitor cells
 MSCs = mesenchymal stroma cells
 NICD = Notch1 intracellular domain
 NO = nitric oxide
 ROS = reactive oxygen species
 Sca-1 = stem cell antigen-1
 SCF = stem cell factor
 SMCs = smooth muscle cells
 SP = side population
 SO = sham-operated
 TGF- β 1R = TGF- β 1 receptor
 VPCs = vascular progenitor cells
 vWf = von Willebrand factor