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Role of stem cells in cardiovascular biology

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Summary

This review article addresses the controversy as to whether the adult heart possesses an intrinsic growth reserve. If myocyte renewal takes place in healthy and diseased organs, the reconstitution of the damaged tissue lost upon pathological insults might be achieved by enhancing a natural occurring process. Evidence in support of the old and new view of cardiac biology is critically discussed in an attempt to understand whether the heart is a static or dynamic organ. According to the traditional concept, the heart exerts its function until death of the organism with the same or lesser number of cells that are present at birth. This paradigm was challenged by documentation of the cell cycle activation and nuclear and cellular division in a subset of myocytes. These observations raised the important question of the origin of replicating myocytes. Several theories have been proposed and are presented in this review article. Newly formed myocytes may derive from a pre-existing pool of cells that has maintained the ability to divide. Alternatively, myocytes may be generated by activation and commitment of resident cardiac stem cells or by migration of progenitor cells from distant organs. In all cases, parenchymal cell turnover throughout lifespan results in a heterogeneous population consisting of young, adult, and senescent myocytes. With time, accumulation of old myocytes has detrimental effects on cardiac performance and may cause the development of an aging myopathy.

Keywords

aging heart; cardiac niches; cardiac stem cells; myocardial regeneration; myocyte turnover

The recognition that the adult heart possesses a stem cell compartment that can regenerate myocytes and coronary vessels has changed the perennial view of the heart as a post-mitotic organ and has formed the basis of a new paradigm in which multipotent cardiac stem cells (CSCs) are implicated in the physiological turnover of myocytes and vascular endothelial cells (ECs) and smooth muscle cells (SMCs) organised in coronary vessels [1,2]. Understanding the mechanisms of cardiac homeostasis would offer the extraordinary opportunity to potentiate this natural occurring process and promote myocardial

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regeneration following injury. However, the field of regenerative cardiology is in its infancy and great caution has to be exercised in the implementation of any cell type in humans. Additionally, in humans, multiple variables may negatively interfere with the therapeutic efficacy of the harvested cells. Ischaemic and non-ischaemic pathologic states, co-morbidities, duration and severity of the cardiac disease may have profound implications on the availability and functional competence of CSCs. Also, the age and gender of patients have to be regarded as important determinants of CSC growth and lineage commitment.

Cell-based cardiac repair

Different cell types have been proposed experimentally for the reconstitution of the damaged heart: skeletal myoblasts [3,4], fibroblasts [5], SMCs [6], foetal myocytes [7,8], embryonic stem (ES) cells [9], bone marrow-derived cells (BMCs) [10–14], and induced pluripotent stem (iPS) cells [15]. Fibroblasts, SMCs, and foetal myocytes form a passive graft, which, by decreasing the stiffness of the scarred portion of the wall, has a transient positive effect on ventricular remodelling and function. Totipotent ES cells appear to engraft into the myocardium but die rapidly because of the lack of vessel formation and immune rejection [16]. Additionally, ES cells give rise to teratomas and teratocarcinomas [17]. Similarly, the recently identified iPS cells have a tumorigenic potential [18]. These cell types have never been employed clinically.

The first attempt to replace infarcted myocardium with a patch of skeletal muscle was performed in the 1930's. Fifty years later, large sheets of skeletal muscle tissue were positioned on the epicardial surface of the ischaemic area and stimulated to contract with a pacemaker. This surgical procedure, known as dynamic cardiomyoplasty, has prompted investigators to utilise individual myogenic cells. The new approach has been called cellular cardiomyoplasty and consists of the direct injection of isolated skeletal myoblasts into the ischaemic area [3,4]. The autologous origin of the cells to be implanted constitutes an obvious advantage of this form of cardiac repair. Moreover, skeletal myoblasts are more resistant to ischaemia than cardiomyocytes, enhancing their possibility of survival in the necrotic myocardium. For these reasons, experimental skeletal myoblast implantation has been translated to the clinical arena, and infarcted patients have been subjected to this kind of treatment [19,20].

However, the lack of integration of skeletal myoblasts within the myocardium represents a reason of concern for the therapeutic implementation of skeletal myoblasts. Analysis of the graft-host myocardium interface has failed to document any evidence of mechanical or electrical coupling between skeletal and cardiac muscle [21]. Connexin 43 and N-cadherin are consistently absent in injected skeletal muscle cells and a layer of dense scar tissue often separates the cardiomyocytes from the skeletal muscle further opposing the incorporation process. As documented in animals [21,22], the absence of synchronous contraction may be one of the factors responsible for the arrhythmic manifestations observed in patients treated with skeletal myoblasts [19,20].

Moreover, skeletal myoblast implantation is preceded by an *in vitro* step of amplification. This necessity represents a limitation for the clinical application of this procedure. Adult

human myoblasts divide only 20–25 times *in vitro*, before becoming senescent [23]. This issue is particularly relevant since it has been documented that the success of skeletal myoblast implantation is directly correlated with the number of donor cells [24]. The need of a large quantity of cells to be implanted is dictated by massive and rapid cell death that occurs over a week after administration. During *in vitro* expansion and following the introduction in the heart, myoblasts withdraw from the cell cycle and form myotubes. The state of terminal differentiation rapidly acquired by skeletal myoblasts opposes any possible proliferation of the implanted cells. Damaged cells within the graft cannot be replaced impairing the mechanical and elastic properties of the graft and, ultimately, its effects on cardiac function. An important argument that speaks against the utilisation of skeletal myoblasts in cardiac repair is that the injured portion of the ventricular wall is replaced by a tissue that is far from being similar to the myocardium. Regenerative medicine should target the restoration of tissue with the same functional and structural properties of the damaged organ. However, transdifferentiation of skeletal myoblast in cardiac myocytes has never been observed [16]. These numerous problems have resulted in an early termination of the enrolment of patients in clinical trials [19,20].

BMCs may translocate to the heart, form temporary niches and participate in the homeostasis of the healthy organ or the regeneration of the injured tissue [25]. The contribution of this cell class to cardiomyogenesis and coronary vasculogenesis is currently unknown and remains an important unanswered question. The involvement of BMCs in cardiac chimerism has been proposed [26]. Interestingly, a comparison has been made between the degree of chimerism in cardiac allografts and in hearts of patients who received allogeneic bone marrow transplantation [27]. In the latter case, only 2–5% chimeric myocytes were detected, while 14–16% of chimeric myocytes and endothelial cells were found in transplanted hearts. These observations suggest the intracardiac origin of the recipient cells in the donor heart and the extracardiac origin of chimeric cells in the resident heart following bone marrow transplantation. In the first case, host cells may have migrated from the residual atrial stumps to the donor heart [28] and, in the second, donor cells may have reached the myocardium because of the high level of blood chimerism [27]. Thus blood-borne cardiac cells may be detected exclusively when the peripheral blood contains a large number of haematopoietic stem cells (HSCs). Experimental results support this contention [10,29].

Whether BMCs drive the regenerative response of the damaged heart remains an unresolved issue. The striking discrepancy between the incidence of heart failure and bone marrow failure and the lack of co-morbidity of these disease stated in the same patient indicates that HSCs do not typically migrate from the bone marrow and repopulate the decompensated heart. If the bone marrow continuously replenishes the heart with new functionally competent HSCs, the decline in myocyte number with cardiac diseases would not occur, and the poorly contracting myocytes would be constantly replaced by a bone marrow-derived progeny.

Shortly after the experimental evidence that HSCs induce myocardial regeneration after infarction [10], unfractionated mononuclear BMCs and CD34-positive cells have been administered to patients affected by acute and chronic myocardial infarction, dilated

cardiomyopathy, and refractory angina [30–34]. Although the individual outcomes have been inconsistent and variability exists among trials, meta-analyses of pooled data indicate that BMC therapy results in a 3–4% increase in ejection fraction [35]. Allogeneic and autologous mesenchymal stromal cells (MSCs) have also been employed in small clinical trials with encouraging results [36–38]. Although the benefits may seem modest, these initial data have favoured the conduct of larger randomised trials designed to critically evaluate the long-term effects of BMC therapy on a broader patient population. The mechanisms involved in the positive impact of BMC therapy on human beings remains to be identified. Measurements of coronary flow suggest that vasculogenesis may be operative while the contribution of *de novo* myocyte formation is uncertain. Additionally, the injected BMCs activate the growth and differentiation of resident CSCs via a paracrine effect, mediated by the release of a multiplicity of cytokines [39,40]. Importantly, the recent identification of CSCs has shifted the attention to endogenous cell mechanisms as a novel target of cell therapy for the failing heart.

Cardiac progenitor cell types

Dynamic cardiomyogenesis characterises the response of the damaged heart prenatally and shortly after birth. Additionally, in the adult zebrafish, cardiac regeneration in the absence of scar formation takes place after resection of up to 20% of the ventricle. Traditionally, muscle reconstitution in this model has been considered to be mediated by cardiomyocyte proliferation [41]. A similar regenerative response has been observed after surgical resection of the apex of the left ventricle in the neonatal mouse heart [42]. Again, cardiomyocyte proliferation was viewed as the crucial cell process, promoting cardiac repair. However, investigators in several laboratories concur with the notion that the adult heart contains a compartment of stem/ progenitor cells [43–53]. Distinct protocols based on the recognition of surface markers and transcription factors, and functional assays have been employed for the isolation of stem cells from the myocardium. The stem cell antigens c-kit and Sca-1 are expressed in partially overlapping pools of cardiac primitive cells. The presence of Sca-1 has been reported [45– 47,51–53] and the expression of c-kit has been detected in multiple studies [44,46,52,53] and found to be absent in another [45]. c-kit-positive cells are an established component of the canine [49] and human [28,48,50,54] heart, and their activation leads to the formation of new myocardium. The regenerative potential of c-kit-positive and Sca-1-positive cells after ischaemic injury differs significantly; a robust regenerative response occurs with c-kit-positive cells [44,55] whereas little engraftment and repair takes place with Sca-1-positive cells [45]. Whether the modality of administration of cells, the animal model, and the properties of the injected cells are responsible for the different outcome is presently unclear.

The developing heart and adult heart typically contains a CSC pool that has the ability to efflux the Hoechst 33342 dye [43,45,47,51,56]. Similar cells, which express an ATP-binding cassette transporter, have been identified in other organs and termed side-population (SP) cells [51,57]. The classicmember of this family is a P-glycoprotein that confers to the cells multidrug resistance by extruding anticancer drugs and rhodamine 123. These putative CSCs are 93% Sca-1-positive and appear to represent a small subset of the Sca-1-positive cells in the mouse heart [45]. According to a different study, however, SP cells comprise 2% of all

cells (2 per 100 cardiac cells) [47]. Cardiac SP cells appear to express CD31 and form haematopoietic colonies *in vitro* [47]. The presence of CD31, common to bone marrow SP cells [29,57] together with the peculiar growth behaviour of these cells *in vitro*, raises questions concerning their actual origin and suggests the possibility of a colonisation to the heart from the haematopoietic system.

More recently, a novel Sca-1-positive, Hoechst 33342 dyelow, and CD31-negative cardiac SP cell has been identified [51,56]. The modest expression of c-kit in these cells was attributed to methodological limitations inherent in the enzymatic cleavage of this receptor during digestion of the myocardium and cell isolation [51]. There is 1 SP cell per 30 000 cardiac cells in the mouse heart. CD31-negative cardiac SP cells form beating cardiomyocytes *in vitro* and acquire the adult phenotype *in vivo* through cellular coupling with differentiated cardiomyocytes [51]. These results strengthen the notion of a functional role for resident SP cells in the heart.

The Isl1 transcription factor is associated with the commitment to the myocyte lineage of cardiac cells that have lost their undifferentiated stem cell state. Isl1 and GATA4 are transcriptional coactivators of the myocyte transcription factor MEF2C [58]. Homozygous deletion of Isl1 alters the development of the heart, affecting the atria, right ventricle, and outflow tract [59,60]. However, these cells disappear after birth, which raises serious questions on the possibility of employing Isl1-positive cells for therapeutic purpose.

Cell culture in serum-free media on non-adhesive substrates has been employed for the isolation of primitive cells in several organs including the brain and the heart [61]. The formation of spherical clusters of cells known as floating spheres is achieved by a suspension culture method which is used for large-scale amplification of stem/progenitor cells as an alternative technique to single-cell deposition and clonal expansion [44,49]. The suspension protocol does not reflect the formation of multicellular clones from single founder cells. Spheres are highly motile structures, prone to fuse [62] and may, therefore, correspond to aggregate non-homogeneous cells. This peculiar form of anchorage-independent growth typically occurs with neural stem cells [63]. A central core of proliferating cells is commonly surrounded by quiescent cells with restricted developmental options. This dynamic phenotypic transition from a ‘mesenchymal’ monolayer state to an ‘epithelial’ floating state is commonly seen in culture of bone marrow MSCs [64].

Cardiospheres possess similar characteristics. Whether cardiospheres are clonal or oligoclonal in nature, cardiosphere-derived cells represent the progeny of the most primitive cells within the aggregates. A fraction of cells located in the core of the cardiospheres express the stem cell antigen c-kit and is surrounded by an outer layer composed of cells positive for CD105, a membrane glycoprotein commonly expressed in bone marrow MSCs [64]. Cardiosphere-derived cells undergo spontaneous maturation toward the myocyte lineage, and the process of commitment can be coaxed by co-culture with neonatal ventricular myocytes [65]. Connexin 43 is expressed between highly dividing cells within the cardiospheres and in the expanded differentiating cardiosphere-derived cells [66]. The presence of gap junctions between uncommitted and differentiated cells mimics the

organisation of stem cell niches *in vivo* raising the possibility that the differentiated cells may function as supporting cells [67,68].

The c-kit receptor tyrosine kinase originally was detected in a class of murine HSCs with long-term reconstituting ability in irradiated recipients [69]. More recently, c-kit has been found in several populations of stem cells in the adult liver, brain, and pancreas [70]. In the heart, this stem cell antigen identifies a pool of resident CSCs that are self-renewing, clonogenic, and multipotent *in vitro* and *in vivo* [44,49]. These CSCs replace infarcts with functionally competent myocardium restoring ventricular performance experimentally. Importantly, an identical cell with similar biological and functional characteristics exists in the human heart (Fig. 1) [2]. c-kit-positive CSCs and cardiosphere-derived cells are currently employed in clinical trials for the treatment of ischaemic cardiomyopathy (ClinicalTrials.gov Identifier: NCT00474461, NCT00893360). Encouraging results have been obtained [71] although the small number of treated patients and the short duration of the follow-up preclude definitive conclusions concerning the efficacy of cardiac progenitors in the treatment of heart failure. Together, these observations are consistent with the notion that the heart possesses an intrinsic capacity for regeneration, but whether differences in the expression of surface antigens reflect CSC subclasses, which are functionally distinct, is difficult to ascertain.

Cardiac stem cell niches

Stem cells are sheltered in specialised structures called niches, which provide a microenvironment designed to preserve the survival and replication potential of the primitive cells [72]. The concept of niches was introduced in 1978 by Schofield, who defined niche as ‘a stable microenvironment that might control haematopoietic stem cell behaviour’ [73]. Currently, the niche is viewed as ‘a subset of tissue cells and extracellular substrates that can indefinitely house one or more stem cells and control their self-renewal and progeny production *in vivo*’ [72]. Interstitial structures with the architectural organisation of niches have been found in the adult heart [1,67]. CSCs, progenitors, precursors and early differentiating cells are clustered together in the niche and are coupled with the surrounding cells through the expression of gap and adherens junctions [74,75]. Gap junctions are intercellular channels formed by individual structural units called connexins [76,77] while adherens junctions are composed of cadherins. Gap junctions allow cells to communicate with each other and to exchange small molecules. CSCs utilise gap junctions to transmit and receive signals from the surrounding tissue for cell survival, proliferation or differentiation [68,74,75,78,79]. Adherens junctions appear to be involved in the preservation of the undifferentiated state of CSCs. The function of gap junctions is probably cell and tissue specific. However, deregulation of the expression of connexins and cadherins, and alterations in the configuration of the junctional complexes affect the growth and commitment of stem cells.

Resident CSCs accumulate in areas exposed to low levels of haemodynamic load and wall stress. These properties are commonly found in the atria and in the apical portion of the ventricle. However, niches have been detected in several sites of the free wall of the left ventricle indicating that CSCs are preferentially but not exclusively present in protected

areas of the heart. The formation of ventricular niches may be dictated by the migration of CSCs from their sites of storage to the regions of damage, which are typically located in the ventricular wall. Physical forces in the ventricular wall can be transduced in intracellular responses that regulate cell behaviour and fate. The consequences of mechanical factors on CSC function are unknown. The low haemodynamic stress in the atria and apex may facilitate the preservation of the CSC pool while the high degree of stress at the base andmid-region of the left ventricular wall may condition CSC commitment. Importantly, abnormal pathologic loads are coupled with the initiation of myocyte regeneration, which is heterogeneous and tends to parallel the alterations in the distribution of stresses in the damaged heart [80,81]. The effects of strain on CSCs are currently unknown. However, the peculiar topographical distribution of CSCs in the heart suggests that a relationship may exist between the function of CSCs and the level of haemodynamic stress.

Growth kinetics of c-kit-positive cardiac stem cells

If the heart is a dynamic organ, the replenishment of its parenchymal and non-parenchymal cells is regulated by a stem cell compartment and by the ability of these primitive cells to self-renew and differentiate. Regeneration conforms to a hierarchical archetype: slowly dividing stem cells give rise to highly proliferating, lineage-restricted progenitor cells, which then become committed precursors that, eventually, reach growth arrest and terminal differentiation. Stem cells divide rarely while committed transient amplifying cells are the actual group of replicating cells in self-renewing organs. The short-lived amplifying cells possess a unique property; they undergo a finite number of doublings and simultaneously differentiate until they withdraw from the cell cycle and reach terminal full maturation.

Stem cells divide symmetrically and asymmetrically. When stem cells divide symmetrically, two self-renewing daughter stem cells may be formed. The purpose of this modality of replication is the expansion of the stem cell pool, which occurs in active phases of growth during prenatal organ development. Stem cells can also divide symmetrically into two committed daughter cells; this type of cytokinesis is triggered by an emergent situation, that requires restoration of the lost tissue upon a pathological insult. This process may decrease the number of primitive cells. With asymmetric division, two differently fated sibling cells are generated: one daughter-stem cell and one daughter-committed cell. The non-stem cell sister is a short-life committed progenitor cell that divides and simultaneously differentiates, i.e. the amplifying cell [70,82–84]. When the amplifying cell acquires complete maturation, the cell cannot divide further and reaches the terminal state of differentiation. The objective of asymmetric division is to maintain a steady state in which organ homeostasis is conditioned by a tight balance between stem cell formation and the production of a committed progeny. The developmental choice made by CSCs at any given time has a direct impact on the number of stem cells, progenitors, precursors, amplifying cells and, ultimately, mature cells. The size of the amplifying myocyte pool conditions the magnitude of the homeostatic and regenerative response in damaged heart.

The organisation of the stem cell compartment in niches is crucial for the maintenance of the primitive cell pool and for the preservation of organ homeostasis [67,72,82,83,85,86]. The niche microenvironment controls the number of stem cells and their progeny by influencing

the pattern of stem cell division. The inhomogeneous intracellular segregation of selective proteins in daughter cells at the time of mitosis constitutes the intrinsic determinants of CSC fate in animals and humans. Genes, including numb, α-adaptin and members of the Notch pathway, interact to enable single primitive cells to produce differently destined sibling cells [87–90]. Numb can segregate to one of the two daughter cells or be equally distributed in the cytoplasm of both daughter cells [87–90]. Numb is expressed during mitosis, from late prophase to telophase, and in the early stages of life of the new daughter cell [91]. Numb localises to endocytic vesicles and binds to the endocytic protein α-adaptin inducing the internalisation and inactivation of the Notch receptor [92].

CSCs that receive Numb become unresponsive to Notch while Numb-negative CSCs retain their responsiveness to Notch and adopt the phenotype associated with Notch activation [87,93]. Signalling through the Notch receptor can occur only between closely adjacent stem cells and supporting cells. The Notch ligands are transmembrane proteins, which, upon binding, cleave the Notch receptor so that its intracellular domain is translocated to the nucleus where it forms complexes with transcription factors of the recombinant DNA binding protein RBP-Jk [94–96]. These effector pathways are operative in the heart and Notch1 activation by the Jagged1 ligand promotes the commitment of CSCs to the myocyte lineage within the cardiac niches in the mouse heart [97]. A similar behaviour has been observed in human CSCs (hCSCs). The progressive dropout of myocytes with aging and pathological states may activate an emergency response of CSCs towards the differentiating pathway to generate rapidly a large number of muscle cells. The long-term outcome of CSC growth is a reduction of the stem cell pool and, ultimately, the exhaustion of their replicating reserve.

Cardiomyogenesis and myocyte turnover in the aging heart

The adult heart is largely composed of terminally differentiated myocytes. Damaged and old units of this highly specialised compartment of contracting cells are constantly replaced by new younger elements. Mitosis and cytokinesis have been recognised in poorly differentiated myocytes with a thin subsarcolemmal halo of myofibrils (Fig. 2) [50], in combination with telomerase activity [50,54,98,99]. These *in vivo* findings, together with observations *in vitro* [44], have indicated that replicating myocytes are transit amplifying cells derived from lineage commitment of CSCs. The ability to replenish old and damaged cells is maintained by the existence of telomerase-competent CSCs. Telomerase activity has been documented in the decompensated old rat heart [100], in the failing canine heart [101], and in the human myocardium. In humans, cardiac hypertrophy with modest ventricular dysfunction [54], and prematurely aged heart with severe functional impairment [102] are characterised by an increase in telomerase activity. This enzyme, however, cannot prevent telomere erosion [54]. In hCSCs, telomere shortening occurs at a rate of approximately 130 base pairs per population doublings, possibly limiting CSC lifespan. Thus, aging effects on CSCs lead to an imbalance between telomerase activity and length of telomeres, resulting in critical telomeric shortening, permanent withdrawal from the cell cycle, and CSC senescence.

The controversy on the growth reserve of the adult human heart has not been resolved, and the extent of myocyte renewal claimed by different groups varies dramatically. A recent study, based on retrospective carbon 14 (^{14}C) birth dating of cells, has claimed that 1% and 0.45% replacement of myocytes occurs annually in the human heart at 25 and 75 years of age, respectively [103]. These findings indicate that only 50% of myocytes are renewed during the entire life of the human heart, from birth to death, whereas an equal number lives as long as the organ and organism, up to 100 years of age and longer. Although the possibility of myocyte regeneration was confirmed, the actual magnitude of the process is in contrast with the level of myocyte apoptosis found in the adult human heart [104] and the progressive increase in myocyte loss that occurs with aging in humans [105]. Myocyte regeneration increases as a function of age, and the age of cardiomyocytes does not coincide with the age of the organ and organism. This discrepancy becomes more apparent in the senescent myocardium in which a large proportion of myocytes is 5 years old or younger in both women and men [106]. The older the human heart, the younger is its myocyte compartment. From 19 to 104 years of age, essentially none of the myocytes present at birth is preserved in the young adult, middle-aged, and senescent heart [106]. These findings question the contention that 50% of cardiomyocytes are not replaced during the entire lifespan in humans [103], suggesting that a large proportion of cells survives and retains its function for more than 100 years.

The presence of hCSCs throughout the lifespan of the human heart is apparently at variance with the limited capacity of endogenous tissue repair after infarction [50]. This phenomenon has been interpreted as the unequivocal documentation of the inability of the adult heart to create cardiomyocytes [107– 110]. A possible explanation for this apparent paradox has been obtained in animal models in which dead stem cells have been found throughout the infarct, indicating that the fate of hCSCs is comparable to that of the other cells located in the ischaemic region [84]. It might come as a surprise, but a similar event occurs in other solid and nonsolid organs including the skin, bone marrow, liver, intestine, and kidney. In all cases, occlusion of a supplying artery leads to scar formation mimicking cardiac pathology [1,84].

hCSC aging conditions myocardial aging; chronological age leads to telomeric attrition in hCSCs, which generate a progeny that rapidly attains the senescent phenotype. Daughter cells acquire the shortened telomeres of maternal hCSCs and, after a few rounds of division and terminal differentiation, express $p16^{INK4a}$ in nearly 2 years [106]. The pool of old cardiomyocytes progressively increases, defining the aging myopathy. Telomere length reflects the past replicative history and cumulative oxidative DNA damage occurring during the life cycle of the cell [111]. Telomerase activity delays but does not prevent telomere erosion, which is mediated by downregulation of telomerase, reactive oxygen species, and loss of telomere-related proteins [50,112]. Shortening of telomeres beyond a critical length triggers cellular senescence, which corresponds to irreversible growth arrest in G1 with loss of specialised functions, including cell proliferation, migration, and differentiation. Suggestive evidence in humans and genetically manipulated mice [113–115] points to shortening of telomeres as a critical determinant of cellular senescence and, possibly, organ aging. In this regard, a lineage relationship was found between hCSCs with short telomeres and the formation of old myocytes. In a mouse model of accelerated aging, the deletion of

the RNA competent of telomerase leads to cardiac hypertrophy, cavitary dilation and heart failure [113]. These pathological alterations are dictated by excessive myocyte apoptosis and defective cardiomyogenesis. In analogy with HSCs [116], forced expression of telomerase in CSCs may prevent replicative senescence. Although it is difficult to establish whether telomeric shortening is a consequence of aging or a primary event conditioning the aging myopathy, telomerase deletion is coupled with telomere shortening and the manifestation of a premature aging cardiac phenotype [113].

Importantly, the female heart possesses a superior ability to sustain the multiple variables associated with the aging process and the development of the senescent myopathy. At all ages, the female heart is equipped with a larger pool of functionally competent hCSCs and younger myocytes than the male myocardium. The replicative potential is higher and telomeres are longer in female hCSCs than in male hCSCs. Animal studies have shown that the insulin-like growth factor (IGF)-1– IGF-1 receptor system is present in CSCs at very old age [117] and overexpression of IGF-1 in cardiomyocytes prevents the manifestations of the senescent cardiac phenotype and heart failure [118]. Additionally, the IGF-1–IGF-1 receptor axis is enhanced in female myocytes [119], and it may condition the favourable outcome of age in this gender. Importantly, premature cardiac aging appears to affect predominantly men than women [102]. Additionally, oestrogens phosphorylate IGF-1 receptors [120] mimicking the effects of IGF-1, a powerful inducer of CSC division, survival, and maintenance of telomere length [117,118]. In postmenopausal women and throughout life in men, oestrogen is synthesised in extragonadal organs including bone, cartilage, adipose tissue, skin, breast, and heart [121]. With aging, oestrogen loses its circulating generalised function and works mainly at the local level as a paracrine, autocrine, or intracrine factor. Oestrogen induces transcription of the catalytic subunit of the telomerase protein (TERT), because an oestrogen response element is present in the TERT promoter [122]. Additional downstream effector pathways of oestrogen involve the activation of phosphatidylinositol 3-kinase/Akt cascade, which exerts multiple beneficial effects on cardiac performance and biology [123].

Myocyte regeneration in the physiologically aging heart takes place at previously unexpected levels in both women and men. In the female heart, myocyte replacement occurs at a rate of 10%, 14%, and 40% per year at 20, 60, and 100 years of age, respectively. Corresponding values in the male heart are 7%, 12%, and 32% per year [106], documenting that myocyte turnover involves a large and progressively increasing number of parenchymal cells with aging. From 20 to 100 years of age, the myocyte compartment undergoes 15 cycles of complete replacement in women, and 11 cycles in men [106].

This high degree of myocyte turnover, which increases further with age, is strikingly different from the data derived from the integration of ^{14}C into the DNA of myocyte nuclei [103]. Retrospective birth dating of human myocardium DNA by ^{14}C mirrors the incorporation of thymidine analogues in animal models, an analysis that has only been rarely possible in human beings and, thus far, has been restricted to the brain [124] and more recently to the heart [125]. An inherent limitation of ^{14}C birth dating is related to the need to introduce mathematical models with assumptions that affect the computed cell turnover values [103]. The scenario chosen presumed that the number of myocytes in the heart was

constant and that cells turned over at a near constant rate. This form of invariant growth defines parenchyma in a steady state in which cell death is compensated by cell regeneration in young healthy individuals. However, this steady-state scenario poorly represents the biology of aging in men where nearly 64×10^6 cardiomyocytes are lost per year. Furthermore, because women do not lose cardiomyocytes as they age like men [105] different models should be used to reflect the changing cell populations. Importantly, myocyte number increases postnatally [125,126] and cell loss typically occurs with cardiac diseases [104].

Another problem with the study of retrospective ^{14}C birth-dating involves the use of troponin I expression as a marker for the isolation of a representative pool of myocyte nuclei. The presence of troponin I almost exclusively identifies a population of $p16^{INK4a}$-positive senescent cells that exhibit marked alterations in the permeability of nuclear pore complexes [125]. Additionally, the percentage of $p16^{INK4a}$-positive myocytes varies dramatically with age and cardiac diseases [50,102]. Multiple markers in a large number of healthy hearts spanning a large age range are required to measure and model the actual degree of cardiac cell turnover [106].

Conclusions

The human heart is a highly dynamic organ regulated by a pool of resident hCSCs that modulate cardiac homeostasis and condition of organ aging. Hopefully, recent findings will resolve the long debate that has divided the scientific community in strong opponents and passionate supporters of the regenerative potential of the human heart, offering a more biologically valid understanding of cardiac homeostasis and repair. A common ground can now be found to translate this different perspective of cardiac biology into the development of novel strategies for the management of the human disease. However, the magnitude of the process, the effects of age on the extent of myocyte renewal, and the origin of newly formed cardiomyocytes remains a matter of controversy.

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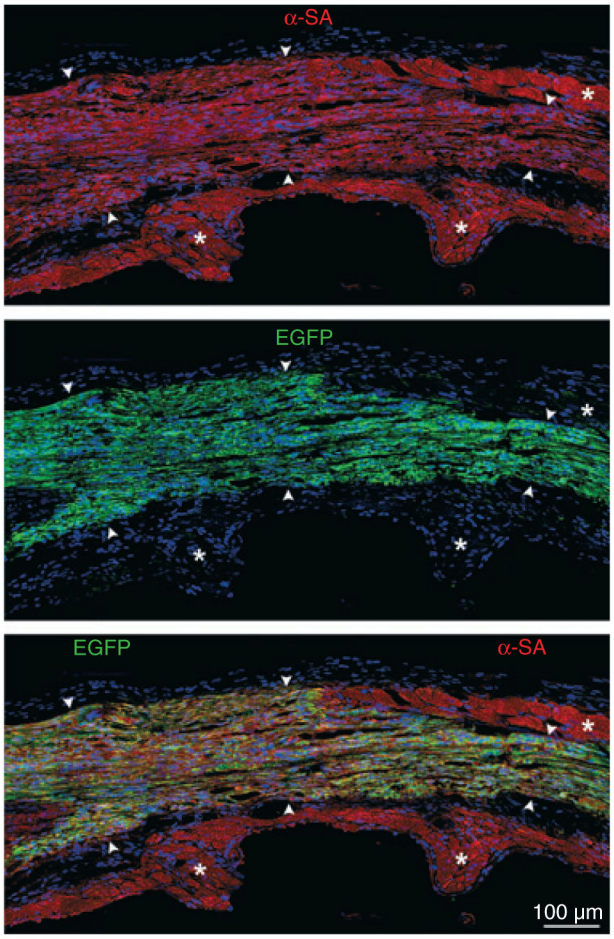

Fig. 1.

Viral tagging of cultured hCSCs and myocardial regeneration. Band of regenerated myocardium (arrowheads) within the infarcted region of the rat left ventricle. Newly formed myocytes express α-SA (upper panel, red) and EGFP (central panel, green). Lower panel, merge of upper and central panel. *Spared myocytes.

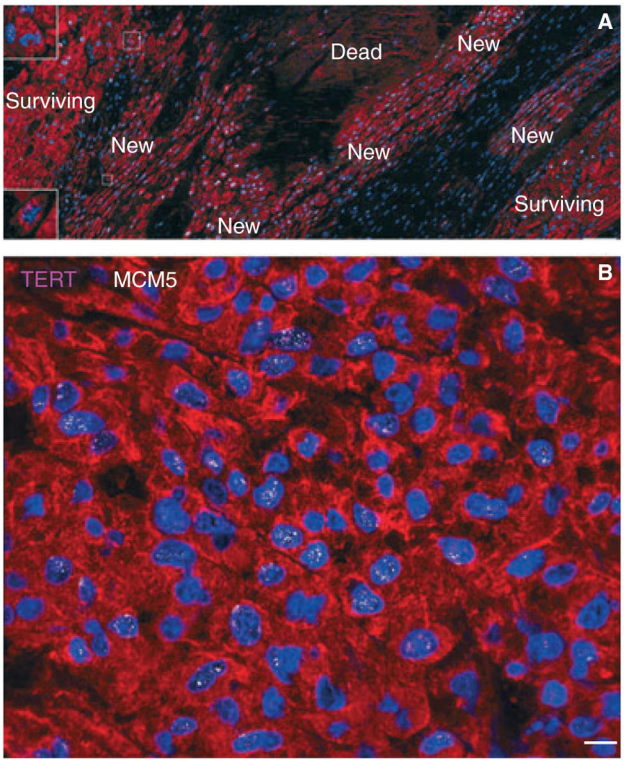

Fig. 2.

Spontaneous myocyte regeneration within the infarcted human myocardium. (A) Clusters of highly proliferating small developing myocytes are visible. Myocytes are labelled by cardiac myosin (red) and nuclei by DAPI (blue). Most of these cells are positive for MCM5 (white). Two dividing small myocytes are shown in insets. (B) Small developing myocytes within the infarct are positive for telomerase (magenta) and MCM5 (white) in the nuclei (Scale bars: A, 100 μm; B, 10 μm).