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Cardiac Aging – Getting to the Stem of the Problem

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Abstract

Cardiac aging is a heterogeneous process caused by a combination of stochastic events which manifests as loss of structure and function in the heart, however several recent studies draw attention to aging being primarily a stem cell problem. This review summarizes findings in support of the “stem cell hypothesis of aging” and discusses the impact of age on cardiac stem cells and the niche.

Keywords

cardiac stem/progenitor cells; aging; heart

Introduction

Heart disease is the leading cause of death in the Western world with advancing age as a predisposing risk factor for heart failure. Recent statistical studies report a 171% increase in the number of deaths caused by cardiovascular diseases in patients between 65 to 85 years of age [1]. Changing this appalling statistic requires a clear comprehension of the genetic basis of age associated-cardiovascular diseases and molecular mechanisms directly causal to the phenotype. Aging is a heterogeneous process characterized by classical hallmarks including genomic DNA damage, telomere shortening, epigenetic modifications leading to accumulation of senescent cells accompanied by defects in protein homeostasis, nutrient sensing mechanisms, mitochondrial function, regenerative potential of stem and progenitor cells and inter- and intra-cellular communication abilities [2]. While the combination of these culpable stochastic events causes organ aging, manifesting as loss of structure and

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None.

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function in the heart, several recent studies draw attention to aging being primarily a stem cell problem [3–5]. This review summarizes findings in support of the “stem cell hypothesis of aging” [6, 7] and discusses the impact of age on stem cells and the niche.

The pioneering study documenting that the adult heart is home to c-kit⁺ clonogenic, multipotent, self-renewing cardiac stem/progenitor cells (CPC) which are capable of repairing the heart after injury, marked the dawn of a new era in cardiac regenerative medicine [8]. Over the years, several labs across the globe have identified various stem cell populations such that the heart has transformed from a non-regenerative organ to one housing several “different” stem and progenitor cells, identified by a myriad of markers such as c-kit, stem cell antigen1, islet1, Abcg2/MDR, Peg3/PW1, platelet derived growth factor receptor or derived from 3 dimensional multicellular clusters known as cardiospheres [9–16]. Despite the claimed abundance of endogenous cardiac progenitor cell types in the heart, repair following myocardial injury is limited by age, prompting the need for exogenously expanded stem cells to regenerate the damaged myocardium. For example, mesenchymal stem cells (MSCs) derived and expanded from the bone marrow have been shown to improve cardiac structure and function post myocardial infarction (MI) in animals and human patients (Clinical Trials- PROMETHEUS [17], TAC-HFT [18], POSEIDON [19]). Similarly, phase I clinical trials with c-kit⁺ CPCs (SCIPIO trial [20]) as well as cardiosphere derived cells (CADUCEUS trial [21]) demonstrate that the autologous infusion of stem cells or isolated and cultured from the patient heart represent a viable, safe, clinically relevant option of therapy; however, only modest improvements in left ventricular ejection fraction and scar size reduction post MI are observed [20, 21]. The patient heart in need of stem cell based therapy typically represents an aged and diseased microenvironment which cripples the functional and regenerative potential of the isolated cardiac stem cells [22, 23]. Understanding the molecular mechanisms of cardiac aging and cellular senescence is therefore critical to antagonize aging and improve the efficacy of stem cell mediated repair and regeneration.

Stem Cell Hypothesis of Aging – You are as old as your stem cells

The “stem cell hypothesis of aging” postulates that the organ and organism’s age is determined by the age of its endogenous stem cells; the decline in regenerative potential of the stem cells at least in part causes aging and an associated loss of organ function and tissue homeostasis [5, 6]. The more youthful stem cells present in an organ, the better its ability to heal following injury. Therefore the biological age of the stem cells supersedes the actual age of the organism and determines the regenerative capacity. Elegant studies using muscle derived stem and progenitor cells validate this hypothesis. The health and life span of progeroid mice with defective stem cells showed significant improvement following transplantation of youthful muscle stem cells isolated from skeletal muscle of young mice [24]. Substantial blunting of degeneration and vascularization were observed in tissues where the transplanted stem cells were not detected suggesting secretion of systemic factors as a mechanistic basis for the beneficial effects mediated by young stem cells [24]. Conversely, restoring levels of systemic factors that decline with age appears to rejuvenate stem cells in the skeletal muscle [25] and brain [26], thus ablating age associated tissue degeneration. A cell autonomous decline in stem cell function has also been demonstrated to

cause age associated skeletal muscle degeneration [27]. Stem cells in aged muscle lose up to two-thirds of their functional capacity and this cell-intrinsic senescence of stem cells is not abrogated even upon transplantation into a youthful environment [27]. Therefore stem cell senescence and loss of function appear to have a causal role in aging thus validating the stem cell hypothesis of aging, and rejuvenation of endogenous stem cells by targeting cell-intrinsic or extrinsic pathways appears to mitigate the functional defects associated with age.

Findings from genetically engineered mice provide corroborative evidence of the stem cell hypothesis of aging in the heart. Transgenic mice overexpressing insulin growth factor 1 (IGF1) show a clear correlation between CPC age and the biological age/health of the animal [28]. CPCs and myocytes displayed increased phenotypic characteristics of senescence in aged non-transgenic mice, eventually leading to cell death and age-associated ventricular dysfunction. However, more youthful stem cells were evident in the transgenic mice, contributing to abrogation of cell death and significant functional improvements. Overall, the antagonism of myocardial aging apparent in the transgenic mice was found to correlate with, and was attributed to, the youthfulness of the cardiac stem cell pool [28]. Consistently, activation of CPCs in aged rats was found to partially reverse myocardial aging and elongate lifespan, suggesting that rejuvenation of stem cells is an effective strategy to combat the aging myopathy and heart failure [29]. In a recent study from our lab, CPC exhaustion was found to precede the early cardiac aging phenotype seen in heterozygous knockout mice for nucleostemin, a nucleolar stem cell associated protein, permitting the conclusion that CPC senescence may, at least in part, cause the loss of structure and function typically associated with cardiac aging in the animal [30]. Although a clear causal relationship between CPC senescence and myocardial aging has not been established in humans, correlative evidences demonstrate the relevance of the stem cell problem to humans. Heart failure in aged humans is associated with exhaustion of the CPC pool and chronological age is a predictor of classical biomarkers of cellular senescence in CPCs [23]. Studies from our group have established that adult human CPCs isolated from old patients with end stage heart failure have molecular characteristics of cellular senescence coinciding with declined functional capacity, as compared to stem cells isolated from fetal human hearts [30, 31]. Consistently, biologically youthful stem cells harbor better potential to repair and mitigate cardiac damage in patients [31, 32]. Corroborating these findings, the growth potential of CPCs, as determined by the proliferation rate, telomere length, telomerase activity and expression of IGF1 receptor, correlates with cardiac anatomy and predicts the clinical outcome in patients with ischemic cardiomyopathy receiving coronary artery bypass graft surgery [33, 34]. Patients with explanted CPCs demonstrating reduced growth characteristics, coinciding with increased senescence, revealed ventricular wall thinning, chamber dilation and a decline in ejection fraction. On the contrary, patients with CPCs bearing molecular characteristics of youthfulness had positive remodeling of the left ventricle, characterized by increase in wall thickness and a reduction in chamber volume and inner diameter [34]. Similarly, differentiation of stem cells into amplifying cardiac myocytes is believed to contribute to cardiomyogenesis and hypertrophy in patients with aortic stenosis [35]. While the amplifying cardiac myocytes are smaller in size and express cell cycle markers, the larger terminally differentiated cardiac myocytes express cell cycle arrest/senescence marker, p16 [35]. That compensatory physiological cardiac hypertrophy ensues

from CPCs with growth potential seems a plausible explanation from these findings. It is also reasonable to speculate that the transition from hypertrophy to heart failure in the pathologically challenged myocardium may be accompanied by, and partly caused by, accumulation of senescent and exhausted stem cells. Diastolic heart failure manifested as heart failure with preserved ejection fraction is another hallmark of cardiac aging [36]; however whether or not it is a stem cell disease with a cure involving stem cell based therapy remains a relatively unexplored area of myocardial biology. Overall these clinically relevant findings suggest that CPC age is a predictor of the pathological outcome, health and age of the human heart.

Alternative Perspectives on Stem Cells and Cardiac Aging

Findings from TAC-HFT and POSEIDON clinical trials using MSCs in patients with ischemic cardiomyopathy demonstrate that the chronological age of the recipient does not affect the efficacy of the treatment [37]. Functional improvements and scar size reduction following cell therapy were comparable between patients older and younger than 60 years of age. Although the cellular and functional characteristics of the donor MSCs as well as the recipient's endogenous CPCs were not studied, this finding demonstrates that stem cell therapy using exogenously expanded MSCs can circumvent chronological age of the recipient and potentiate cardiac repair [37]. Treatment with MSCs also enhances proliferation and differentiation of endogenous CPCs, suggesting that stem cell interactions may be an important mechanism for success in stem-cell based therapy [38]. Indeed, combinatorial cell therapy using MSCs and CPCs has been demonstrated to enhance the reparative effects by decreasing scar size and restoring ventricular function following MI [39].

How the stem cells and niche respond to aging

The fundamental properties of stem and progenitor cells such as quiescence, proliferation, self-renewability and lineage commitment are altered as a consequence of age (Figure 1). CPCs reside in a niche or microenvironment encompassing cardiac myocytes, fibroblasts and endothelial cells and exist in a quiescent, metabolically dormant state of reversible cell cycle arrest (G_0 state) to preserve their functional properties and adapt to metabolic stress [40, 41]. The stem cells communicate with their niche and systemic milieu and the bidirectional flow of information determines their ability to respond to intercellular cues, exit the quiescent state following injury and become activated to either enter the cell cycle or undergo lineage commitment (Figure 1). Prior to entering the activated state, skeletal muscle satellite cells have been demonstrated to transition to an 'alert' phase (G_{Alert}) in a mechanism dependent upon mTORC1 [42]. With increased aging, the niche secretes signaling factors which disrupts the quiescence of stem cells causing increased activation even in the absence of an injury stimulus [43]. Age-associated alterations in signaling impairs the communication between the niche and the stem cells accounting for lack of activation in response to injury [43]. Conversion from quiescence to a senescent state caused by activation of signaling cascades such as p16 also contributes to lack of stem cell activation upon injury [44]. The non-beneficial, premature activation of stem cells in the

absence of injury seems likely to result in exhaustion of the stem cell pool leading to impaired repair and regeneration following injury.

In the context of the heart, the number of CPCs increases with age. However, the number of functionally competent CPCs decrease drastically as senescent stem cells with short telomeres progressively accumulate [28, 45]. The oxygen content of the stem cell niche also appears to determine the efficiency of CPC activation. Both normoxic and hypoxic cardiac stem cell niches exist in the heart; normoxia favours stem cell activation, proliferation and commitment, eventually leading to senescence and exhaustion; hypoxia on the other hand favors a more quiescent state. In the aged heart, the pool of hypoxic, quiescent niches which fail to activate following injury increases, leaving the load of cell turnover on the small pool of normoxic CPCs, thus accounting for the decline in tissue regeneration [46, 47]. Findings from our lab demonstrate that phenotypic characteristics underlying CPC maintenance, such as morphology, expression of molecular markers of stemness and multipotency, proliferation potential and multinucleation are also altered with aging [30]. In addition, the migratory potential of old CPCs is impaired, associated with cell autonomous defects in Ephrin signaling [32].

Aged CPCs appear to undergo lineage commitment in the absence of a differentiation stimulus. Our findings seem to suggest that CPCs isolated from mice with critically short telomeres undergo cell cycle arrest, lose stemness and become increasingly differentiated even at basal conditions, when cultured in media favoring proliferation and stem cell maintenance (Hariharan N, Nguyen N and Sussman MA, unpublished observations). However upon addition of dexamethasone, a non-specific inducer of differentiation, senescent CPCs inefficiently undergo commitment and have reduced expression of differentiation markers [30]. Stem cell self-renewal is another important characteristic that is influenced by aging. Dividing CPCs undergo asymmetric or non-random chromatid segregation as well as random segregation [48, 49]. As a consequence of non-random segregation, the daughter cells generated are typically one stem cell which maintains the stem cell pool, and one committed cell which is likely to differentiate into a cardiomyogenic cell. Random segregation typically results in 2 daughter stem cells or 2 daughter committed cells [47]. However, if as a consequence of non-random segregation, 2 daughter stem cells result, then one stem cell will retain the template or immortal DNA and exhibit elevated regenerative potential typical of a primitive stem cell, and the other stem cell will bear the nascent DNA and is likely to become the senescent stem cell [49–51]. This mechanism at least partly accounts for the age associated decline in the pool of functionally competent stem cells and the heterogeneity observed in the stem cell population.

Epigenetic and epigenomic modifications occurring during aging also dictate stem cell fate. In hematopoietic stem cells, the epigenomic modifications enforce increased self-renewal and decreased differentiation [52]. The epigenetic signature of CPCs is not clearly known, although the epigenetic mechanisms underlying differentiation of cardiac stem cells during development of the heart has been well documented [53].

Controversies and Limitations

Like all intellectual ideas that invoke debate, the stem cell field is ridden with controversies from believers and non-believers of CPCs and its role in cardiomyogenesis. Research labs have employed multifarious strategies to prove and disprove the existence of CPCs and their ability to form cardiac myocytes. Mouse models developed for genetic lineage mapping have demonstrated that new cardiac myocytes are formed from division of pre-existing myocytes in the aging heart [54] and that c-kit⁺ CPCs minimally contribute to cardiac myocyte formation following MI [55]. On the contrary, several studies spanning nearly a decade, elegantly demonstrate that c-kit⁺ CPCs are both necessary and sufficient for cardiac repair and regeneration following injury [8, 56]. Given the ongoing controversies [57–61], the use of CPCs in clinical trials is also being questioned. However, fundamental limitations of the above mentioned studies [54, 55] make it misleading to extrapolate the conclusions to clinical use: A) conclusions drawn from genetically modified mice do not accurately translate into humans. The basic biology of mouse and human CPCs appear to be very different. As a simple example, findings from our group demonstrate that human CPCs undergo replicative senescence while those from mice do not arrest in culture easily owing to their long, hypervariable telomeres [30, 31, 62]. B) The afore-mentioned studies examine the role of endogenous CPCs in cardiomyogenesis. However, clinical trials use exogenously expanded stem cells which behave very differently from endogenous stem cells. *In vitro* culturing is likely to favor growth and survival of the fittest cells, in an artificial environment that is highly conducive to their growth, whereas the oppressive environment of the aged, damaged heart does not support massive expansion of the endogenous CPCs. C) Cardiomyogenesis may not be the only mechanism through which the exogenous CPCs contribute to enhanced repair. Decline in cell death and improvement of cardiac function are seen in the recipient heart following stem cell infusion, despite poor engraftment and retention of the cells, suggesting that secretion of pro-survival and pro-angiogenic paracrine factors is likely to be a mechanism through which stem cells heal the heart [63, 64].

Conclusion and Future Directions

Despite the controversies surrounding the efficiency of CPC-based cardiomyogenesis, copious incontrovertible evidences in animal models and humans prove the presence of CPCs and also demonstrate its ability to mitigate damage and mediate myocardial repair. The outstanding question remains – how do we make it better? Both exogenous and endogenous stem cell-mediated repair needs to be improved greatly. The stem cells should be potentiated to competently thrive in the coercive environment of the heart which is both aged and damaged. Changes to cardiac stem cells and the niche is both a cause and consequence of biological aging. An in-depth understanding of the mechanisms by which stem cells age and the molecular targets that either accelerate or delay aging need to be identified. Strategies aimed at rejuvenating aged or senescent stem cells need to be the next step forward, so as to turn back the clock and antagonize the myocardial aging process. The field is also in need of animal models with early cardiac aging to understand the influence of biological age on stem cell-mediated myocardial regeneration. Marked disparities in cardiac stem cell biology, phenotypic characteristics of cellular aging and telomere dynamics between current mouse models of cardiac aging and humans underscore the need for naturally occurring

animal models that more closely recapitulate human aging. Comparing aged stem cells from animal models and human patients will enable identification of novel molecular mechanisms that can be used for stem-cell based therapy to treat age associated cardiovascular diseases in the clinic.

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Highlights

- Age is a predisposing risk factor for heart disease
- Aging is accompanied by, and may be caused by, exhaustion of the stem cell pool
- The age of an organism is determined by the age of its endogenous stem cells
- Both stem cells and the niche alter in response to aging
- Studying factors causing aging is essential to improve success of stem cell therapy

Figure 1.

Hypothetical schematic of cardiac stem cells in a young (**A**) and aged (**B**) heart. **A.** Cardiac stem cells exist in a quiescent, dormant state of cell cycle arrest in the young, uninjured heart. Following cardiac injury, the stem cells exit the quiescent state and become activated to undergo proliferation, self-renewal and differentiation. **B.** In the aged heart, cardiac stem cells lose quiescence and are defectively activated even in the absence of an injury stimulus leading to exhaustion of the stem cell pool. The lack of competent stem cells accounts for the limited, inefficient regenerative response to injury in the aged heart.