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Rejuvenating the senescent heart

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Abstract

Purpose of review—The purpose of this review is to provide an update on the cardiac stem cell field with an emphasis on aging and to suggest some relevant strategies directed toward rejuvenation of the senescent heart.

Recent findings—Stem cells were long considered as a fountain of youth and were assumed to be equipped against any form of aging effect. However, it is now clear that stem cells suffer the consequences of aging as well. With the discovery that cardiac stem cells reside in the heart comes the question whether these cells are also impaired upon aging. As cardiac stem cell properties are also altered with age, autologous stem cell-based therapy to treat heart failure will benefit from new improved strategies.

Summary—With the goal to improve stem cell properties that are impaired upon aging, some strategies are highlighted. Genetic modification of adult human cardiac progenitor cells prior to autologous stem cell-based therapy, delivery of the next generation of stem cells such as CardioChimeras and CardioClusters, and improvement of the myocardial environment with rejuvenating factors constitute some of the possibilities and are discussed in more detail in this review.

Keywords

aging; rejuvenation; senescence; stem cells

Introduction

Aging is ineluctable and impacts upon every tissue and organ of the body. As we age, our body accumulates damage because of increased oxidative stress, mitochondrial dysfunction, genome instability, and telomeric dysfunction. Ultimately, accumulation of damage and shortening of telomeres lead to cellular senescence, which is defined as a state of irreversible growth arrest. Rejuvenation, the long-sought ‘*Holy grail*’ in the aging field, is defined as the reversal of the aging process to provide youthful properties to tissue and organ function to improve longevity. The contribution of stem cells to the rejuvenation process is beginning to emerge as a viable strategy to bring youthful properties to tissue and organs of the body. Stem cells are clonogenic cells capable of self-renewal and multilineage differentiation [1].

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These cells were long considered as a fountain of youth and were assumed to be equipped against any form of aging effect. However, it is now clear that stem cells suffer the consequences of aging as well. The discovery that cardiac stem cells reside in the heart has challenged the dogma that the myocardium is a postmitotic organ incapable of self-renewal, opening new possibilities and therapeutic options to patients suffering from heart failure. However, aging negatively impacts upon cardiac stem cell function. Aged cardiac stem cells have decreased ability to self-renew and to properly differentiate, leading to stem cell pool exhaustion [2]. Manipulating cardiac stem cells to improve their properties and rejuvenate them is the focus of intense research in our laboratory and we will discuss some of our recent studies as well as others with the goal to suggest relevant strategies to rejuvenate the senescent heart.

Aging, a Matter of Environmental Cues

Cardiac stem cells are located in niches inside the heart. The niches consist of a specific microenvironment where stem cells reside and they are responsible for dictating the stem cells' fate. When stem cells are inside this microenvironment, they are in a quiescent state, but once they leave it, stem cells are prone to differentiate, leading to exhaustion of the compartment. Therefore, the niches control the physiologic turnover of cardiac stem cells, regulating the growth, migration, and commitment of the cells that leave the microenvironment [3]. Interestingly, Sanada *et al.* [4] have shown that the niches are composed of hypoxic c-kit-positive cardiac progenitor cells (CPCs) and accumulate preferentially in the atria and apex of the left ventricle. They further demonstrated that hypoxic CPCs are unable to reenter the cell cycle and divide, so that normoxic CPCs are forced to undergo intense proliferation and differentiation, resulting in progressive telomere erosion, formation of senescent dysfunctional cardiomyocytes, and stem cell pool exhaustion. Therefore, the balance between hypoxic and normoxic CPCs is critical for the regulation of cardiac homeostasis and the preservation of the stem cell pool during the organ lifespan.

The environment surrounding the niches can influence cardiac stem cell properties and behavior as well. Stem cell behavior depends on their contact with other cells and on the release of paracrine factors from neighboring cells. Mounting evidence suggests that stem cell function is impaired with aging and that niche aging contributes to the age-related decline in stem cell function. This is mediated in part by the senescence-associated secretory phenotype. Accumulation of senescent cells alters the microenvironment by secreting various inflammatory factors, thus altering stem cell function. For example, old myocytes nested in proximity to the cardiac stem cell niches may secrete a variety of inflammatory molecules and factors, such as interleukin-6, favoring senescence and apoptosis [5]. This illustrates how important environmental cues impact upon cardiac stem cell properties, whether they are coming from the niche or from released factors, and how an aged environment has the potential to negatively impact upon cardiac stem cell function.

Altered Cardiac Progenitor Cell Properties Upon Aging

Myocardial aging is dictated by stem cell function and properties. According to Anversa and Leri [2], aging effects on myocytes, smooth muscle cells, endothelial cells, and fibroblasts may be only an epiphenomenon dictated by cardiac stem cell aging. Therefore, any impairment in stem cell properties may disturb the structural integrity and function of the aged heart.

Proliferation

Human cells have a limited capacity to divide, also referred to as the Hayflick limit. Once cells reach this limit, they stop proliferating and become senescent. It is now clear that telomere shortening plays a key role in the senescent fate of the cell. Human CPCs (hCPCs) have telomeres of approximately 8–10 kilobase pairs. With each cell division, CPCs lose around 130 base pairs of telomeric DNA [3]. When hCPCs have reached their limit in population doublings, telomeres shorten to a critical length, growth arrest occurs, and senescence is acquired [6]. Therefore, because of progressive shortening of telomeres with age, proliferative capacity is significantly impaired in aged CPCs. Nevertheless, the possibility that a pool of hCPCs with intact telomeres in the senescent heart exists is not to be excluded [7]. This is of utmost importance when selecting cardiac stem cells for cell-based regenerative therapy.

Migration

Alterations in the migration of CPCs from the niche toward damaged areas have been observed upon cardiac aging. The family of Eph receptor tyrosine kinases and ephrin ligands play a critical role in the migration of stem cells. In their study, Goichberg *et al.* demonstrated that in senescent cells, the tyrosine kinase activity of the EphA2 receptor is inadequately activated. They showed that the accumulation of reactive oxygen species in aged CPCs induces modifications of the EphA2 receptor, which severely influences its function in CPC migration [8]. Loss of migratory ability of old CPCs affects their translocation within the heart, impairing cell turnover in the aging myocardium.

Self-renewal/differentiation

Stem cells can divide symmetrically or asymmetrically. Through symmetric division, stem cells repopulate and expand their numbers by giving rise to two self-renewing daughter cells. When under going asymmetric division, a stem cell generates one daughter stem cell and one daughter cell committed to differentiation. Asymmetric division is the predominant form of cardiac stem cell renewal, accounting for 65% of proliferating cells [3]. Upon aging, loss of CPC self-renewal through increased asymmetric division pushes the fate of stem cells toward lineage commitment, resulting in stem cell pool exhaustion. CPCs have the potential to differentiate into cardiac myocytes, smooth muscle cells, and endothelial cells. However, it is clear that, following significant damage and cell loss such as after myocardial infarction, CPCs are not efficient enough to regenerate the injured tissue. Perhaps one of the most memorable events in the cardiac stem cell field this past year comes from the debate involving two studies. Van Berlo *et al.* [9■■■] showed that endogenous c-kit-positive cells are capable of generating new cardiac myocytes, albeit at a functionally insignificant level.

However, Ellison *et al.* [10] previously demonstrated that endogenous c-kit-positive cells are necessary and sufficient for the regeneration and repair of myocardial damage. Many commentaries coming from both groups are referenced herein to provide the reader with a more thorough view on the controversy [11–17]. This ongoing debate suggests that it is not a settled issue yet and that a real consensus needs to be reached before clear conclusions can be drawn. Nevertheless, the common ground in all this controversy is that cardiac stem cells have indeed the potential to form new cardiac myocytes and that a collaborative effort has to be made to bring a significant change in this field.

Telomere Shortening as an Indicator of Aging

As we age, shortening of telomeres occurs as a consequence of cell turnover to replace damaged cells. Telomeres consist of specialized DNA structures made up of tandem repeats located at the end of chromosomes. The main function of telomeres is to protect chromosomal ends from DNA damage and prevent chromosomal end-to-end fusion, degradation, and instability [18]. Because DNA polymerase does not replicate chromosomal ends completely, telomeres shorten with each round of cell division, and their diminished length has been used as an indicator of cellular senescence. Telomerase is a ribonucleoprotein with reverse transcriptase activity that preserves and restores telomere length and is found primarily in germline cells, cancer cells, and immortalized cell lines. Telomerase maintains telomere length by adding hexameric repeats to the telomeric ends of the chromosomes, thus compensating for the continued shortening of telomeres that would otherwise occur. However, even if telomerase is expressed and active in stem cells, it does not prevent telomere erosion. Thus, telomeres still get shorter with age and telomere shortening influences stem cell aging. Daughter cells inherit the shortened telomeres of the maternal cells. This means that cardiac stem cells displaying an old phenotype will form myocytes that will inherit the shortened telomeres of the maternal cells and will rapidly acquire the senescent cell phenotype.

Strategies to Rejuvenate the Senescent Heart

With the current view that aging cannot be avoided, increasing mortality caused by heart failure is expected. Therefore, efficient strategies to rejuvenate an old heart need to be considered. Focusing on strategies that will circumvent deleterious effects on both the stem cell niches and stem cell properties is the most viable approach.

Genetically engineered human cardiac progenitor cells

hCPCs derived from aged failing hearts display a senescent phenotype with shortened telomeres and limited proliferation capacity. Thus, a viable strategy to consider is enhancing CPC intrinsic properties to rejuvenate them *in vitro*. Our group has a legacy of work on Pim1 in the myocardium, a serine/threonine kinase with a pro-proliferative and antiapoptotic role. In a recent study, we demonstrated that Pim-1 modification of human adult CPCs reverses their senescent phenotype by increasing telomere length, improving growth kinetics, and delaying replicative senescence [19]. Interestingly, we also observed that beneficial effects of Pim-1 overexpression in human adult CPCs depend on Pim-1 intracellular localization. In particular, we showed that overexpressing Pim-1 in the nuclear

compartment reversed the senescent phenotype to a higher extent than overexpression of Pim-1 in mitochondria (Samse K, unpublished). Another recent study from Hariharan *et al.* [20] demonstrated that overexpression of nucleostemin in human adult CPCs antagonizes their senescence by decreasing the number of senescent and multinucleated cells, and by restoring a youthful cell morphology. Nucleostemin is a nucleolar protein activated downstream of Pim-1 and is known to regulate ribosomal biogenesis, proliferation and growth of stem cells [21]. Given their efficacy in antagonizing hCPC senescence, Pim-1 and nucleostemin constitute promising candidates for future interventional approaches to restore reparative potential in the aged failing heart.

CardioChimeras and CardioClusters

The second strategy we are suggesting is based upon enhancing CPC environment by promoting a better communication between CPCs and other types of stem cells. Two novel stem cell products have been engineered and are currently being studied in our laboratory: CardioChimeras and CardioClusters [22]. CardioChimeras are formed by fusing together CPCs with mesenchymal stem cells (MSCs). This fusion allows the direct influence of MSCs through their powerful secretory phenotype on the regenerative and differentiation potential of CPCs [23]. It has been previously demonstrated that the interaction between exogenously delivered bone marrow MSCs and endogenous c-kit-positive CPCs enhances CPC proliferation and differentiation [24]. Furthermore, combined administration of human MSCs and human c-kit-positive CPCs into an infarcted heart significantly improved myocardial structure and function [25]. However, combinatorial cell therapy is a limited approach to support cellular communication because of marked differences in proliferation rates and survival after injection into the damaged heart. Thus, CardioChimeras constitute the next generation for combining the beneficial effects of MSCs with CPCs. CardioClusters consist of a 3D microenvironment composed of specific cell types isolated from the human heart: CPCs, MSCs, endothelial progenitor cells (EPCs), and fibroblasts. They are rationally layered to promote efficient myocardial repair. Thus, CPCs are mixed in the CardioCluster core with MSCs and fibroblasts, which are responsible for secreting cell adhesion molecules to help maintain the CardioCluster structure. The core is protected by a layer of EPCs, which provide for endothelial-specific differentiation and production of tubular networks. CardioClusters' uniqueness lies in the fact that they can be delivered without the necessity to dissociate them from their 3D architecture [26]. An ex-vivo cardiac microenvironment allowing enhanced cellular communication and physical proximity, known as Cardiospheres, has previously been reported [27]. Cardiospheres are 3D spontaneous aggregations of heterogeneous stem cells and have been created to enhance communication between cardiogenic stem cells and the cardiac environment after adoptive transfer. However, clinical application of Cardiospheres as reported in the CADUCEUS trial requires dissociation of the spheres, destroying the 3D architecture and communication [28]. Therefore, because of their unique characteristics to potentially enhance stem cell microenvironment and properties, delivery of CardioClusters or CardioChimeras in an aged failing heart is considered as the next generation of stem cell therapy to rejuvenate the senescent heart.

Systemic growth factors

Emerging evidence indicates that a youthful environment enhances endogenous regeneration and that systemic factors have a significant impact upon tissue aging. Loffredo *et al.* [29] identified the rejuvenating factor GDF11 and demonstrated that this systemic factor reverses age-related cardiac hypertrophy. They further showed that GDF11 was ineffective in preventing cardiac hypertrophy in the context of pressure overload, suggesting that the antihypertrophic effect of GDF11 may not apply to all forms of cardiac hypertrophy. The identification of rejuvenating growth factors such as GDF11 opens new avenues and therapeutic possibilities to reverse the effects of aging on the heart.

Conclusion

Initial studies testing stem cell-based therapy in young animals to treat an injured heart provided promising results showing improved heart structure and function. However, the benefits of autologous cell therapy are greatly dampened in the elderly, who constitute the target population for regenerative medicine. This is mainly because adult stem cell functionality decreases with age, thus impairing tissue regeneration. Rejuvenation of the senescent heart with strategies focusing on improving intrinsic properties of cardiac stem cells as well as the microenvironment surrounding cardiac stem cells constitutes the next step, bringing us closer to the ultimate goal of finding the ‘Holy grail’ in the treatment of the aging failing heart.

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References and Recommended Reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■■ of outstanding interest

1. Fuchs E, Segre JA. Stem cells: a new lease on life. *Cell*. 2000; 100:143–155. [PubMed: 10647939]
2. Anversa P, Leri A. Innate regeneration in the aging heart: healing from within. *Mayo Clin Proc*. 2013; 88:871–883. [PubMed: 23910414]
3. Leri A, Rota M, Hosoda T, et al. Cardiac stem cell niches. *Stem Cell Res*. 2014; 13:631–646. [PubMed: 25267073]
4. ■ Sanada F, Kim J, Czarna A, et al. c-Kit-positive cardiac stem cells nested in hypoxic niches are activated by stem cell factor reversing the aging myopathy. *Circ Res*. 2014; 114:41–55. This study is the first to show that CPCs are nested in a hypoxic niche and that a balance between the hypoxic and normoxic milieu is necessary to maintain cardiac homeostasis and stem cell pool. [PubMed: 24170267]

5. Siddiqi S, Sussman MA. Cardiac hegemony of senescence. *Curr Transl Geriatr Exp Gerontol Rep*. 2013; 4:2.
6. Cesselli D, Beltrami AP, D'Aurizio F, et al. Effects of age and heart failure on human cardiac stem cell function. *Am J Pathol*. 2011; 179:349–366. [PubMed: 21703415]
7. Rota M, Leri A, Anversa P. Human heart failure: is cell therapy a valid option? *Biochem Pharmacol*. 2014; 88:129–138. [PubMed: 24239645]
8. Goichberg P, Kannappan R, Cimini M, et al. Age-associated defects in EphA2 signaling impair the migration of human cardiac progenitor cells. *Circulation*. 2013; 128:2211–2223. [PubMed: 24141256]
- 9■■■. van Berlo JH, Kanisicak O, Maillet M, et al. c-kit+ cells minimally contribute cardiomyocytes to the heart. *Nature*. 2014; 509:337–341. This study challenges the cardiac stem field by showing that c-kit cells are capable of forming new cardiomyocytes, but at a functionally insignificant level. [PubMed: 24805242]
- 10■■■. Ellison GM, Vicinanza C, Smith AJ, et al. Adult c-kit(pos) cardiac stem cells are necessary and sufficient for functional cardiac regeneration and repair. *Cell*. 2013; 154:827–842. This study supports the notion that c-kit cells make a significant contribution to myocardial regeneration and repair. [PubMed: 23953114]
11. Molkentin JD, Houser SR. Are resident c-Kit+ cardiac stem cells really all that are needed to mend a broken heart? *Circ Res*. 2013; 113:1037–1039. [PubMed: 24115067]
12. Torella D, Ellison GM, Nadal-Ginard B. Adult c-kit(pos) cardiac stem cells fulfill Koch's postulates as causal agents for cardiac regeneration. *Circ Res*. 2014; 114:e24–e26. [PubMed: 24526679]
13. Molkentin JD, Houser SR. Response to Torella *et al*. *Circ Res*. 2014; 114:e27. [PubMed: 24526680]
14. Nadal-Ginard B, Ellison GM, Torella D. Absence of evidence is not evidence of absence: pitfalls of cre knock-ins in the c-Kit locus. *Circ Res*. 2014; 115:415–418. [PubMed: 24965482]
15. Molkentin JD. Letter by Molkentin regarding article, 'The absence of evidence is not evidence of absence: the pitfalls of Cre Knock-Ins in the c-Kit Locus'. *Circ Res*. 2014; 115:e21–e23. [PubMed: 25258403]
16. Nadal-Ginard B, Ellison GM, Torella D. The cardiac stem cell compartment is indispensable for myocardial cell homeostasis, repair and regeneration in the adult. *Stem Cell Res*. 2014; 13:615–630. [PubMed: 24838077]
17. van Berlo JH, Molkentin JD. An emerging consensus on cardiac regeneration. *Nat Med*. 2014; 20:1386–1393. [PubMed: 25473919]
18. Blackburn EH. Switching and signaling at the telomere. *Cell*. 2001; 106:661–673. [PubMed: 11572773]
- 19■. Mohsin S, Khan M, Nguyen J, et al. Rejuvenation of human cardiac progenitor cells with Pim-1 kinase. *Circ Res*. 2013; 113:1169–1179. This study shows that Pim-1 overexpression in aged hCPCs reverses their senescent phenotype. This observation suggests that genetic modification of hCPC properties may improve autologous stem cell-based therapy. [PubMed: 24044948]
20. Hariharan N, Quijada P, Mohsin S, et al. Nucleostemin rejuvenates cardiac progenitor cells and antagonizes myocardial aging. *J Am Coll Cardiol*. 2015; 65:133–147. [PubMed: 25593054]
21. Hariharan N, Sussman MA. Stressing on the nucleolus in cardiovascular disease. *Biochim Biophys Acta*. 2014; 1842:798–801. [PubMed: 24514103]
22. Quijada P, Sussman MA. Making it stick: chasing the optimal stem cells for cardiac regeneration. *Expert Rev Cardiovasc Ther*. 2014; 12:1275–1288. [PubMed: 25340282]
23. Quijada PJ, Cubillo JD, Staub C, Sussman MA. Enhanced myocardial repair with CardioChimeras. *Circ Res*. 2013; 113:E157–E1157.
24. Hatzistergos KE, Quevedo H, Oskouei BN, et al. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. *Circ Res*. 2010; 107:913–922. [PubMed: 20671238]
25. Williams AR, Hatzistergos KE, Addicott B, et al. Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. *Circulation*. 2013; 127:213–223. [PubMed: 23224061]

26. Monsanto MM, Mohsin S, Fisher K, Sussman M. Enhanced myocardial repair with CardioClusters. *Circ Res*. 2013; 113:E156–E164.
27. Smith RR, Barile L, Cho HC, et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation*. 2007; 115:896–908. [PubMed: 17283259]
28. Malliaras K, Makkar RR, Smith RR, et al. Intracoronary cardiosphere-derived cells after myocardial infarction: evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trial (CARDiosphere-Derived aUtologous stem CELls to reverse ventricUlar dySfunction). *J Am Coll Cardiol*. 2014; 63:110–122. [PubMed: 24036024]
29. Loffredo FS, Steinhauser ML, Jay SM, et al. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell*. 2013; 153:828–839. This article identifies for the first time a rejuvenating factor from the systemic circulation. This study opens new avenues for the identification of other factors potentially capable of reversing aging effects on other tissues and organs. [PubMed: 23663781]

Key Points

- Myocardial aging can be reversed at the stem cell level by improving their intrinsic properties and by optimizing their microenvironment.
- Understanding cardiac stem cell behavior in the aging environment is necessary for the development of new strategies.
- Strategies to consider include genetic engineering of hCPCs, delivery of next-generation stem cells such as CardioChimeras and CardioClusters, and rejuvenating the environment with systemic factors.