Stem Cell Aging and Age-Related Cardiovascular Disease: Perspectives of Treatment by Ex-vivo Stem Cell Rejuvenation

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Abstract: Aging affects endogenous stem cells in terms of functionality and numbers. In particular, during aging, the stemness property can decrease because of enhanced apoptotic cell death and senescence. In addition, aging and aging-related co-morbidities affect the paracrine activity of stem cells and the efficiency of their transplantation. Collectively, this leads to a reduction of the capacity of organs to repair themselves, possibly due to a reduced functional capability of stem cells. Therefore, major efforts have been invested to improve the repair capability of stem cells in aged individuals by overexpressing antisenescence and antiapoptotic genes. In this review, we describe critical genes and signaling pathways in stem cell aging and discuss ex vivo genetic modification approaches aimed at stem cell rejuvenation that are of interest for the cardiovascular system.

Keywords: Aging, diabetes, gene therapy, Hippo, hyperlipidemia, myocardin, Notch, Pim-1, YAP, stem cell, rejuvenation, telomerase.

Potential of Stem Cells in the Treatment of Age-Related Cardiovascular Diseases

Diseases of aging, such as metabolic syndrome, diabetes, hyperlipidemia, atherosclerosis, neurodegenerative diseases, osteoporosis and cancer, represent a major problem for all societies. Despite contemporary medical treatments, heart failure continues to be an important cause of morbidity and mortality amongst the elderly of developed countries [1]. In addition, the incidence of cardiovascular disease (CVD) is rapidly increasing in developing countries. Both type 1 and type 2 diabetes are associated with aging and increased risk of micro- and macrovascular disease, which can lead to ischemic heart disease, heart failure and critical limb ischemia. Hyperglycemia leads to and aggravates the reduction of blood flow in cardiovascular tissues. This is believed to occur in a cascade in which ischemia induces oxidative stress, which initiates fibrosis, thereby increasing the thickness of microvasculature walls [2]. Other consequences of oxidative stress associated with hyperglycemia include: alteration of energy metabolism, organ dysfunction, limited exercise tolerance, and greatly increased vulnerability to a super-imposed ischemic stressor (i.e., following atherosclerotic occlusion of a main artery).

Valid therapeutic strategies that repair damaged heart muscle and ischemic tissue have not yet been developed. Heart transplantation and mechanical support devices remain the only effective remedy for severe cardiac dysfunction. However, because of the existence of major limitations including the limited number of donor organs, immune rejection and infections, research interest has increased towards alternative treatments.

Additionally, critical limb ischemia represents an important cause of diabetes-associated cardiovascular complications [3], and still represents the most common cause of amputation in diabetic patients. Transplantation of stem cells, progenitor cells, or stem-cell derived engineered tissues could be an alternative treatment for tissue repair [4-6]. For the application of progenitor cells, different cell sources have been suggested, including pluripotent and adult cell populations. Unlike adult stem cells, concerns remain in regard to the harvesting of human embryonic stem cells, because of ethical concern, allogenicity, neoplasm and teratomas formation [7]. Muscle, bone marrow, blood, epidermis, brain, liver and adipose tissue are the major sources for adult stem cells.
In addition, skin has been utilized to generate induced pluripotent stem cells [10]. Besides the transplantation of exogenous stem/progenitor cells, it might be possible to enhance the endogenous regenerative capacity of the heart. Recent data has suggested that the adult heart retains some capacity for self-healing and self-renewal due to the presence of resident cardiac stem (CSCs) and progenitor cells (CPCs) [11-14]. These observations suggest the opportunity to take a multilateral approach for cardiac regeneration, consisting of boosting the endogenous regenerative capability of the heart in addition to stem cell transplantation. Despite these remarkable advances, however, mortality and morbidity of patients with heart failure, remains high.

Several other potential cardiac progenitor cell types have been reported in recent years, such as c-kit(+) cells. However, the potential of c-kit(+) cells has become controversial [15, 16]. Recent lineage tracing experiment suggest that endogenous c-kit(+) cells contribute only minimally to the formation of new cardiac myocytes [17]. There are also data that epicardium-derived progenitor cells are capable of regenerating the adult heart when stimulated [18]. Although these different progenitor cell types appear to be distinct from each other in situ [19], cell culture expanded adult cardiac-derived cardiac progenitor cell populations exhibit a high degree of transcriptome similarity [20]. This suggests that more effort needs to be invested on optimizing the regenerative potential of these cells and less on the different isolation methods of these cells.

The use of adult stem cells is limited by the high degree of morbidity due to the isolation procedure itself. An example of this is represented by the isolation of bone marrow-derived stem cells, which requires general or spinal anesthesia associated with limited cell yield and further steps for in vitro cell expansion, which in turn introduce a risk of bacterial contamination [21, 22]. Some aspects of these problems have been solved by the use of more easily available stem cell types such as adipose tissue-derived stem cells [23], or by somatic reprogramming of fibroblasts into pluripotent stem cells [10]. These types of sources can be considered as the ideal stem cell source, since they allow easy stem cell isolation, higher cell yield and lower patient morbidity. However, the following limitations of current approaches have still to be solved: 1) inadequate recruitment of circulating or resident cardiac stem cells; 2) poor capability of adult stem cells to differentiate into cardiomyocytes; 3) elevated mortality of transplanted stem cells; 4) abnormal electromechanical behavior of transplanted cells after stimulation and the eventual onset of arrhythmias; 5) improper structure of newly formed heart tissue; and 6) diminished functionality and number of both resident and circulating stem/progenitor cells or even induced pluripotent stem cells [24, 25] with the onset of aging and age-related CVD [26-28].

Stem cell aging is of particular importance in patients with CVD. Even though CVD can affect people of all ages, the risk of CVD increases significantly with age [29]. Over the last decade, it has become clear that vascular wall integrity is maintained by circulating cells, named vascular stem/progenitor cells dedicated to endothelial repair and angiogenesis (reviewed in [30]). However, patients with severe obstructive vascular disease, usually caused by atherosclerotic plaque narrowing of arteries, are often aged and have tissue-resident and circulating vascular stem/progenitor cells with diminished functionality [31, 32]. These functional deficits may determine poor angiogenic activity in response to hypoxia or ischemia, associated with lower collateral vessel formation and impaired microcirculation [33]. Thus, the regenerative properties of cardiac stem cells might also deteriorate with age resulting in a decreased repair capacity upon injury. Furthermore, in aged tissues, myogenic or angiogenic stem cells may transform into fibroblasts, which contribute to enhanced fibrosis [34, 35]. These combined deficits associated with aging are likely to be the major cause of decreased muscle and weakened vessel regeneration after injury as well as facilitation of atherosclerosis and its sequelae in older individuals [26].

In recent years, evidence has accumulated that it might be possible to reverse stem cell aging at the organism level [36, 37]. Thus, replenishing the function of stem cells either by rejuvenating aged cells or by transplanting young stem/progenitor cells from young donors has been considered an appropriate therapy for age-related diseases. In this review, we describe critical genes and signaling pathways in stem cell aging that are of interest for the cardiovascular system, and discuss ex vivo genetic modification approaches aimed at stem cell rejuvenation.

**LIMITATIONS OF STEM CELLS IN THE TREATMENT OF AGE-RELATED CARDIOVASCULAR DISEASE**

Aging determines the reduction in the capacity for organs, including the heart, to undergo self-repair [31, 38, 39]. Aged organs become compromised after ischemic injury, partially due to the reduced functional capabilities of stem cells [31]. Major obstacles for stem cell therapy in aged people are the decreased functionality of autologous stem cells and difficulties in the engraftment and survival of transplanted stem cells in the pathological microenvironment of the host tissue. A plausible reason for this unfavorable microenvironment is the ischemic host tissue with acidic pH, scar formation, as well as a vascularisation [40]. Moreover, stem cells may be more susceptible to ischemia/reperfusion injury than more developed cells; e.g. cells of the embryonic stem cell-derived cardiac myocyte lineage have been shown to be more sensitive to ischemia/reperfusion-induced injury than neonatal cardiac myocytes [41, 42]. In addition, aging determines the reduction in number, function and paracrine activity of both resident and circulating stem/progenitor cells, as well as the reduction of stem cell resistance to senescence and apoptosis [43]. Furthermore, aging and aging-related cardiovascular risk factors such as diabetes [44] and hyperlipidemia negatively influence endogenous cardioprotective pathways [45, 46] and somatic reprogramming [47, 48]. Collectively, this results in the problem that transplantation of autologous stem cells into adult patients, as well as the transplantation of non-autologous stem cells from young donors into old recipients, is often affected by age-related disease. Moreover, aged or diseased people might not be good donors as aging and/or disease may lead to a poor quality of the stem cell preparation, and may impair the source for stem cells [48]. In this context, the reintegration of stem cell function and paracrine activity by stem cell rejuvenation or transplantation of functional competent...
stem/progenitor cells, or by injecting factors that are usually secreted by the cells, can represent possible strategies to treat age-related CVD.

CRITICAL GENES AND SIGNALING PATHWAYS IN STEM CELL AGING AND REJUVENATION

Parabiotic pairings between mice at different ages via shared circulatory system, which expose old mice to factors present in young serum, indicated in 2005 that aging of skeletal muscle stem cells can be reversed [36, 37, 49]. In recent years, it has been suggested that aging can also be reversed in cardiovascular stem cells. For example, Pim-1 kinase has been identified as an anti-senescence factor in cardiac stem cells. Pim-1 enhances proliferation [50], metabolic activity [51] and differentiation [52, 53] of CSCs and mesenchymal stem cells (MSCs) in neovessels and new cardiac myocytes. Pim-1 also serves as a pro-survival mediator by preserving mitochondrial integrity [54] and antagonizing intrinsic apoptotic cascades [55]. Moreover, Pim-1 preserves telomere length and telomerase activity of CSCs [56]. Finally, Mohsin and colleagues have shown that genetic modification of aged human CPCs with Pim-1 kinase results in remarkable rejuvenation of the CPCs associated with enhanced proliferation, increased telomere lengths, and decreased susceptibility to replicative senescence [51] (Fig. 1).

Several signaling pathways have been identified that revert the process of cardiac senescence. For example, the activation of Notch has been shown to restore the myogenic differentiation capacity of satellite cells, bringing it to a level similar to that of young cells from 20 year old humans [57]. Notch also plays important roles in cardiac differentiation and regeneration. In mice it has been shown that Notch is required for cardiac development and promotes the expansion of cardiac precursor cells [58-60]. Moreover, in zebrafish it has been demonstrated that Notch signaling is required for cardiac regeneration [61] (Fig. 1). Another pathway involved in stem cell aging is the telomere-telomerase axis. In 1998 it was suggested that the life-span of human cells can be extended by telomerase activation [62]. Since then great effort has been invested to translate this finding to cardiac cells [63, 64]. Telomerase has been demonstrated to maintain telomere length, to contribute to cell survival and proliferation, and to prevent cellular senescence [65, 66] (Fig. 1). A subpopulation of adipose tissue-derived mesenchymal stromal cell MSCs (AT-MSCs) was recently identified that expresses high levels of myocardin (MYOCD) and the catalytic subunit of telomerase (i.e., telomerase reverse transcriptase or TERT) with antisenesence properties [67, 68]. MYOCD is a key regulator of cardiovascular myogenic development [67, 69-71] and nuclear co-transcription factor for myogenic genes, as well as genes involved in muscle regeneration and protection against apoptosis [69, 72, 73]. AT-MSCs have been shown to contain a population of adult multipotent mesenchymal stem cells with high cardiovascular regenerative potential [67, 74-77]. AT-MSCs that co-express TERT and MYOCD are characterized by high endogenous levels of octamer-binding transcription factor 4 (Oct-4), MYOCD, myocyte-specific enhancer factor 2c (Mef2c), and homeobox protein Nkx2.5 [67, 68, 78], as well as decreased cell death both in the form of spontaneous cell death and Fas-induced apoptosis [48]. Therefore TERT and MYOCD may act together to protect AT-MSCs from apoptosis and to enhance their cardiovascular myogenic development [48, 68, 78].

EX VIVO GENE MODIFICATION APPROACH FOR STEM CELL REJUVENATION

Ex vivo cell-based gene delivery represents the most-used strategy to augment regeneration of old and diseased cardiovascular tissues. It consists of removing the cells of interest from the donor, infecting them with a viral vector encoding...
for the therapeutic transgene and then injecting them into the recipient organ [50, 79]. Although this approach requires more cell manipulation with some risk of cell contamination, accumulation of mutations during in vitro culture or insertional activation of other genes, it avoid the direct injection of viral vectors in vivo [80]. Therefore, transient modulation of cell specification and thereby enhancing myogenic differentiation via e.g. microRNAs could be beneficial approaches. For this, miR-1 and 499 are excellent candidates that enhance both differentiations in vitro [81] as in vivo and thereby enhance cardiac performance [82]. However, after the overexpression of the gene encoding for the rejuvenating factor followed by in vitro proliferation and expansion, genetically modified cells may secrete high amount of the regenerating factor, either transiently or permanently, in the site where they have been transplanted [83, 84]. Recently, the research group of Madonna examined the interplay in MSCs rejuvenation between TERT and MYOCD [48]. They determined the role of TERT and MYOCD in the rejuvenation of aged MSCs [48]. It was found that delivery of the TERT and MYOCD genes can rejuvenate MSCs from aged mice by increasing cell survival, proliferation, and smooth muscle myogenic differentiation in vitro [48]. Furthermore, the improved efficacy of these rejuvenated cells was demonstrated in an in vivo hindlimb ischemia model [48].

**PERSPECTIVE AND OPEN QUESTIONS**

Ex-vivo genetic modification of stem cells may offer an effective strategy for rejuvenating aged stem cells and diseased organs. Additionally, lack of cellular retention is an ongoing problem [85] that needs attention but might be tackled via cardiac tissue engineering and 3D bioprinting [86], as highlighted elsewhere [87, 88]. Further studies, particularly more bench-to-bedside translational work, are needed to clarify the impact of aging and aging-related cardiovascular risk factors on stem cell regeneration and help identifying the genetic as well as pharmacological tools that can rescue aged/sick stem cells as part of personalized medicine. In particular, future research in this field should aim at achieving the following goals:

1. add fundamental novel information on the pathophysiology of aged stem cells, isolated from aged, atherosclerosis-prone or cardiac infarct patients; (2) design new protocols for stem cell rejuvenation that allow improved preparation and clinical application of stem cells harvested from aged tissues and their products, and (3) design new protocols for in vivo application of rejuvenated stem cell therapies.

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflict of interest.

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**REFERENCES**


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Table 1. Preclinical studies of stem cell rejuvenation.

<table>
<thead>
<tr>
<th>Cell Signaling</th>
<th>Rejuvenation Method</th>
<th>In vivo/In vitro Model</th>
<th>Effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pim-1 kinase</td>
<td>Lentiviral Pim-1 overexpression</td>
<td>Human CSCs</td>
<td>Anti-sentences, anti-apoptosis, proliferation, differentiation</td>
<td>[51,53,54]</td>
</tr>
<tr>
<td>Notch</td>
<td>Notch hyperactivation</td>
<td>Cardiac amputation in double Tg (hsp70:Gal4); Tg(UAS:NICD) Zebrafish</td>
<td>Cardiac development, differentiation</td>
<td>[58-62]</td>
</tr>
<tr>
<td>TERT</td>
<td>Lentiviral-mediated TERT expression</td>
<td>Murine CSCs</td>
<td>Anti-senescence, proliferation</td>
<td>[63,64-67]</td>
</tr>
<tr>
<td>MYOCD</td>
<td>Lentiviral-mediated MYOCD overexpression</td>
<td>Murine CSCs</td>
<td>Anti-apoptosis</td>
<td>[68-78]</td>
</tr>
<tr>
<td>YAP</td>
<td>Adeno-associated-mediated YAP overexpression</td>
<td>AMI in adult murine myocardium</td>
<td>Cardiomyocyte proliferation</td>
<td>[97]</td>
</tr>
<tr>
<td>Hippo</td>
<td>Hippo down-regulation</td>
<td>AMI or amputation in Hippo-deficient adult mouse heart</td>
<td>Cardiomyocyte proliferation</td>
<td>[98]</td>
</tr>
<tr>
<td>VEGF and bFGF</td>
<td>Porous collagen scaffolds releasing cytokines</td>
<td>Human CSCs</td>
<td>Proliferation</td>
<td>[99]</td>
</tr>
</tbody>
</table>

Legend: VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; TERT, telomerase reverse transcriptase; MYOCD, myocardin; YAP, Yes-associated protein; Hippo, protein kinase Hpo; CSCs, cardiac stem cells; MSCs, mesenchymal stem cells; AMI, acute myocardial infarction; Pim-1, pro-viral integration site 1.
Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease); endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. Circulation 2006; 113(11): e635-654.


