Stem cell aging and age-related cardiovascular disease: Perspectives of treatment by ex-vivo stem cell rejuvenation

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Abstract

The number and function of stem cells decline with aging, reducing the ability of stem cells to contribute to endogenous repair processes. Particularly, *stemness* property of stem cells is susceptible to age-related changes, including increased rates of apoptosis and senescence, and decreased efficiency of paracrine activity. Also, aging detrimentally affects the effectiveness of stem cell transplantation and the injection of the collected types of molecules released by them, leading to reduction in stem cell ability to contribute to endogenous repair processes. The repair capacity of stem cells in aged individuals may be improved by genetically reprogramming the stem cells to exhibit delayed senescence and enhanced regenerative properties. 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.

Key words: gene therapy; myocardin; Notch; Pim-1; stem cell aging; stem cell rejuvenation; telomerase

INTRODUCTION

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 functions [1, 2]. These functional deficits may cause a poor angiogenic response to hypoxia or ischemia, with impaired collateral vessel formation and microcirculation [3]. Likewise with age, which is a major risk factor for cardiovascular disease, regenerative properties deteriorate and consequently resident stem/progenitor cells in elderly humans may have a decreased capacity for repair in response to tissue injury. Also in aged tissues, myogenic or angiogenic stem cells may transform into fibroblasts which contribute to enhanced fibrosis [4, 5]. These combined age-related deficits likely contribute to decreased muscle, weakened vessel regeneration after injury and facilitation of atherosclerosis and its sequelae in older individuals [6]. Replenishing stem cell function either by rejuvenating existing aging cells or transplanting stem/progenitor cells from donors capable of supplying the ischemic tissue with new vessels and preventing ischemic tissue damage, have been considered an appropriate therapy for this condition. 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.

POTENTIAL OF STEM CELLS IN THE TREATMENT OF AGE-RELATED CARDIOVASCULAR DISEASES

Diseases of aging, such as metabolic syndrome, diabetes, atherosclerosis, neurodegenerative diseases, osteoporosis and cancer, constitute a huge burden for all societies, both in terms of economics and quality of life. Despite contemporary medical treatments, heart failure remains a major cause of morbidity and mortality in old people of developed countries [7]. 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 initiating fibrosis and, thereby, increasing the thickness of microvasculature walls [8]. Some consequences of oxidative stress-associated 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 remains the only effective remedy for heart failure. Meanwhile critical limb ischemia represents a major cause of diabetes-associated morbidity [9], still representing the most common cause of amputation in diabetic patients. Recent seminal reports have indicated that the adult heart is self-healing and self-renewing. Specifically, these studies demonstrated that there is a pool of resident cardiac stem cells (CSCs) that are clonogenic and multipotent and are capable of differentiating into new blood vessels or into new myocytes [10]. This suggests the opportunity to boost the endogenous regenerative approach to complement other treatments (e.g., stem cell transplantation) that facilitate myocardial repair. However, despite recent progress in applying the approaches of regenerative medicine to the treatment of these diseases, valid strategies aimed at repairing the infarcted heart and, in general, at treating end-organ ischemia continue to be elusive [11].

In recent years, efforts to use the alternative strategies of regenerative medicine as for treating cardiovascular disease have been partially successful. The limitations, still not completely understood, include: 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) anomalous electro-mechanical behavior of

transplanted cells after stimulation and the eventual onset of arrhythmias; 5) formation of new heart tissue structure differing from that of normal heart; and 6) diminished function of both resident and circulating stem/progenitor cells with the onset of aging and age-related cardiovascular disease.

BOUNDARIES OF STEM CELLS IN THE TREATMENT OF AGE-RELATED CARDIOVASCULAR DISEASE

The difficulty in providing functionally competent autologous stem cells isolated from aged patients with disease, that are specifically effective in myocardial repair, and in the engraftment and survival of transplanted stem cells in the harmful microenvironment of host tissue, represent major obstacle for stem cell therapy in aged people. The capacity of organs, including the heart, to self-repair decreases with age and becomes compromised after ischemic injury, partially resulting from the reduced functional capabilities of stem cells [1]. Aging is a major risk factor for cardiovascular disease. With the onset of age-related cardiovascular disease, which often occurs secondarily to atherosclerosis, the function of both resident and circulating stem/progenitor cells and their paracrine activities is diminished [12]. Furthermore, aging and risk factors might largely affect endogenous cardioprotective pathways [13]. Stem cells are susceptible to age-related changes, including increased rates of apoptosis and senescence, that reduce their ability to contribute to endogenous repair processes. Furthermore, with aging the effectiveness of stem cells after transplantation diminishes [14]. Therefore, concerns remain with regard to the potentially lower potency of stem cells from patients, in whom aging and/or disease may lead to a poor quality of the stem cell preparation, as aging or the disease may impair the source for stem cell or create a hostile microenvironment for implanted stem cells [14]. The combination of these disease and age-related deficits may contribute to decreased muscle and vessel regeneration after injury and facilitate the development of

atherosclerosis and its sequelae in aged individuals. In this context, an appropriate therapy for age-related vascular disease may be to replenish stem cell function and the collected types of molecules released by the stem cells, by rejuvenating existing cells or transplanting functional competent stem/progenitor cells or injecting their cellular products that will supply the ischemic tissue with new vessels to prevent ischemic tissue damage.

CRITICAL GENES AND SIGNALING PATHWAYS IN STEM CELL AGING AND REJUVENATION

Although relatively little has been accomplished for “turning back the clock” in the myocardial context, there are signaling pathways that seem connected to reversing the cardiac senescence. For example, experimental activation of Notch restored “youthful” myogenic responses to satellite muscle cells isolated from 70-year-old humans, rendering them similar to cells from 20-year-old humans [15]. Pim-1 kinase is another example of antisenescence pathway that works in the context of cardiac stem cells. Pim-1 enhances proliferation [16], metabolic activity [17] and differentiation [18, 19] of CSCs and mesenchymal stem cells (MSCs) in neovessels and new myocytes. Pim-1 also serves as a prosurvival role by preserving mitochondrial integrity [20] and antagonizing intrinsic apoptotic cascades [21]. Moreover, Pim-1 preserves telomere length and telomerase activity of CSCs [17]. Mohsin and collegues have recently showed that genetic modification of aged human cardiac progenitor cells (CPCs) with Pim-1 kinase results in remarkable rejuvenation of them, with enhanced proliferation, increased telomere lengths, and decreased susceptibility to replicative senescence [22]. Manipulation of the telomere-telomerase axis was suggested in 1998, when 2 different human cell lines, retinal pigment epithelial cells and foreskin fibroblasts, were transfected with vectors encoding for human telomerase catalytic subunit. Since then much research has been done on the heart,

telomeres and telomerase. We recently identified a subpopulation of adipose tissue–derived mesenchymal stromal cells MSCs (AT-MSCs) that expresses high levels of the catalytic subunit of telomerase (ie, telomerase reverse transcriptase or TERT) and myocardin (MYOCD) [23, 24]. AT-MSCs have been shown to contain a population of adult multipotent mesenchymal stem cells with high cardiovascular regenerative potential [23, 25-28]. MYOCD is a key regulator of cardiovascular myogenic development [23, 29, 30] and acts as a nuclear transcription cofactor for myogenic genes, as well as genes involved in muscle regeneration and protection against apoptosis [31, 32]. Telomerase maintains telomere length, contributes to cell survival and proliferation, and prevents cellular senescence [33, 34]. We have shown that AT-MSCs that co-express TERT and MYOCD have increased endogenous levels of octamer-binding transcription factor 4 (Oct-4), MYOCD, myocyte-specific enhancer factor 2c (Mef2c), and homeobox protein NKx2.5. These observations suggest that TERT and MYOCD may act together to enhance cardiovascular myogenic development [24, 35].

EX VIVO GENE MODIFICATION APPROACH FOR STEM CELL REJUVENATION

Although traditional *in vivo* gene delivery approach via direct injection of viral vectors by attaching the delivery vector to the scaffold [36], is still a candidate strategy in laboratory-based trials, the most frequently investigated cell engineering method to augment regeneration of old and diseased cardiovascular tissues, to date, is ex vivo cell-based gene therapy. This therapy typically relies on transplanting cells, such as stem cells, lymphocytes, fibroblasts, or – alternatively – the cells of interest, that are removed from the body and injected after therapeutic transgene modifications [16, 37]. This *ex vivo* approach allows for targeting of specific cells for gene delivery, supplies rejuvenated cells that may directly participate in the regenerative process, and avoids the safety risks of directly

injecting viral vectors or transfection reagents *in vivo*. This approach, however, involves an extra step to manipulate and expand cells in tissue culture, and has the risk of contamination. Additionally, the *ex vivo* approach does not eliminate the possibility of retroviral vectors causing insertional activation of other genes, the over-expression of which may cause cancer, as experienced when using *ex vivo* gene therapy for the treatment of children with X-linked severe combined immune deficiency [38]. Progress in the field of gene therapy has been limited by safety concerns related to delivery vectors. Genetically modified cells are potentially able to provide a stable source of rejuvenating factors at a level that is sufficient to elicit a biological response. Autologous cells may also be used in this approach via the isolation of a small number of differentiated adult cells or stem cells, followed by *in vitro* expansion to produce an appropriate supply. The cells may naturally secrete or be genetically modified *in vitro* to overexpress the rejuvenating factor, either transiently or permanently. After their genetic modification, the cells are allowed to grow *in vitro* and increase in number, so as to synthesize and secrete the desired rejuvenating factors at the site where they have been transplanted. Recently, our research group examined the interplay in mesenchymal stem cells (MSCs) between two genes, one coding for the catalytic subunit of telomerase (i.e., telomerase reverse transcriptase or TERT), that has antisenescence properties, the other coding for myocardin (MYOCD), a nuclear transcription cofactor for myogenic genes, as well as for genes involved in muscle regeneration and protection against apoptosis [14, 23, 24, 35] . We have examined the role of TERT and MYOCD in the conversion of aged MSCs to rejuvenated anti-apoptotic, promyogenic stem cells [14]. We have shown that the delivery of the *TERT* and *MYOCD* genes can restore MSCs from aged mice by increasing cell survival, proliferation, and smooth muscle myogenic differentiation in vitro [14]. Furthermore, we have demonstrated the therapeutic efficacy of these rejuvenated cells in an in vivo hindlimb ischemia model [14].

LENTIVIRAL AND NON-LENTIVIRAL VECTORS FOR GENE DELIVERY INTO STEM CELLS

The introduction or the overexpression of rejuvenating genes in stem cells can be performed by using viral or non-viral vectors. In the choice of using viral vectors, important experimental variables for a successful gene therapy include Multiplicity Of Infection (MOI) time length for viral incubation and medium used for viral incubation. An optimal combination of such experimental conditions would increase gene transfer efficiency and possibly obviate the need for selective antibiotic-based enrichment and long-term culture, which may contribute to senescence or compromise the long-term engraftment efficiency and/or multipotency of grafted cells [39]. In addition, by increasing gene transfer efficiency, fewer cells may be required to achieve a therapeutic effect. This justifies the use of lentiviral vectors for transducing adult stem cells, by virtue of their ability to transduce both dividing and non-dividing cells and their relative ease of use and comparable nature to adeno-associated viral (AAV) vectors, which are clinically preferred. For the transduction of adult stem cells, lentivirus-based systems are virtually ideal, since they overcome most problems, including the short duration of gene expression and the occurrence of significant inflammatory responses, which plague other types of gene vectors (such as adenoviruses). Lentiviruses are a subgroup of retroviruses that include the human type 1 immunodeficiency virus (HIV). While retroviral systems are inefficient in transducing non-dividing or slowly dividing cells, lentivirus-based vectors, after being pseudotyped with vesicular stomatitis virus glycoprotein G (VSV-G) (i.e., using the glycoprotein envelope from the vesicular stomatitis virus to package recombinant retroviruses) [40], can mediate genome integration into both non-dividing and dividing cells. There is evidence that lentiviral vectors can also transduce more primitive, quiescent progenitors with stable transgene integration [41]. In comparison with other retroviral vectors, lentiviral systems

allow the immediate transduction without prior expansion, or with growth factor stimulation for only short exposure times. Compared with adenoviral vectors, lentiviral vectors also offer the major advantages of causing little or no disruption of the target cells and of not promoting any inflammatory response [42]. AAV vectors represent an alternate type of vector that may also be used for long-term transgene expression in the heart through cell-based therapy [43]. Like lentiviruses, AAV can stably integrate into the host genome providing long-term transgene expression, with a minimum inflammatory response. However, AAV can cause insertional mutagenesis and can only carry genes which are less than 5 kb [44]. A possible drawback of the use of lentiviral and AAV vectors for delivering genes that encode for growth factors might be that they can cause a chronic overexpression of the protein, with an uncertain therapeutic effect. Short-term gene expression of the rejuvenating factor gene would be desirable if the goal is to deliver an anti-senescent protein, such as TERT, without neoplastic modification or immortalization of the stem cell target. On the contrary, long-term expression would be preferable if the goal is to express membrane proteins such as receptors for growth factors that require stable expression, or promyogenic transcription factors. Possible strategies to induce short-term gene expression of the transgene include plasmid transfection or the use of adenoviral vectors [45]. Limitations of these strategies are the low transfection efficiency with plasmids and the immunogenic response of the host with adenoviruses.

PERSPECTIVE AND OPEN QUESTIONS

Ex-vivo genetic modification of stem cells may offer an effective strategy for rejuvenating aged stem cells and diseased organs. Further studies, particularly more bench-to-bedside translational work, are needed to clarify the impact of aging and cardiovascular disease on stem cell generation 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 pathobiology of aged stem cells, isolated from aged, atherosclerosis-prone or cardiac infarct patients; (2) design new protocols for aged stem cell rejuvenation capable to lead to improved preparation and clinical application of stem cells harvested from aged tissues and their products, and (3) design new protocols for in vivo transplantation of rejuvenated stem cell therapies.

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