Commentary

Aging of mesenchymal stem cells: Implication in regenerative medicine

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Abstract

Multipotent mesenchymal stem cells (MSCs) represent a great candidate for various clinical applications including regenerative medicine. However, aging both in vivo and in vitro can significantly compromise MSC characteristics and performance. This paper highlights current thoughts on senescence-induced damage to MSCs that should be considered prior to their use for regeneration of different cells, tissues or organs.

1. Multipotent mesenchymal stem cells

MSCs which can be derived from different origins such as adipose tissues, bone marrow, skeletal muscle, spleen, synovial membrane, and umbilical cord blood present great potential for regenerative medicine due to multipotency and self-renewal capacity [1,2]. MSCs are usually characterized using a colony-forming unit-fibroblasts approach and are capable of expressing a variety of surface antigens. Specifically, human MSCs are commonly positive for Stro-1, CD13, CD29, CD44, CD73, CD105 and CD106, but are negative for CD11b, CD31, CD34 and CD45 [3]. However, not a single MSC expresses all the identified surface antigens and expression of certain markers such as CD10, CD90 and Flk-1 can be variable depending on the origin of the cells as well as species [4,5]. Ever since their initial identification and characterization by Friedenstein et al., in 1976 [6], many studies have demonstrated that, under proper stimulation, uncommitted MSCs can differentiate into a variety of cell types of mesodermal origin, including but not limited to adipocytes, cardiomyocytes, chondrocytes, myocytes, osteoblasts, and tendon cells [3,7]. Several clinical benefits of MSCs have also been reported. Treatment with allogeneic bone marrow transplantation from human leukocyte antigen-compatible donors has led to functional engraftment of MSCs that promote bone regeneration and alleviate bone fracture in children with osteogenesis imperfecta [8]. In cancer research, Anklesaria et al. discovered that transplantation of clonal bone marrow MSCs into mice exposed to total body or hind limb irradiation allowed hematopoietic recovery and further corrected the defective marrow stroma [9]. A follow-up study conducted by Koç et al. showed that co-infusion of autologous bone marrow-derived MSCs with peripheral blood progenitor cells effectively restored hematopoietic function in advanced breast cancer patients who received high-dose chemotherapy, suggesting that MSC transplantation after myeloablative therapy would have a positive impact on hematopoiesis [10]. In addition, MSCs are a rich source of cytokines and growth factors and can release specific trophic signals in response to external stimuli from the adjacent cells and tissues [11]. It is thought thatMSCs represent a promising cell type for delivery of proteins of interest in the treatment of various diseases. For example, conditioned media derived from unmodified [12,13] or Akt-modified [14] MSCs have been shown to contain paracrine factors that contribute to myocardial protection from ischemic injury. Similar approaches were also applied to lung [15] and bone [16,17] regeneration as well as wound healing [18].
Immunologically, Yang et al. reported that MSCs could effectively downregulate the secretion of interferon-γ and tumor necrosis factor-α by T cells [19]. Furthermore, infusion of retroviral vector-modified MSCs delivered biologically active coagulation factor IX to the circulation at therapeutic levels in a canine model and thus could potentially be a feasible treatment of hemophilia B [20].

2. Donor age-related effects on MSC fitness

Despite positive outcome derived from MSC clinical research, its properties and functionalities can be influenced by several intrinsic factors including aging [21] and thereby should be reviewed on a case-by-case basis. MSCs extracted from young and elderly individuals have been shown to have diverse properties. Upon isolation, MSC numbers obtained by bone marrow aspiration decline with donor age [22]. A reduction in colony forming efficiency, proliferative capacity and osteogenic potential has also been observed in aged MSCs in comparison with juvenile cells [23]. Moreover, Choudhery et al. demonstrated that when harvested from adipose tissues, aged MSCs exhibited senescent features concomitant with more senescence-associated β-galactosidase-positive cells, ultimately leading to cell apoptosis and thus decreased cell viability [24]. The Choudhery’s study also revealed that while chondrogenic and osteogenic differentiation potentials of MSCs extracted from older individuals were compromised, adipogenesis and neurogenesis seemed to be independent of donor age. Mechanistically, elevated expression of p53 gene and its pathway genes, p21 and BAX, has been detected in aged MSCs [23]. These markers have been shown to accelerate senescence and degradation of different cells and tissues such as fibroblasts [25], endothelial cells [26], myocardium [27], and bone [28]. Furthermore, reactive oxygen species and nitric oxide are thought to be involved in the aging process, whose levels have been determined to be significantly higher in aged MSCs [22] and the induced oxidative stress can potentially impair normal biological functions of organisms [29,30]. Reduced superoxide dismutase activity, an antioxidant enzyme that catalyzes the conversion of superoxide radical anions to hydrogen peroxide and later to oxygen and water, has also been found in aged MSCs, suggesting an inferior antioxidant defense mechanism [24]. The combination of all the aforementioned factors may contribute to the age-related loss of overall MSC performance and fitness.

3. Aging of MSCs in vitro

MSCs are a rare cell population in the body whose number is usually no more than 0.01% of the total cell count in its origins [31]. Because of the low frequency, use of MSCs in various clinical applications including regenerative medicine relies on their strong proliferative ability in vitro to produce later generations with comparable regenerative capabilities. A process called “passage” is usually implemented to expand MSC populations in vitro. In many cases, however, senescence accompanies the passage process and potentially deteriorates MSC fitness to the point where the residual stem cell features are compromised and insufficient to support long-term tissue or organ regeneration [32]. Number of cell population doubling (NCPD) has been used as an indicator to track the age of a cell population in vitro and is calculated based on the equation $N_{t+1} = N_t \times 2^{C_{t}}$ where $N_t$ and $N_{t+1}$ are the cell counts at the terminal and initial time points, respectively. Several groups have suggested that the maximal NCPD of human MSCs is approximately around 15–30 depending on donor age such that the cells completely lose the ability to replicate in vitro [32–36]. The results also indicated that during in-vitro aging, MSC proliferative rate gradually decreased with increasing passage number and MSC morphology shifted from the traditional fibroblast-like spindle shape to a more heterogeneous and enlarged geometry over time. In addition, a large portion of passaged MSCs could lose tri-differentiation potential (adipogenic, chondrogenic and osteogenic) when the cultures reached the upper limit of NCPD [34–36]. A recent study further demonstrated that MSCs subjected to extensive in-vitro passage could undergo morphological, phenotypic and genetic changes even before the cells ceased growing [37]. Specifically, altered expression of specific MSC surface markers and genetic instability were detected in MSCs at late passages and these observed variations were regulated by medium formula. The outcome also suggests that while in-vitro senescence negatively impacts both adipogenesis and osteogenesis of MSCs, the reduction in adipogenic potential is less significant regardless of expansion condition.

4. Concluding remarks

Stem cell aging is a complex process, yet the underlying mechanisms remain elusive. Early preservation of autologous MSCs is recommended to avoid age-related damage due to the fact that MSCs can be cryogenically stored for decades without devastating their proliferative and differentiation capabilities [38]. Several attempts to prevent MSCs from in-vitro senescence have been made. For example, since it has been shown that aging contributes to shorter telomere length in passaged cells [32,35,37], a genetic engineering method has been developed by introducing a retroviral vector that contains the gene for the catalytic subunit of telomerase to bone marrow-derived [39,40] or umbilical cord blood-derived [41] MSCs such that the cells can maintain normal proliferative lifespan and differentiation capacity throughout the prolonged expansion process in vitro. Mori and colleagues further demonstrated that transduction with additional genes such as bmi-1, E6 or E7 could extend the lifespan of MSCs extracted from a 91-year-old donor while maintaining their neurogenic potential [42]. Additionally, a defined xenofree medium supplement has been prepared from human plasma and its use in lieu of commonly used animal serum has led to preservation of MSC phenotype, multipotency and genetic stability during in-vitro passage [43]. The

Table 1

| Observations | Potential solutions |
|--------------|---------------------|
| **In-vitro senescence** | Early cryopreservation [38] |
| Cell number [22] | | |
| Cell viability or ↑ apoptosis [24] | | |
| Colony forming efficiency [22,23] | | |
| Propagation [22–24] | | |
| Adipogenesis [24] | | |
| Chondrogenesis [24] | | |
| Osteogenesis [23,24] | | |
| Neurogenesis [24] | | |
| Reactive oxygen species, nitric oxide, oxidative stress [22,30] | | |
| Superoxide dismutase activity [24] | | |
| p21, p53, BAX genes [23] | | |
| **In-vitro senescence** | Genetic modifications [39–42] |
| Non-spindle morphology [32–37] | | |
| Proliferative capacity [32–37] | | |
| Adipogenesis [34–37] | Xeno-free expansion media [43] |
| Chondrogenesis [34–36] | Senolytic drugs: navitoclax* [44] |
| Osteogenesis [34–37] | | |
| Genetic instability [37] | | |
| Telomere length [32,35,37] | | |

* Limited effects.
immediate next step is to examine the efficiency and effectiveness of these approaches to generation of large MSC populations for clinical purposes. More recently, four senolytic drugs (navitoclax, quercetin, nicotinamide riboside, and danazol) were tested on bone marrow-derived MSCs undergoing in-vitro expansion, yet only navitoclax had slightly anti-aging effects and none of them rejuvenated passaged MSCs [44]. Taken altogether, it is crucial to consider the influence of not only in-vivo but also in-vitro aging on MSC characteristics and performance (Table 1) when developing relevant stem cell therapeutic strategies for regenerative medicine.

Conflicts of interest

The author declares no financial conflicts.

Acknowledgments

This work was partially supported by the Professional Staff Congress-City University of New York Research Award (Grant number 61584-00 49).

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