Regenerative Therapy 9 (2018) 120–122

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Commentary

Aging of mesenchymal stem cells: Implication in regenerative medicine

Yueh-Hsun Kevin Yang*

[a] Grove School of Engineering, The City University of New York – the City College, New York, NY 10031, USA

ARTICLE INFO

Article history:
Received 5 August 2018
Received in revised form
30 August 2018
Accepted 13 September 2018

Keywords:
Mesenchymal stem cell
Aging
Senescence
Regenerative medicine
Proliferation
Differentiation

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 that MSCs 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].

Abbreviations: MSC, Mesenchymal stem cell; NCPD, Number of cell population doubling.

* Grove School of Engineering, The City University of New York – the City College, 160 Convent Avenue, Steinman Hall 581, New York, NY 10031, USA. Fax: +1 212 6505768.

E-mail address: kyang@ccny.cuny.edu.

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2018.09.002

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 elder 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 $NCPD = 3.33 \times \log(N_t/N_i)$ where N_t and N_i 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 xeno-free 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
Key highlights of *in-vivo* and *in-vitro* senescence of mesenchymal stem cells.

	Observations	Potential solutions
In-vitro senescence	↓ 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]	Early cryopreservation [38]
In-vivo senescence	↑ Non-spindle morphology [32–37] ↓ Proliferative capacity [32–37] ↓ Adipogenesis [34–37] ↓ Chondrogenesis [34–36] ↓ Osteogenesis [34–37] ↑ Genetic instability [37] ↓ Telomere length [32,35,37]	Genetic modifications [39–42] Xeno-free expansion media [43] Senolytic drugs: navitoclax[a] [44]

[a] 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).

References

[1] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.
[2] Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 1997;276:71–4.
[3] Kolf CM, Cho E, Tuan RS. Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: regulation of niche, self-renewal and differentiation. Arthritis Res Ther 2007;9:204–13.
[4] Colter DC, Sekiya I, Prockop DJ. Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. Proc Natl Acad Sci U S A 2001;98:7841–5.
[5] Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ. Adult stem cells from bone marrow isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood 2004;103:1662–8.
[6] Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 1976;4:267–74.
[7] Yang Y-H, Lee AJ, Barabino GA. Coculture-driven mesenchymal stem cell-differentiated articular chondrocyte-like cells support neocartilage development. Stem Cells Transl Med 2012;1:843–54.
[8] Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WWK, Gordon PL, Neel M, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 1999;5:309–13.
[9] Anklesaria P, Kase K, Glowacki J, Holland CA, Sakakeeny MA, Wright JA, et al. Engraftment of a clonal bone marrow stromal cell line in vivo stimulates hematopoietic recovery from total body irradiation. Proc Natl Acad Sci U S A 1987;84:7681–5.
[10] Koç ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol 2000;18:307–16.
[11] Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem 2006;98:1076–84.
[12] Timmers L, Lim SK, Arslan F, Armstrong JS, Hoefer IE, Doevendans PA, et al. Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium. Stem Cell Res 2008;1:129–37.
[13] Angoulvant D, Ivanes F, Ferrera R, Matthews PG, Nataf S, Ovize M. Mesenchymal stem cell conditioned media attenuates in vitro and ex vivo myocardial reperfusion injury. J Heart Lung Transpl 2011;30:95–102.
[14] Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005;11:367–8.
[15] Goolaerts A, Pellan-Randrianarison N, Larghero J, Vanneaux V, Uzunhan Y, Gille T, et al. Conditioned media from mesenchymal stromal cells restore sodium transport and preserve epithelial permeability in an in vitro model of acute alveolar injury. Am J Physiol Lung Cell Mol Physiol 2014;306:L975–85.
[16] Osugi M, Katagiri W, Yoshimi R, Inukai T, Hibi H, Ueda M. Conditioned media from mesenchymal stem cells enhanced bone regeneration in rat calvarial bone defects. Tissue Eng A 2012;18:1479–89.
[17] Ogata K, Katagiri W, Osugi M, Kawai T, Sugimura Y, Hibi H, et al. Evaluation of the therapeutic effects of conditioned media from mesenchymal stem cells in a rat bisphosphonate-related osteonecrosis of the jaw-like model. Bone 2015;74:95–105.
[18] Walter MNM, Wright KT, Fuller HR, MacNeil S, Johnson WEB. Mesenchymal stem cell-conditioned medium accelerates skin wound healing: an in vitro study of fibroblast and keratinocyte scratch assays. Exp Cell Res 2010;316:1271–81.
[19] Yang ZX, Han Z-B, Ji YR, Wang YW, Liang L, Chi Y, et al. CD106 identifies a subpopulation of mesenchymal stem cells with unique immunomodulatory properties. PLoS One 2013;8:e59354.
[20] Cherington V, Chiang GG, McGrath CA, Gaffney A, Galanopoulos T, Merrill W, et al. Retroviral vector-modified bone marrow stromal cells secrete biologically active factor IX in vitro and transiently deliver therapeutic levels of human factor IX to the plasma of dogs after reinfusion. Hum Gene Ther 1998;9:1397–407.
[21] Chen FH, Rousche KT, Tuan RS. Technology insight: adult stem cells in cartilage regeneration and tissue engineering. Nat Clin Pract Rheum 2006;2:373–82.
[22] Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. Mech Ageing Dev 2008;129:163–73.
[23] Zhou S, Greenberger JS, Epperly MW, Goff JP, Adler C, LeBoff MS, et al. Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell 2008;7:335–43.
[24] Choudhery MS, Badowski M, Muise A, Pierce J, Harris DT. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. J Transl Med 2014;12:8.
[25] Atadja P, Wong H, Garkavtsev I, Veillette C, Riabowol K. Increased activity of p53 in senescing fibroblasts. Proc Natl Acad Sci U S A 1995;92:8348–52.
[26] Rosso A, Balsamo A, Gambino R, Dentelli P, Falcioni R, Cassader M, et al. p53 mediates the accelerated onset of senescence of endothelial progenitor cells in diabetes. J Biol Chem 2006;281:4339–47.
[27] Song H, Conte Jr JV, Foster AH, McLaughlin JS, Wei C. Increased p53 protein expression in human failing myocardium. J Heart Lung Transpl 1999;18:744–9.
[28] Wang X, Kua H-Y, Hu Y, Guo K, Zeng Q, Wu Q, et al. p53 functions as a negative regulator of osteoblastogenesis, osteoblast-dependent osteoclastogenesis, and bone remodeling. J Cell Biol 2006;172:115–25.
[29] Renault V, Thornell L-E, Butler-Browne G, Mouly V. Human skeletal muscle satellite cells: aging, oxidative stress and the mitotic clock. Exp Gerontol 2002;37:1229–36.
[30] Stolzing DA, Sethe S, Scutt AM. Stressed stem cells: temperature response in aged mesenchymal stem cells. Stem Cells Dev 2006;15:478–87.
[31] Wexler SA, Donaldson C, Denning-Kendall P, Rice C, Bradley B, Hows JM. Adult bone marrow is a rich source of human mesenchymal 'stem' cells but umbilical cord and mobilized adult blood are not. Br J Haematol 2003;121:368–74.
[32] Baxter MA, Wynn RF, Jowitt SN, Wraith JE, Fairbairn LJ, Bellantuono I. Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion. Stem Cells 2004;22:675–82.
[33] Colter DC, Class R, DiGirolamo CM, Prockop DJ. Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. Proc Natl Acad Sci U S A 2000;97:3213–8.
[34] DiGirolamo CM, Stokes D, Colter D, Phinney DG, Class R, Prockop DJ. Propagation and senescence of human marrow stromal cells in culture: a simple colony-forming assay identifies samples with the greatest potential to propagate and differentiate. Br J Haematol 1999;107:275–81.
[35] Bonab MM, Alimoghaddam K, Talebian F, Ghaffari SH, Ghavamzadeh A, Nikbin B. Aging of mesenchymal stem cell in vitro. BMC Cell Biol 2006;7:14.
[36] Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci 2000;113:1161–6.
[37] Yang Y-HK, Ogando CR, Wang See C, Chang T-Y, Barabino GA. Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro. Stem Cell Res Ther 2018;9:131.
[38] Hayflick L. Antecedents of cell aging research. Exp Gerontol 1989;24:355–65.
[39] Shi S, Gronthos S, Chen S, Reddi A, Counter CM, Robey PG, et al. Bone formation by human postnatal bone marrow stromal stem cells is enhanced by telomerase expression. Nat Biotechnol 2002;20:587–91.
[40] Simonsen JL, Rosada C, Serakinci N, Justesen J, Stenderup K, Rattan SIS, et al. Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat Biotechnol 2002;20:592–6.
[41] Terai M, Uyama T, Sugiki T, Li X-K, Umezawa A, Kiyono T. Immortalization of human fetal cells: the life span of umbilical cord blood-derived cells can be prolonged without manipulating $p16^{INK4a}$/RB braking pathway. Mol Biol Cell 2005;16:1491–9.
[42] Mori T, Kiyono T, Imabayashi H, Takeda Y, Tsuchiya K, Miyoshi S, et al. Combination of hTERT and bmi-1, E6, or E7 induces prolongation of the life span of bone marrow stromal cells from an elderly donor without affecting their neurogenic potential. Mol Cell Biol 2005;25:5183–95.
[43] Blázquez-Prunera A, Díez JM, Gajardo R, Grancha S. Human mesenchymal stem cells maintain their phenotype, multipotentiality, and genetic stability when cultured using a defined xeno-free human plasma fraction. Stem Cell Res Ther 2017;8:103.
[44] Grezella C, Fernandez-Rebollo E, Franzen J, Ventura Ferreira MS, Beier F, Wagner W. Effects of senolytic drugs on human mesenchymal stromal cells. Stem Cell Res Ther 2018;9:108.