Mechanisms of Ageing and Development 133 (2012) 215–225

Contents lists available at SciVerse ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Review

Age-related alterations in mesenchymal stem cells related to shift in differentiation from osteogenic to adipogenic potential: Implication to age-associated bone diseases and defects

MiJung Kim [a,b,c,d,e,f,*], ChanWha Kim [e], Yu Suk Choi [d], MinHwan Kim [d], ChanJeoung Park [c], Yousin Suh [a,b,f]

[a] Department of Genetics, Albert Einstein College of Medicine, 1301 Morris Park Avenue, Bronx, NY 10461, USA
[b] Department of Medicine, Albert Einstein College of Medicine, 1301 Morris Park Avenue, Bronx, NY 10461, USA
[c] Department of Laboratory Medicine, University of Ulsan College of Medicine, Seoul, Republic of Korea
[d] Asan Institute for Life Sciences, Seoul, Republic of Korea
[e] School of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea
[f] Institute for Aging Research, Diabetes Research and Training Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA

ARTICLE INFO

Article history:
Received 14 August 2011
Received in revised form 17 March 2012
Accepted 28 March 2012
Available online 26 April 2012

Keywords:
Mesenchymal stem cells (MSC)
Aging
Adipogenesis
Osteogenesis
Cell therapy

ABSTRACT

Mesenchymal stem cells (MSC) have attracted considerable attention in the fields of cell and gene therapy due to their intrinsic ability to differentiate into multiple lineages. The various therapeutic applications involving MSC require initial expansion and/or differentiation *in vitro* prior to clinical use. However, serial passages of MSC in culture lead to decreased differentiation potential and stem cell characteristics, eventually inducing cellular aging which will limit the success of cell-based therapeutic interventions. Here we review the age-related changes that occur in MSC with a special focus on the shift of differentiation potential from osteogenic to adipogenic lineage during the MSC aging processes and how aging causes this preferential shift by oxidative stress and/or energy metabolism defect. Oxidative stress-related signals and some microRNAs affect the differentiation potential shift of MSC by directly targeting key regulatory factors such as Runx-2 or PPAR-γ, and energy metabolism pathway is involved as well. All information described here including transcription factors, microRNAs and FoxOs could be used towards development of treatment regimens for age-related bone diseases and related defects based on mutually exclusive lineage fate determination of MSC.

1. Introduction to mesenchymal stem cell aging

The core of adult stem cell research over the last 25 years has been based on the finding that mesenchymal stem cells (MSC) have the capacity to develop into fully differentiated mesenchymal lineages, including bone, cartilage, tendon, muscle, and adipose tissue (Awad et al., 1999; Ferrari et al., 1998; Pittenger et al., 1999). MSC have been isolated from many tissues, including bone marrow, umbilical cord blood, adipose tissue, synovial fluid, periosteum, and fetal tissue. MSC were originally described as colony forming unit fibroblasts (CFU-F), as they can be isolated from bone marrow as plastic-adherent fibroblastic cells (Friedenstein et al., 1974). MSC represent a promising cell source for cellular therapy and tissue engineering, mainly due to their intrinsic ability to proliferate and differentiate into various cell types (Gerson, 1999).

MSC are already known to be efficacious in various cellular therapeutic applications (Kumar et al., 2008). However, although MSC are effective tools for cell therapy, stem cell therapy cannot yet be used reliably because these cells show differential behavior across patients and/or MSC sources (*i.e.* by donor age or disease). Many researchers have studied the effects of donor age on MSC properties as well as proliferation and differentiation capacities *in vitro* (Justesen et al., 2002; Stenderup et al., 2003; Tokalov et al., 2007). Their findings indicate that *in vitro* aging (passage number in culture) is more important than *in vivo* aging (donor age) when considering the proliferation and differentiation potential of MSC.

Age-related changes in MSC include loss of differentiation potential, loss of proliferation potential, increases in senescent cell numbers, and loss of *in vivo* bone formation (Banfi et al., 2000; Digirolamo et al., 1999; Stenderup et al., 2003). We describe here the key physiological, functional and molecular parameters of aging MSC in culture known to date including changes in cell morphology, proliferation capacity, differentiation capacity, telomere length, and telomerase activity, which are also summarized in Tables 1 and 2 (Celebi and Elçin, 2009; Kretlow et al., 2008; Mareddy et al., 2009).

* Corresponding author at: Current address: Columbia University, College of Physicians ad Surgeons, Department of Ophthalmology, Harkness Eye Institute, 635 w 165st., New York, NY10032, USA.
E-mail address: mjkimhsc@korea.com (M. Kim).

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http://dx.doi.org/10.1016/j.mad.2012.03.014

Table 1
In vitro aging of mesenchymal stem cells characterized by passage number.

Species	Passage number	Proliferation (with passage)	Morphology	Telomere length/ telomerase activity	Osteogenesis	Adipogenesis	References
Human (young, old)	Early, late	Maximal life span: young (41 ± 10 PD[b]), old (24 ± 11 PD[b])			Early p[a] > late p[a]	Early p[a] > late p[a]	Stenderup et al. (2003)
Human	p1[a] → p10[a] (Mean passage number: p9[a])	Early > late	Granular, round shape in late passage	Early > late	Early p[a] > late p[a]	Early p[a] > late p[a]	Bonab et al. (2006)
Rat	p1[a] → p10[a]	p1[a]–p4[a]: drop p5[a]: increase, then stay	No differences				Celebi and Elçin (2009)
Human	p1[a] → p7[a]	p1[a] > p3[a] > p5[a] > p7[a]	Cuboidal or flatten in late passage		Early p[a] > late p[a]		Sun et al. (2006)
Human	Fast growing clone, Slow growing clone	Fast growing clone > slow growing clone	No differences				Mareddy et al. (2009)

[a] Passage.
[b] Population doubling.

Table 2
In vivo aging of mesenchymal stem cells characterized by donor age.

Species	Age (years)	Proliferation	Maximal life span	Morphology	Telomere length/ telomerase activity	Osteogenesis	Adipogenesis	References
Human	Young: 18–29 years Old: 68–81 years	Young > Old	Young > Old		• No difference in early passage • Telomere shortening rate: young > old	No differences	No differences	Stenderup et al. (2003)
Human	Young:0–18 years Old: 59–75 years	Young > old	Young > old		Young > old	Young > old	Old: reduced adipogenic capacity	Baxter et al. (2004)
Human	Young: 7–18 years Adult: 19–40 years Aged: >40 years	Young and Adult > Aged		Cell size: young < adult < aged		Young > adult > aged	Aged < young < adult	Stolzing et al. (2008)
Human	Young: 18–42 years Old: 66–78 years Osteoporosis patients: 58–76 years					No differences	No differences	Justesen et al. (2002)
Mice	Post-natal: 6 days Adult: 6 weeks Aged: 1 years	Post natal > adult > aged				No statistical significant interaction among donor age, passage	Post natal > adult > aged	Kretlow et al. (2008)

1.1. Changes in cell morphology

Two distinct MSC morphologies are correlated with progressive changes from multilineage to unilineage potential during aging. Type I cells are spindle-shaped, grow rapidly, and are associated with maximal potential to expand in culture, while type II cells are flat, wide, and proliferate slowly (Colter et al., 2001). Human MSC from aged donors were shown to have reduced numbers of type I MSC and a predominance of type II cells even at an initial passage (Baxter et al., 2004). *In vitro* aged MSC exhibit more podia, spread further, contain more actin stress fibers, and show increased cell size associated with cellular senescence (Dimri et al., 1995; Stolzing et al., 2008). MSC from older patients show no spindle-shaped morphology in culture (Baxter et al., 2004), whereas MSC from young donors exhibit a spindle-shaped morphology in early passages in culture, which is gradually lost over time (Sethe et al., 2006). Bonab et al. (2006) observed granulated cytoplasm and debris at increasing level from the 3rd month of *in vitro* aging.

1.2. Changes in proliferation capacity

While subpopulations of MSC retain their multipotential capacity through a number of passages, most cultures progressively lose their proliferative ability (Awad et al., 1999; Ferrari et al., 1998; Pittenger et al., 1999). Loss of proliferative capacity, one of the hallmarks of aging cells, is manifested in MSC at 30–40 population doubling (PD) in contrast to embryonic stem cells (ESC), which show no such loss *in vitro* (Banfi et al., 2000; Rogenberger, 1995; Sethe et al., 2006). In addition, there are clear differences in the growth patterns of MSC obtained from young *vs.* old donors, including donor age-related decreases in the maximal life span and proliferation rate (Stenderup et al., 2003; Baxter et al., 2004). These results suggest that loss of proliferative capacity occurs over time in culture, and with increasing donor age. Of note, the observed decline in lifespan related to donor age (Baxter et al., 2004) was not reproduced in a separate study (Liu et al., 2004).

1.3. Changes in telomerase activity and telomere length

Germ cells or embryonic stem cells (ESC) have unlimited proliferative capacity and high telomerase activity (Thomson et al., 1998; Yang et al., 2008). However, telomerase activity is down regulated during the differentiation of murine embryonic stem cells. And also telomerase activity is rapidly down regulated in somatic cells as well as in non-embryonic stem cells such as progenitor or normal stem cells after a finite number of cell division (Armstrong et al., 2000; Forsyth et al., 2002). Similarly, in differentiated or fate-committed cells derived from MSC, including