Effect of aging on stem cells

Abu Shufian Ishtiaq Ahmed, Matilda HC Sheng, Samiksha Wasnik, David J Baylink, Kin-Hing William Lau

Abstract

Pluripotent stem cells have the remarkable self-renewal ability and are capable of differentiating into multiple diverse cells. There is increasing evidence that the aging process can have adverse effects on stem cells. As stem cells age, their renewal ability deteriorates and their ability to differentiate into the various cell types is altered. Accordingly, it is suggested aging-induced deterioration of stem cell functions may play a key role in the pathophysiology of the various aging-associated disorders. Understanding the role of the aging process in deterioration of stem cell function is crucial, not only in understanding the pathophysiology of aging-associated disorders, but also in future development of novel effective stem cell-based therapies to treat aging-associated diseases. This review article first focuses on the basis of the various aging disease-related stem cell dysfunction. It then addresses the several concepts on the potential mechanism that causes aging-related stem cell dysfunction. It also briefly discusses the current potential therapies under development for aging-associated stem cell defects.

Key words: Aging; Biological aging; Cellular aging; Adult stem cells; Premature aging; Mesenchymal stem cell; Stem cell renewal; Tissue regeneration

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Stem cells have the remarkable self-renewal capability and the amazing ability to differentiate into all cell types. It is generally believe that stem cells are the main source that provides cells to repair and regenerate damaged tissues and organs. However, there is now compelling evidence that the aging process has a deleterious effect on stem cells, and that the aging effects on stem cells may have play essential roles in the pathophysiology of the various aging-associated diseases. This review discusses briefly the relationship of aging-
associated stem cell dysfunction and the various aging-associated ailments, and several proposed concepts on the molecular mechanism of aging-related stem cell dysfunction.

Ahmed ASI, Sheng MHC, Wasnik S, Baylink DJ, Lau KHW. Effect of aging on stem cells. World J Exp Med 2017; 7(1): 1-10 Available from: URL: http://www.wjgnet.com/2220-315X/full/v7/i1/1.htm DOI: http://dx.doi.org/10.5493/wjem.v7.i1.1

INTRODUCTION

Aging is an unavoidable physiological consequence of the living animals. Mammalian aging is mediated by the complex cellular and organismal processes, driven by diverse acquired and genetic factors\textsuperscript{[1-5]}. Aging is among the greatest known risk factors for most human diseases\textsuperscript{[6-8]}, and of roughly 150000 people who die each day across the globe, about two thirds die from age-related causes\textsuperscript{[9]}. In modern era, one of the emerging fields in treating human diseases is the "stem cells" research, as stem cells have the remarkable potential for use to treat a wide range of diseases. Accordingly, stem cells research has become a focal point of biomedical research since 1998, when Dr. James Alexander Thomson made the scientific breakthrough of successful generation of several embryonic stem cell lines from human blastocysts\textsuperscript{[10,11]}. Stem cells are undifferentiated pluripotent cells that can give rise to all tissue types and serve as a sort of internal repair system\textsuperscript{[9,12]}. Until the recent advance in development of induced pluripotent stem cells (iPSCs), scientists primarily worked with two kinds of pluripotent stem cells from animals and humans: Embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and non-embryonic "somatic" or "adult" stem cells, which are found in various tissues\textsuperscript{[13]}. Because of potential ethical issues, "adult" stem cells have become the primary target.

Although stem cell science promises to offer revolutionary new ways of treating diseases, it is identified that aging affect the ability of stem (and progenitor) cells to function properly, which ultimately can lead to cell death (apoptosis), senescence (loss of a cell’s power of division and growth), or loss of regenerative potential\textsuperscript{[11,12]}. Aging may also shift gene functions, as reported for some genes such as, p53 and mammalian target of rapamycin (mTOR), which are beneficial in early life, but becomes detrimental later in life\textsuperscript{[13-15]}. In this regard, a novel theory, namely "stem cell theory of aging", has been formulated, and it assumes that inability of various types of pluripotent stem cells to continue to replenish the tissues of an organism with sufficient numbers of appropriate functional differentiated cell types capable of maintaining that tissue’s (or organ’s) original function is in large part responsible for the aging process\textsuperscript{[1]}. In addition, aging also compromises the therapeutic potentials of stem cells, including cells isolated from aged individuals or cells that had been cultured for many passages \textit{in vitro}. Nevertheless, in either case, understanding the molecular mechanism involved in aging and deterioration of stem cell function is crucial in developing effective new therapies for aging- as well as stem cell malfunction-related diseases. In fact, given the importance of the aging-associated diseases, scientists have developed a keen interest in understanding the aging process as well as attempting to define the role of dysfunctional stem cells in the aging process.

In this review, we will first focus on the various aging disease-related stem cell dysfunction and then address the several concepts on potential mechanisms that cause aging-related stem cell dysfunction. We will also discuss current strategies for reversing age-related stem cell dysfunction. Finally, we will discuss up-to-date therapies for aging-associated stem cell defects, available-drugs, growth factors, etc.

DISEASES OF AGING FROM OLD STEM CELLS

Adult stem cells, also known as somatic stem cells, are found throughout the body in every tissues and organs after development and function as self-renewing cell pools to replenish dying cells and regenerate damaged tissues throughout life\textsuperscript{[16]}. However, adult stem cells appear to age with the person. As stem cells age, their functional ability also deteriorates\textsuperscript{[12,17]}. Specifically, this regenerative power appears to decline with age, as injuries in older individuals heal more slowly than in childhood. For example, healing of a fractured bone takes much longer time in elderly than in young individuals\textsuperscript{[18-21]}. There is a substantial amount of evidence showing that deterioration of adult stem cells in the adult phase can become an important player in the initiation of several diseases in aging\textsuperscript{[22,23]}. The following is some of the examples of aging-associated effects on stem cells.

Neural stem cells (NSCs) are multipotent and self-renewing cells and located primarily in the neural tissues. In response to a complex combination of signaling pathways, NSCs differentiate into various specific cell types locally in the central nervous system (CNS), like neurons, astrocytes, and oligodendrocytes\textsuperscript{[24]}. NSCs in humans maintain brain homeostasis and it continuously replenishes new neurons, which are important for cognitive functions\textsuperscript{[25,26]}. However, there is now strong evidence for the aging-associated cognitive deficits, such as olfactory dysfunction, spatial memory deficits, and neurodegenerative disorders, which are caused by deterioration of NSC proliferation and differentiation and enhanced NSC senescence as a consequence of aging\textsuperscript{[27,28]}. Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into cells of mesenchyme tissues, including osteoblasts (bone cells)\textsuperscript{[29]}, chondrocytes (cartilage
cells\textsuperscript{30}, myocytes (muscle cells)\textsuperscript{31} and adipocytes (fat cells)\textsuperscript{32}. MSCs were first isolated from the bone marrow of guinea pigs in 1970’s and after that it was isolated from almost every organ in mice including fat, liver, spleen, pancreas, kidney, lung, muscle, and brain\textsuperscript{32}. Human MSCs have also been isolated from umbilical cord tissue and cord blood, placenta, bone and joints\textsuperscript{33}. However, the major sources of MSCs are the bone marrow-derived MSCs (BM-MSCs) and the adipose tissue-derived MSCs (A-MSCs); and they are currently the most studied MSCs\textsuperscript{32,34}. Aging also affects MSCs in humans and in animal models as indicated by the decrease in the bone marrow MSC pool and also shifts their lineage differentiation from one that usually favors osteoblastic differentiation to one that prefers adipogenic differentiation\textsuperscript{35}, which is largely responsible for the gradual and aging-associated shift of hematopoietic (red) marrows to fatty (yellow) marrows, and which also contributes significantly to the etiology of senile osteoporosis. It is also evident that with increasing donor age, MSCs from both bone marrow and adipose tissues have been shown to have reduced capacity to handle oxidative stress\textsuperscript{36-38}. During the aging process, oxidative stress leads to hyperactivity of pro-growth pathways, such as insulin/IGF-1 and mTOR pathways, and the subsequent accumulation of toxic aggregates and cellular debris ultimately lead to apoptosis, necrosis, or autophagy\textsuperscript{39}. In addition, in some non-skeletal tissues, particularly the hematopoietic system, MSCs is a key niche component for hematopoietic cells. Aging of MSCs has been shown to be detrimental with respect to this important function\textsuperscript{30}.

Adult skeletal muscle stem cells (satellite cells) have a remarkable capacity to regenerate\textsuperscript{36,41}. Similarly, their regeneration capacity declines with aging, although it is not clear whether this is due to extrinsic changes in the environment and/or to cell-intrinsic mechanisms associated to aging. This impaired regenerative capacity of skeletal muscle during aging is due to accumulation of the altered progeny, which leads to progressive deterioration of tissue structure and function, manifesting after injury or in response to the depletion of memory B cells and naive T cells in the hematopoietic system in the elderly\textsuperscript{41-44}.

Hematopoietic stem cells (HSCs) are the blood-forming stem cells through the process of hematopoiesis\textsuperscript{45}. They are located in the red bone marrow within marrow cavity of most bones. HSCs also produce immune cells of the body. Since blood cells are responsible for constant maintenance and immune protection of every cell type of the body, the constant production of billions of new blood cells each day by HSCs is very important for mammal life. HSC-derived monocytes can give rise to osteoclasts, macrophage and granulocyte. Osteoclasts are giant cells with numerous nuclei that work in synergy with osteoblasts through complicated bone coupling mechanisms to maintain bone homeostasis\textsuperscript{35,46}. All these activities of HSCs are carefully modulated by a complex interplay between cell-intrinsic mechanisms and cell-extrinsic factors produced by the microenvironment; and aging altered this fine-tuned regulatory network, leading to aberrant HSC cell cycle regulation, degraded HSC function, and hematological malignancy\textsuperscript{47}.

**MECHANISM FOR FUNCTIONAL DETERIORATION OF STEM CELL IN AGING**

There are several potential mechanisms that are believed to contribute to the aging-associated stem cell dysfunction; and they probably are in part responsible for many aging-associated diseases. Figure 1 proposes some of the contributing factors/mechanisms that could be responsible for the aging-induced deterioration of stem cell functions and aging-associated diseases. This section summarizes some of these contributing factors/mechanisms and their potential roles in the aging effect on stem cells.

**Microenvironment**

Aging is characterized by common environmental conditions, such as hormonal, immunologic, and metabolic disorders\textsuperscript{48-50} and these are considered as the critical microenvironmental factors affecting stem cell functions. Changes in these microenvironmental factors in response to aging are believed to be responsible for the changes in stem cell function with aging\textsuperscript{51}. It has been shown that potentially underlying aging-related tissue degeneration, such as osteoporosis, could be due to impaired MSCs by surrounding micro-environmental pathologic factors\textsuperscript{52,53}. It has also been shown that in mammals, metabolic alterations of hyperglycemia and hyperinsulinemia are important pathologic factors in aging and in MSC dysfunction\textsuperscript{54}. However, the molecular mechanism in mediating stem cells dysfunction by microenvironmental signals is not yet fully understood.

Cells produce soluble (endocrine or paracrine) factors necessary for information exchange among cells of distant tissues and/or within the same organ\textsuperscript{51}. Aging cells can influence an organ or tissue by secreting soluble endocrine or paracrine factors. Accordingly, aging of the endocrine glands has known to result in hormonal disturbances\textsuperscript{50,55}, which ultimately affects normal function and or differentiation of the stem cells. In humans, sex hormones, especially estrogen, are the most prominent endocrine factors that change with aging, and sex hormones discordance often leads to several significant diseases. Estrogen insufficiency also induces the biased differentiation of MSCs to adipocytes over osteoblasts\textsuperscript{56,58,57}. Aging-related elevation in circulating levels of proinflammatory cytokines, such as interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α), can also cause differentiation disorders of MSCs\textsuperscript{50,59}.

**DNA damage and telomere shortening**

In mammals, spontaneous and extrinsic mutational
events occur on DNA on daily basis. While most of the damaged DNAs are repaired by normal DNA repair mechanism, some of the mutated DNAs appear to escape from the repair mechanism and accumulate over time. Accordingly, there would be a significant accumulation of mutated or damaged DNAs in aging cells compared to young cells. The accumulation of damaged DNA may in part be responsible for the various cellular events of the aging process. In fact, this “mutational theory” is one of the earliest theories of the aging process [15]. DNA damage can be caused by environmental factors, like UV irradiation, and also can be the consequence of the cell’s own metabolic processes [e.g., generating reactive oxygen species (ROS)] that tend to accumulate with time [60]. DNA damage impaired stem-cell function in aging, which has been documented by the study that HSCs derived from aged mice harbored significant alterations in their DNA repair response [1,17]. DNA-repair proteins, such as FANCD1 [61], MSH2 [62] or ERCC1 [63], are found to be deficient in adult mice with significant functional defects of HSCs and the dysfunction of MSCs in aging led to leukemia and aging-associated remodeling [64]. In addition, measures of DNA damage in HSCs, such as histone H2AX phosphorylation and comet tails, were also found to be increased with advancing age [65,66]. In satellite cells, H2AX phosphorylation was also accumulated with increasing age [67].

Premature aging can be resulted from defects in the DNA repair and telomerase pathway components in humans and mice [68]. In aging diseases, there has been significant interest in the telomere shortening that is now being used as a hallmark of aging, to which even stem cells are not immune [1,17]. A telomere is a region of repetitive nucleotide sequences at each end of a chromosome. It protects genome from nucleolytic degradation, unnecessary recombination, repair, or fusion with neighboring chromosomes [69]. Although stem cells express telomerase, the telomeres of HSCs, MSCs, NSCs, HFSCs and GSCs do shorten with age [70-72]. When telomeres become critically short, the cell becomes senescent, it ceases to divide and may undergo apoptosis. In fact, many aging-associated diseases, like the increased cancer risk [73,74], coronary heart disease [75-77], heart failure [78], diabetes [79], and osteoporosis [80], are caused by accelerated telomere shortening. Despite considerable evidence that telomere shortening causes reduction in life span, the telomere shortening concept of aging is still somewhat controversial, since laboratory mice lacking telomerase RNA component (TERC) showed no obvious abnormal phenotypes even after five generations [81,82].

**Mitochondrial dysfunction**

Mitochondria are ubiquitous intracellular organelles in mammals and are the main source of cellular adenosine triphosphate (ATP) that plays a central role in a variety of cellular processes. As mitochondria produce about 90% of cellular energy, the aging-related ROS generation, disruption in Ca²⁺ homeostasis, and increased cell apoptosis are three causes of mitochondria dysfunction that directly affects aging-related diseases [83]. In fact, there have been many studies suggesting a direct relationship between mitochondrial dysfunction and human stem cell aging [84-87]. Accordingly, in several cell systems, mitochondrial dysfunction has been shown to lead to respiratory chain dysfunction, which may be the result of the accumulation of mutations in mitochondrial DNA.
Epigenetic alteration

Epigenetics refer to changes in gene expression, which are heritable through modifications without affecting the DNA sequence. It has also been defined more broadly as the dynamic regulation of gene expression by sequence-independent mechanisms, including but not limited to changes in DNA methylation and histone modifications[90-94]. Epigenetic marks in stem cells are transmitted heritably to their daughter cells, priming lineage-specific loci for modification in downstream progenies[95]. Stem cell fates are regulated by epigenetic modifications of DNA that establish the memory of active and silent gene states[96,97]. Aberrant epigenetic regulation affects the organismal aging[98], age-associated dysfunction of stem cells, and predisposition to hematological cancers development[99]. For instance, DNA methylation specific to regions of the genome that are important for lineage-specific gene expression increased in aging HSCs[100] and the perturbations of their histone modifications (H3K4me3) may impair its self-renewal genes[101]. It has also been reported that mutations in epigenetic regulators, such as DNMT3a, TET2, and ASXL1, are frequently found in myeloid neoplasia[102]. Since most of the chromatin changes are intrinsically reversible, epigenetic alterations are therefore considered good therapeutic targets for molecular effectors and thereby are potential therapies for certain distinct pathologies[103,104]. Therefore, there has been immense interest in understanding these genome-scale regulatory mechanisms that lead to impaired gene expression, and that contribute to the decline of stem cell and tissue function with age.

MicroRNAs (miRNAs) are another key class of epigenetic mediators of stem cell dysfunction. They are a class of small ncoding RNAs composed of 18- to 25-bp nucleotides[105] that functions in RNA silencing and post-transcriptional regulation of gene expression[106-109]. It plays an important role in regulating self-renewal and differentiation by repressing the translation of selected mRNAs in stem cells and differentiating daughter cells[105]. In fact, non-coding RNA-mediated regulatory events as a part of the epigenetic mechanism to modulate mRNA degradation and/or protein translation that play important role in development and disease state[110]. miRNAs, such as miR-17, regulates osteoblast differentiation of MSCs[112,114]. MiR-290–295 cluster seems to promote embryonic stem cell differentiation, self-renewal, and maintenance of pluripotency[115]. Moreover, recent findings show the involvement of miRNAs in senescence manipulation. These findings have led to the suggested use of these miRNAs as clinical biomarkers of stem cell senescence and their potentiality[116].

Therapeutic Approaches for the Treatment of Aging-Induced Stem Cell Dysfunction

In recent years with increasing understanding of stem cell behavior in different niche of the body offers promise for the development of potential therapeutic approaches to treat aging-associated dysregulation of adult stem cells and aging-related diseases. Some of the potential therapeutic approaches for the treatment of age-related stem cell dysfunction are discussed below.

Parabiosis

The concept of parabiosis is not new; however, in the past decade its role in reversing the effects of aging and enhancing rejuvenation has gathered substantial momentum. Recent findings suggest that aging-related cellular dysfunctions can be repaired successfully by modulating the molecular architecture of the tissue environment rather than inducing cell intrinsic changes alone[117,118]. Therefore, the effects of aging in an old individual can be modulated or reversed by the circulatory or systemic factors derived from the young blood through an anatomical joining, parabiosis[60]. The fascinating results of parabiosis have been reported to rejuvenate brain[118], muscles[67], and liver tissues in the aged animals[119]. In skeletal muscle regeneration, serum derived from young mice activated the Notch signaling pathway and regulated the satellite cells proliferation of old mice in vitro[119]. In aged mice, through the parabiosis approach, systemic factors from young mice successfully reversed inefficient CNS remyelination, a regenerative process of CNS that produces new myelin sheaths from adult stem cells[118]. Despite the promising outcomes in animal models, there is persistence of contradiction in functions of factors identified in prominent parabiosis studies, rendering the concept highly controversial for use in humans. For instance, growth differentiation factor 11 (GDF-11) has been reported to show both positive[67] and negative correlations[120] with stem cell aging.

Retrotransposons

Retrotransposons are mobile DNA elements that can induce genetic instability and have been reported to be a cause of cellular dysfunction during aging[21]. The long interspaced nuclear elements (L1) are 6-kb long retrotransposons that code for RNA binding protein and endonuclease protein. There are 500000 copies of L1 elements in the human genome, and approximately 100 of such active elements replicated to induce genomic instabilities and to increase the risk of DNA damage. Elevated activity of L1 has been reported in aging-related pathological conditions[122]. The link between SIRT-6 (an important marker of longevity) and L1 offered more direct evidence for the role of L1 in aging-related genomic complications. SIRT6 are
known to repress the activity of L1 retrotransposons\textsuperscript{123}. DNA damage-induced mobilization of SIRT6 to the site of repair and subsequent repression of L1 have been contemplated in the development of therapeutics for age-related neurological pathologies, such as dementia and cancer\textsuperscript{124}. Suppression of L1 activity by overexpression of SIRT6 in senescence cells delayed the onset of L1-induced pathological conditions. High caloric diet activated the SIRT1 activity and has been reported to protect the animal from premature aging in Cockayne syndrome\textsuperscript{125} whereas in the case of the mouse Alzheimer's disease model the caloric restriction slowed down the disease progression\textsuperscript{126}. Other than modulation of SIRT6 expression, inhibition of reverse transcriptase (a critical enzyme for the L1 replication) is another way to attenuate L1 activity\textsuperscript{127}. Several small non-coding RNAs, such as pi-RNAs, si-RNAs and L1 specific small RNAs, have also been reported to regulate the silencing of retrotransposons element activity in mouse germ cells and in aging human somatic cells\textsuperscript{127}.

**Cellular reprogramming towards iPSCs**

iPSCs are a type of pluripotent stem cell that can be generated directly from adult cells and the recent advances in this area have opened up many gateways for the research in cell-based therapeutics\textsuperscript{128}. Cellular reprogramming of aged somatic cells towards iPSC enables the editing and resetting of the cellular clock by removing the characteristic feature of aging. The ability to derive iPSCs from aging-related pathological cells have enabled investigators to develop recombination-based therapeutic approaches to edit genetic defects responsible of premature and accelerated aging. The reprogramming of aged somatic cells to target stem can be used as an alternative source to get cells for transplantation and for genetic editing. Recent studies show encouraging effects of reprogramming in rejuvenation of senescent cells, as evident by elongated telomeres and reduced oxidative stress\textsuperscript{129}. Human iPSC-based models for aging-related degenerative diseases have been tested to understand the disease dynamics in Parkinson's disease, Alzheimer's disease and in progeroid laminopathies\textsuperscript{130}. Valuable information from these studies has resulted in the first clinical trial for progeroid patients\textsuperscript{131}. In a mouse model of skeletal defect, human iPSC designed to express PAX7 were able to be differentiated into muscle progenitor cells that engrafted and repaired the defective dystrophin-positive myofibers formation. In case of Hutchinson-Gilford progeria syndrome (HGPS), reprogramming of HGPS fibroblasts by transduction with vectors expressing Oct4, Sox2, Klf4 and c-Myc has been reported to revert aging-associated markers, such as Lamin, to a "young" state\textsuperscript{129}.

**Telomere lengthening**

As discussed above, the telomere length is inversely linked to the chronical age, and thus it is believed that increasing the length of telomere may increase life span. Many advanced approaches are being developed to efficiently increase the telomere length and to protect cells from chromosome shortening. In *in vitro* cultured human cells, the delivery of RNA coding for telomere-extending protein has been reported to increase the cell proliferation rate\textsuperscript{133}. In telomere-deficient mice, genetic editing to reactivate telomerase activity has been reported to reverse the aging symptoms\textsuperscript{134}. Telomerase activation drugs and telomerase gene therapy are also alternative approaches that aim to increase the telomere length to protect the cells from premature aging\textsuperscript{135,136}.

**CONCLUSION**

From the various advances in stem cell research, it is clear that we grow old partly because our stem cells grow old with us. The functions of aged stem cells become impaired as the result of cell-intrinsic pathways and surrounding environmental changes. With the sharp rise in the aging-associated diseases, the need for effective regenerative medicine strategies for the aged is more important than ever. Fortunately, rapid advances in stem cell and regenerative medicine technologies continue to provide us with a better understanding of the diseases that allows us to develop more effective therapies and diagnostic technologies to better treat aged patients. However, there is a big ethical concern regarding the use of human embryos to procure embryonic stem cells and many countries already currently restrict experiments on embryos to the first 14 d. Additionally, the International Society for Stem Cell Research has issued guidelines advising researchers across the globe to stick with this 14-d window. Nevertheless, it seems that the human stem cell research in the next decade will likely bring enormous progress in the aging-associated disease therapies but may also reach a step closer to the edge of ethical concern of creation of "Frankenstein".

**REFERENCES**

\begin{enumerate}
  \item Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol* 2007; 8: 703-713 [PMID: 17717515 DOI: 10.1038/nrm2241]
  \item Dillin A, Gottschling DE, Nystrom T. The good and the bad of being connected: the integrins of aging. *Curr Opin Cell Biol* 2014; 26: 107-112 [PMID: 24529252 DOI: 10.1016/j.cceb.2013.12.003]
  \item Shane Anderson A, Loeser RF. Why is osteoarthritis an age-related disease? *Best Pract Res Clin Rheumatol* 2010; 24: 15-26 [PMID: 20129196 DOI: 10.1016/j.berh.2009.08.006]
  \item Reeve A, Simcox E, Turnbull D. Ageing and Parkinson’s disease: why is advancing age the biggest risk factor? *Aging Res Rev* 2014; 14: 19-30 [PMID: 24503040 DOI: 10.1016/j.arr.2014.01.004]
  \item Nocetti T, Partridge L. Ageing as a risk factor for disease. *Curr Biol* 2012; 22: R741-R752 [PMID: 22975005 DOI: 10.1016/j.cub.2012.07.024]
  \item De Grey AD. Life span extension research and public debate: societal considerations. *Studies in Ethics, Law, and Technology, 2007* [DOI: 10.2202/1941-6008.1011]
  \item Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282: 1145-1147 [PMID: 9804556]
  \item Vogel G. Breakthrough of the year. Capturing the promise of youth. *Science* 1999; 286: 2238-2239 [PMID: 10636772]
\end{enumerate}
Ahmed ASI et al. Stem cells and aging

9 Biehl JK, Russell B. Introduction to stem cell therapy. J Cardiovasc Nurs 2009; 24: 98-103; quiz 104-105 [PMID: 19242274 DOI: 10.1097/JCN.0b013e318197a0af]

10 Marchetto MC, Gage FH. Your brain under the microscope: the promise of stem cells. Cerebrum 2014; 2014: 1 [PMID: 25006961]

11 Jones DL, Rando TA. Emerging models and paradigms for stem cell ageing. Nat Cell Biol 2011; 15: 506-512 [PMID: 21540846 DOI: 10.1038/nclb0511-506]

12 Oh J, Lee YD, Wagers AJ. Stem cell ageing: mechanisms, regulators and therapeutic opportunities. Nat Med 2014; 20: 870-880 [PMID: 25106552 DOI: 10.1038/nm.3651]

13 Kirkwood TB. Understanding the odd science of ageing. Cell 2005; 120: 437-447 [PMID: 15734677 DOI: 10.1016/j.cell.2005.01.027]

14 Blagosklonny MV. Revisiting the antagonistic pleiotropy theory of ageing: TOR-driven program and quasi-program. Cell Cycle 2010; 9: 3151-3156 [PMID: 20724817 DOI: 10.4161/cc.9.16.13120]

15 Medawar P. An Unsolved Problem in Biology. London, Lewis. Reprinted in Medawar PB (1981). The Uniqueness of the Individual. New York: Dover, 1952

16 Boyette LB, Tuan RS. Adult Stem Cells and Diseases of Ageing. J Clin Med 2014; 3: 88-134 [PMID: 24757526 DOI: 10.3390/jcm30100088]

17 Schultz MB, Sinclair DA. When stem cells grow old: phenotypes and mechanisms of stem cell ageing. Development 2016; 143: 2-14 [PMID: 27632838 DOI: 10.1242/dev.136033]

18 Ho AD, Wagner W, Mahlknecht U. Stem cells and ageing. The potential of stem cells to overcome age-related deteriorations of the body in regenerative medicine. EMBO Rep 2005; 6 Spec No: S53-S58 [PMID: 15995569 DOI: 10.1038/sj.embr.7400436]

19 Sousounis K, Baddour JA, Tsonis PA. Ageing and regeneration in vertebrates. Curr Top Dev Biol 2014; 108: 217-246 [PMID: 24512711 DOI: 10.1006/B978-0-12-391498-9.00008-5]

20 Paxson JA, Grumtant A, Parkin CD, Mazan MR, Davis A, Ingenuity EP, Hoffman AM. Age-dependent decline in mouse lung regeneration with age. Proc National Acad Sci 2011; 6: e23232 [PMID: 21912590 DOI: 10.1073/pnas.0902323]

21 Keller K, Engelhardt M. Strength and muscle mass loss with ageing. Ageing and stress loss. Muscles Ligaments Tendons J 2013; 3: 346-350 [PMID: 24596700]

22 Wagner W, Bork S, Horn P, Krunic D, Walenda T, Diehlmann A, Benes V, Blake J, Huber FX, Eckstein V, Bokamp P, Ho AD. Aging and replicative senescence have related effects on human stem and progenitor cells. PLoS One 2009; 4: e3846 [PMID: 19513108 DOI: 10.1371/journal.pone.0003846]

23 Manilla E, Díaz Aquino V, Zambón D, Marin GH, Mártire K, Roque G, Iñiguez T, Rodrán NH, Patel A, Sturla L, Larsen G, Spreitz R, Núñez L, Soratti C, Ibar R, van Leeuwen M, Tau JM, Drago H, Maceira A. Could metabolic syndrome, lipodystrophy, and aging be mesenchymal stem cell exhaustion syndromes? Stem Cells Int 2011; 2011: 943216 [PMID: 21716667 DOI: 10.4061/2011/943216]

24 Alenzi FQ, Bahkali AH. Stem cells: Biology and clinical potential. Afr J Biotechnol 2011; 10: 1999-2040

25 Zhu L, Dong C, Sun C, Ma R, Yang D, Zhu H, Xu J. Rjuveneration of MPITP-induced human neural precursor cell senescence by activating autophagy. Biochem Biophys Res Commun 2015; 464: 526-533 [PMID: 26519917 DOI: 10.1016/j.bbrc.2015.06.174]

26 Winner B, Kohl Z, Gage FH. Neurodegenerative disease and adult neurogenesis. Eur J Neurosci 2011; 33: 1139-1151 [PMID: 21395858 DOI: 10.1111/j.1460-9568.2011.07613.x]

27 Ewure E, Shingo T, Gregg C, Fujikawa H, Ohta S, Weiss S. Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. J Neuroscience 2004; 24: 8354-8365 [PMID: 15385618 DOI: 10.1523/JNEUROSCI.2751-04.0001]

28 Ming GL, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 2011; 70: 687-702 [PMID: 21609825 DOI: 10.1016/j.neuron.2011.05.001]

29 Brighton CT, Hunt RM. Early histological and ultrastructural changes in medullary fracture callus. J Bone Joint Surg Am 1991; 73: 832-847 [PMID: 2071617]
M, Rübe C. Accumulation of DNA damage in hematopoietic stem cell aging and mutations? Mol Cell Biol 2006; 26: 1459-1464 [PMID: 17397675 DOI: 10.1093/mcj/16.6.1459-1464]

56 Brouillette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CJ, Samani NJ. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. Lancet 2007; 369: 107-114 [PMID: 17223473 DOI: 10.1016/S0140-6736(07)60071-3]

57 Zee RY, Michaud SE, Gerner S, Ricker PM. Association of shorter mean telomere length with risk of incident myocardial infarction: a prospective, nested case-control approach. Circ Cardiovasc Qual Outcomes 2009; 2: 139-141 [PMID: 19217888 DOI: 10.1161/CIRCOUTCOMES.108.780200]

58 van der Harst P, van der Steege G, de Boer RA, Voors AA, Hall AS, Mulder MJ, van Gilst WH, van Veldhuisen DJ. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. J Am Coll Cardiol 2007; 49: 1459-1464 [PMID: 17397675 DOI: 10.1016/j.jacc.2007.01.027]

59 Sampson MJ, Winterbone MS, Hughes JC, Doazio N, Hughes DA. Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. Diabetes Care 2006; 29: 283-289 [PMID: 16443874]

60 Valdes AM, Richards JB, Gardner JP, Swaminathan R, Kimura M, Richards JB, Gardner JP, Psaty BM, Jenny NS, Tracy RP, Walston J, Kimura M, Aviv A. Leukocyte telomere length correlates with bone mineral density and is shorter in women with osteoporosis. Osteoporos Int 2007; 18: 1203-1210 [PMID: 17347788 DOI: 10.1007/s00198-007-0357-5]

61 Ju Z, Jiang H, Javorkis M, Rathmann G, Crompton A, Klein C, Trumpp A, Rudolph KL. Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. Nat Med 2007; 13: 742-747 [PMID: 17486688 DOI: 10.1038/nm1578]

62 Lee HW, Blasco MA, Gottlieb GI, Horner JW, Greider CW, DePinho RA. Essential role of mouse telomerase in highly proliferative organs. Nature 1998; 392: 569-574 [PMID: 9560155 DOI: 10.1038/333435a]

63 Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wolfhagen GM, De Vries J, Ruff JS, Sullivan J, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamazaki T, Tanokura M, Weinrich DR, Leeuwenburgh C, Prolla TA. Mitochondrial DNA mutations, oxidative age-related dysfunction in mouse skeletal muscle. Science 2014; 344: 1269-1273 [PMID: 25402537 DOI: 10.1126/science.1251152]
stress, and apoptosis in mammalian aging. Science 2005; 309: 481-484
[PMD: 160207380 DOI: 10.1126/science.1112125]

84 Bratit A, Larsson NG. The role of mitochondria in aging. J Clin Invest 2013; 125: 1246-1257
[PMD: 23454375 DOI: 10.1172/JCISO4125]

85 Taylor RW, Barzilai M, Borthwick G, Camacho DF, Cherny PF, Samuels DC, Taylor GA, Plussa SM, Needham SJ, Greaves LC, Kirkwood TB, Turnbull DM. Mitochondrial DNA mutations in human colonic crypt stem cells. J Clin Invest 2003; 112: 1351-1360
[PMD: 14957761 DOI: 10.1172/JCI9435]

86 McDonald SA, Greaves LC, Gutierrez-Gonzalez L, Rodriguez-Justo M, Dehergadora M, Leedham SJ, Taylor RW, Lee CY, Preston SL, Lovell M, Hunt T, Elia G, Oukif K, Harrison R, Novelli MR, Mitchell I, Sulk DM, Turnbull DM, Jankowski JA, Wright NA. Mechanisms of field carcinization in the human stomach: the expansion and spread of mutated gastric stem cells. Gastroenterology 2008; 134: 500-510
[PMD: 18242216 DOI: 10.1053/j.gastro.2007.11.035]

87 Fellous TG, Islam S, Tadrous PJ, Elia G, Kocher HM, Bhattacharya S, Mears L, Turnbull DM, Taylor RW, Greaves LC, Chinnery PF, Taylor G, McDonald SA, Wright NA, Alison MR. Locating the stem cell niche and tracing hematopoietic lineages in human liver. Hepatology 2009; 49: 1655-1663
[PMD: 19309719 DOI: 10.1002/hep.22791]

88 Miquel J, Economos AC, Fleming J, Johnson JE. Mitochondrial role in cell aging. Exp Gerontol 1980; 15: 575-591
[PMD: 7009178]

89 Pervaiz S, Tanaka R, Ghaffari S. Oxidative stress regulation of stem and progenitor cells. Antioxid Redox Signal 2009; 11: 2777-2789
[PMD: 19056089 DOI: 10.1089/ars.2009.2084]

90 Bonawitz ND, Chatterjee-Lapointe M, Pan Y, Shadel GS. Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. Cell Metab 2007; 5: 265-277
[PMD: 17403371 DOI: 10.1016/j.cmet.2007.02.009]

91 Choi CS, Befroy DE, Codella R, Kim S, Reznick RM, Hwang YJ, Liu ZX, Lee HY, Distefano A, Samuel VT, Zhang D, Cline RJ, Liu W, Hu C, Xue Z, Wang G, Ding B, Luo H, Tang L, Kong X, Chen X, Liu N, Ding Y, Yin J. MiR-17 modulates osteogenic differentiation through a coherent feed-forward loop in mesenchymal stem cells isolated from periodontal ligaments of patients with periodontitis. Stem Cells 2011; 29: 1804-1816
[PMD: 21896965 DOI: 10.1002/stem.728]

92 Jia J, Feng X, Wu Y, Yang S, Zhang Q, Liu X, Feng Y, Dai Z. MiR-17-5p modulates osteoblastic differentiation and cell proliferation by targeting SMAD7 in non-traumatic osteonecrosis. Exp Mol Med 2014; 46: e107
[PMD: 25060766 DOI: 10.1038/emm.2014.43]

93 Liu W, Qi M, Konstantinou A, Zhang L, Jin F, Yin J. The p53/miR-17-5p pathway mediates skeletal deformities in an age-related phenotype model via inhibiting the function of mesenchymal stem cells. Aging (Albany NY) 2015; 7: 205-218
[PMD: 25855145 DOI: 10.18632/aging.100728]

94 Houbavty HB, Murray MF, Sharp PA. Embryonic stem cell-specific MicroRNAs. Dev Cell 2003; 5: 351-358
[PMD: 12919684 DOI: 10.1016/S1534-5807(03)00227-2]

95 Bilsland AE, Revie J, Keith W. MicroRNA and senescence: the senectome, integration and distributed control. Crit Rev Oncog 2013; 18: 373-390
[PMD: 23641622 DOI: 10.1615/CritRevOncog.2013007197]

96 Eggel A, Wyss-Coray T. A revival of parabiosis in biomedical research. Swiss Med Wkly 2014; 144: w13914
[PMD: 24906774 DOI: 10.4444/smw.2014.13914]

97 Ruck JM, Zhao JW, Sladrack JL, van Wijngaarden P, Rao TN, Wagers AJ, Franklin JR. Rejuvenation of regeneration in the aging central nervous system. Cell Stem Cell 2012; 10: 96-103
[PMD: 2226359 DOI: 10.1016/j.stem.2011.11.019]

98 Conboy IM, Conboy MJ, Wagers AJ, Girman ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 2005; 433: 760-764
[PMD: 15716955 DOI: 10.1038/nature03260]

99 Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE, Mallozzi C, Jacob C, Jennings LL, Clay I, Laurent G, https://www.wjgnet.com
Ma S, Brachta S, Lach-Trifilieff E, Shavlakadze T, Trendelenburg AU, Brack AS, Glass DJ. GDF11 Increases with Age and Inhibits Skeletal Muscle Regeneration. *Cell Metab* 2015; 22: 164-174 [PMID: 26001423 DOI: 10.1016/j.cmet.2015.05.010]

De Cecco M, Criscione SW, Peterson AL, Neretti N, Sedivy JM, Kreiling JA. Transposable elements become active and mobile in the aging mammalian somatic tissues. *Aging* (Albany NY) 2013; 5: 867-883 [PMID: 24323947 DOI: 10.18632/aging.100621]

St Laurent G, Hammell N, McCaffrey TA. A LINE-1 component to human aging: do LINE elements exact a longevity cost for evolutionary advantage? *Mech Ageing Dev* 2010; 131: 299-305 [PMID: 20346965 DOI: 10.1016/j.mad.2010.03.008]

Van Meter M, Kashyap M, Rezaazadeh S, Geneva AJ, Morello TD, Seluanov A, Gorbunova V. SIRT6 represses LINE1 retrotransposons by ribosylating KAP1 but this repression fails with stress and age. *Nat Commun* 2014; 5: 501 [PMID: 25247314 DOI: 10.1038/ncomms6011]

Coulal NG, Garcia-Perez JL, Peng GE, Yeo GW, Mu Y, Lovci MT, Morell M, O’Shea KS, Moran JV, Gage FH. L1 retrotransposition in human neural progenitor cells. *Nature* 2009; 460: 1127-1131 [PMID: 19657334 DOI: 10.1038/nature08248]

Scheibye-Knudsen M, Mitchell SI, Fang EF, Iyama T, Wang J, Dunn CA, Singh N, Veith S, Hasban-Olive MM, Mangerich A, Wilson MA, Mattison MP, Pershing BH, Legger CG, Warren A, Le Couteur DG, Moaddel R, Wilson DM, Croteau DL, de Cabo R, Bohr VA. A high-fat diet and NAD(+) activate Sirt1 to rescue premature aging in cockayne syndrome. *Cell Metab* 2014; 20: 840-855 [PMID: 25440059 DOI: 10.1016/j.cmet.2014.10.005]

Braidy N, Jayasena T, Poljak A, Sachdev PS. Sirtuins in cognitive ageing and Alzheimer’s disease. *Curr Opin Psychiatry* 2012; 25: 226-230 [PMID: 22327552 DOI: 10.1097/YCO.0b013e32835112e1]

Goodier JL. Restricting retrotransposons: a review. *Mob DNA* 2016; 7: 16 [PMID: 27525044 DOI: 10.1186/s13000-016-0670-z]

Singh VK, Kalsan M, Kumar N, Saini A, Chandra R. Induced pluripotent stem cells: applications in regenerative medicine, disease modeling, and drug discovery. *Front Cell Dev Biol* 2015; 3: 2 [PMID: 25699255 DOI: 10.3389/fcell.2015.00002]

Freije JM, López-Otín C. Reprogramming aging and progeria. *Curr Opin Cell Biol* 2012; 24: 757-764 [PMID: 22959961 DOI: 10.1016/j.ceb.2012.08.009]

Liu GH, Barkho BZ, Ruiz S, Diep D, Qu J, Yang SL, Panopoulos AD, Suzuki K, Kurian L, Walsh C, Thompson J, Bose S, Fang H, Sancho-Martinez I, Zhang K, Yates J, Izpisua Belmonte JC. Recapitulation of premature ageing with iPSCs from Hutchinson-Gilford progeria syndrome. *Nature* 2011; 472: 221-225 [PMID: 21346760 DOI: 10.1038/nature09879]

Gordon LB, Rothman FG, López-Otín C, Misteli T. Progeria: a paradigm for translational medicine. *Cell* 2014; 156: 400-407 [PMID: 24485450 DOI: 10.1016/j.cell.2013.12.028]

Miller JD, Ganat YM, Kishinevsky S, Bowman RL, Liu B, Ta EY, Mandal PK, Vera E, Shum JW, Kriks S, Taldone T, Fasuki N, Tomishima MJ, Kraic D, Milner TA, Rossi DJ, Studer L. Human iPSC-based modeling of late-onset disease via progerin-induced aging. *Cell Stem Cell* 2013; 13: 691-705 [PMID: 24315443 DOI: 10.1016/j.stem.2013.11.006]

Ramunas J, Yakubov E, Brady JI, Corbel SY, Holbrook C, Brandt M, Stein J, Santiago JG, Cooke JP, Blau HM. Transient delivery of modified mRNA encoding TERT rapidly extends telomeres in human cells. *FASEB J* 2015; 29: 1930-1939 [PMID: 25614443 DOI: 10.1096/fj.14-259531]

Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, Sahin E, Kost-Alimova M, Protopopov A, Cadiñanos J, Horner JW, Maratos-Flier E, Depinho RA. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature* 2011; 469: 102-106 [PMID: 21113150 DOI: 10.1038/nature09603]

Bernardes de Jesus B, Schneeberger K, Vera E, Tejera A, Harley CB, Blasco MA. The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer. *Nature* 2011; 472: 1474-9726.2011.0700.x]

Bernardes de Jesus B, Vera E, Schneeberger K, Tejera AM, Ayuso E, Bosch F, Blasco MA. Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. *EMBO Mol Med* 2012; 4: 691-704 [PMID: 22585599 DOI: 10.1002/emmm.201200245]

P. Reviewer: Jun Y, Kiselev SL, Zaminy A S. Editor: Ji FF L. Editor: A E. Editor: Lu YJ
