Aging-Related Genes in Mesenchymal Stem Cells: A Mini-Review

Kyung-Rok Yu a, b  Kyung-Sun Kang a, b

a Adult Stem Cell Research Center and b Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, Korea

Key Words
Mesenchymal stem cells · Aging · Histone deacetylase · DNA methyltransferase · p16 INK4A · High-mobility group A2 · Lamin A · ZMPSTE24

Abstract
Adult stem cells in mammalian organs play pivotal roles in the maintenance and repair of these organs throughout the life of the adult and maintain the proper homeostasis of a tissue or organ. Among the adult stem cells described to date, mesenchymal stem cells (MSCs) are highlighted for clinical applications because MSCs have many advantages for cell therapy, including multilineage differentiation, homing, immune modulation and wound-healing effects. However, as the aging of MSCs leads to an age-associated decline in their number and function, it is important to clarify the age-associated factors and regulatory mechanism associated with the MSC aging process. In this review, we amassed and discuss the recent data related to age-associated genes in MSCs. In particular, the activities of epigenetic regulatory factors, including histone acetylase and DNA methyltransferase, modulate gene expression and crosstalk with each other during the MSC senescence process. p16 INK4A and high-mobility group A2 play important age-associated roles in the regulation of MSC stemness, and lamin A- and prelamin A-dependent nuclear abnormalities have significant biological relevance in MSC aging. Taken together, the information described here, including the epigenetic regulatory factors, transcription factors and cell signaling, could be used toward the development of treatments for MSC aging and related defects.

Introduction

Aging is an extremely complex process that affects most of the biological functions of an organism, generally culminating in disease and death due to the accumulated actions of different types of stresses. Among these stresses, oxidative reactions, telomere attrition and the decline of the DNA repair and protein turnover systems have been proposed to contribute to aging. Indeed, the downregulation of the regenerative ability of tissues and organs, along with an increased susceptibility to infections and cancers, are prominent hallmarks of senescence [1]. Similar to normal somatic cells, adult stem cells are exposed to stressors during the life span, leading to an age-dependent decline in their number and function. Given that the senescence-induced loss of adult stem cell stemness results in the impairment of tissue homeostasis, regeneration and repair [2], it is important to understand the aging-related genes and the mechanisms that underlie this work was supported by the Research Institute for Veterinary Science, Seoul National University.

KARGER
© 2013 S. Karger AG, Basel
0304–324X/13/0596–0557$38.00/0
E-Mail karger@karger.com
www.karger.com/ger

Prof. Kyung-Sun Kang, DVM, PhD
Adult Stem Cell Research Center
College of Veterinary Medicine, Seoul National University
599 Gwanakno, Sillimi-Dong, 151-742 Seoul (Republic of Korea)
E-Mail kangpub@snu.ac.kr
the regulation of adult stem cell stemness and senescence.

In this review, we have included the latest data on genes that regulate MSC aging (fig. 1).

**Cellular Properties of Mesenchymal Stem Cells**

Mesenchymal stem cells (MSCs), also referred to as multipotent stromal cells or mesenchymal stromal cells, have been isolated from a variety of connective tissues, including bone marrow, adipose tissue, dental pulp, amniotic fluid, Wharton’s jelly and umbilical cord blood. Recently, umbilical cord blood was shown to represent a good alternative source of MSCs, as the use of this material, which is routinely discarded as waste after the delivery of a baby, would be advantageous because it is an abundant source of MSCs, obtainable by noninvasive painless means, and harmless to the mother and baby.

To date, MSCs are widely defined as having a fibroblastic spindle shape and comprising a plastic-adherent cell population that can be directed to differentiate in vitro into cells of osteogenic, chondrogenic, adipogenic, myogenic, tenogenic or hematopoietic-supportive stromal lineages. Furthermore, it is generally accepted that MSCs are positive for CD73, CD90 and CD105, but negative for the hematopoietic markers CD11b, CD14, CD19, CD34, CD45 and HLA-DR. MSCs have been widely described to modulate T cell and B cell functions, including cell activation, proliferation and chemotaxis, by direct cell-to-cell contact or the secretion of soluble factors. Furthermore, it is worth investigating the therapeutic value of MSCs by targeting them to inflamed tissues because their immunosuppressive properties could be enhanced when they are exposed to an inflammatory environment with increased levels of local tumor necrosis factor-α and interferon-γ [3]. The most fascinating properties of MSCs are that the cells are considered immunoprivileged because of their low immunogenicity, they lack the expression of the major histocompatibility complex class II and express very low levels of major histocompatibility complex class I. However, recent studies have suggested that MSCs are not intrinsically immunoprivileged, and under certain conditions, allogeneic MSCs are lysed by activated NK cells and induce memory T cell responses, which results in the rejection of allogeneic MSC grafts [4]. In contrast, other studies have demonstrated that the constitutive expression of the serine protease inhibitor in MSCs is a major defense mechanism against their granzyme B-

![Fig. 1. Schematic diagram of age-related genes in MSCs. The diagram illustrates how age-related genes regulate gene expression and cell signaling in MSCs, leading to MSC senescence. In young MSCs, RB is hyperphosphorylated and PcG (EZH2, SUZ12 and BMI1)-induced H3K27Me3 enrichment leads to the inhibition of p16INK4A expression. The PI3K/AKT/mTOR/p70S6K pathway is activated by HMG2 expression, resulting in the inhibition of p16INK4A expression. In contrast, the inhibition of PcG expression levels and the increase in JMJD3 expression levels leads to the demethylation of H3K27 and the activation of p16INK4A expression in senescent MSCs. The decrease in ZMPSTE24 expression levels in senescent MSCs leads to the upregulation of prelamin A. The accumulation of farnesylated and methylated prelamin A in the nucleus of senescent MSCs results in DNA damage, growth defects and an abnormal nuclear envelope.](image-url)
mediated destruction by NK cells, which allows the cells to escape host immunosurveillance. The partial immunogenicity of MSCs suggests that the cells have the mechanisms that allow allogeneic MSCs to evade (in part) the host immune responses [5].

It is crucial to isolate and populate MSCs in vitro before using them for therapeutic purposes. However, MSCs undergo replicative senescence in vitro after 20–40 rounds of division that involves the ‘Hayflick phenomenon (limit)’, which is characterized by enlargement, changes in morphology, senescence-associated β-galactosidase expression and, ultimately, the arrest of proliferation. It has been reported that the replicative senescence of MSCs encompasses a progressive loss of proliferation ability and a declining differentiation potential [6]. Furthermore, replicative stress contributes to the accumulation of DNA damage during adult stem cell aging. Several lines of evidence have demonstrated that replication stress from replication fork stalling can induce telomeric DNA damage because telomerase exhibits sensitivity to fragile DNA sites that are subject to replication-induced DNA damage [7].

**Histone Deacetylase/DNA Methyltransferase**

The posttranslational modification of histones alters the expression status of individual genes with regard to their locations on chromosomes. Histone deacetylases (HDACs) are a class of enzymes that catalyze the removal of acetyl groups from the ε-amino group of lysine residues in the histone tail. HDACs act as transcription regulators by sustaining the balance between the chromatin status of acetylated and deacetylated histones. As a result of HDAC activity, the DNA status can be modulated as euchromatin, the relaxed and transcriptionally active form, or heterochromatin, the condensed and tightly packed form [8].

HDAC activities can regulate stem cell properties. In embryonic stem cells (ESCs), HDAC inhibition by an HDAC inhibitor, such as trichostatin A, induces morphological and gene expression changes promoting differentiation, with changes in activating histone H3K4 trimethylation and repressive H3K27 trimethylation [9]. However, another HDAC inhibitor, sodium butyrate (NaBu), was reported to support the extensive self-renewal of human and mouse ESCs while inducing convergence toward a common developmental intermediate. Furthermore, NaBu strongly induced several embryonic and germ-cell-associated transcripts (*Dppa5*, *Ddx43* and *Rcn3*), with a corresponding increase in H3K9 acetylation [10]. The reason that these studies described opposite effects of HDAC inhibitors on ESC stemness might be explained by the concentrations used. The application of relatively low doses of HDAC inhibitors (trichostatin A, 10 nM; NaBu, 0.2 mM) inhibits ESC differentiation and further supports ESC self-renewal, whereas a relatively high dose (trichostatin A, 50 nM) induces differentiation.

It has been proposed that histone acetylation modulates gene expression in MSCs, thereby regulating aging and behavior. MSCs undergo aging and spontaneous osteogenic differentiation with the epigenetic dysregulation of histone H3 acetylation on K9 and K14, without affecting the methylation of their promoter sites [11]. Valproic acid and NaBu flattened the morphology and inhibited the growth of human adipose tissue- and umbilical cord blood-derived MSCs. Valproic acid and NaBu further increased the transcription of p21<sup>CIP1/WAF1</sup>, with elevated histone H3 and H4 acetylation, resulting in cell cycle arrest at the G2/M phase. In agreement with these data, the induction of cellular senescence by the decreased expression of HDACs was proposed for MSCs. During the progression of MSC senescence, HDAC inhibitors downregulated such polycomb group genes (PCGs) as BMI1, EZH2 and SUZ12, and upregulated jumonji domain-containing 3 (JMJD3). The expression of EZH2 and SUZ12 is regulated by the phosphorylation status of the retinoblastoma (RB) protein following HDAC inhibitor treatment [12]. HDAC inhibitor-mediated senescence in MSCs is associated with microRNAs (miRNAs) in addition to PCGs, and HDAC inhibitors activate the transcription of a set of miRNAs (let-7a1, let-7d, let-7f1, miR-23a, miR-26a and miR-30a) by altering the histone modification patterns within the vicinity of miRNA and RNA polymerase coding regions. Activated miRNAs strongly repress the translation of high-mobility group A2 (HMGA2), which in turn regulates cellular senescence genes, including p16<sup>INK4A</sup> [13]. The age-associated roles of HMGA2 and p16<sup>INK4A</sup> in MSCs will be discussed later in this review.

DNA methylation plays an important role in biological processes, including the addition of a methyl group to the CpG dinucleotide in DNA. Gene expression can be regulated by DNA methylation through direct interference with transcription factors or methyl-CpG-binding proteins, leading to the silencing of the respective promoter regions [14]. During the replicative senescence of MSCs, highly significant changes were observed at specific CpG sites in genes involved in cell differentiation and in homeobox genes. These data demonstrated that the modification of DNA via methylation is involved...
in replicative senescence and aging in vivo in MSCs. Recently, a correlation between histone acetylation and DNA methylation has been suggested, whereby the pattern of methylation is directed by the histone acetylation state, suggesting that histone acetylation possibly determines DNA methylation [15]. Conversely, DNA methyltransferase (DNMT) 1 and 3B modulate the patterns of polycomb-mediated histone acetylation and methylation. Therefore, it is worth determining the DNMT activities in the cellular aging process of MSCs. The expression of DNMT1 and DNMT3B is significantly decreased during the replicative process of MSCs [16]. Because of the reduction in the DNMT expression level, the DNA methylation level decreased gradually during cellular senescence, reflecting global genome hypomethylation, which is a distinct feature of senescent cells. However, the DNMT3a expression increased during replicative senescence, participating in the new methylation associated with senescence. The inhibition of DNMTs by a DNMT inhibitor, 5-azacytidine, or small interfering RNAs (siRNAs) results in the upregulation of cyclin-dependent kinase (CDK) inhibitors, p16\(^{INK4A}\) and p21\(^{CIP1/WAF1}\), and the induction of cellular senescence in MSCs. DNMT inhibition regulates active/inactive histone marks at the promoter regions of the p16\(^{INK4A}\) and p21\(^{CIP1/WAF1}\) and miRNAs targeting EZH1, which is the key factor that methylates histone H3 lysine 9 and 27 [16]. Taken together, the functions of the epigenetic regulatory factors HDAC and DNMT appear to involve crosstalk with each other during the cellular senescence process.

**p16\(^{INK4A}\)**

The INK4a/ARF locus on chromosome 9p21 encodes two tumor suppressor proteins, p16\(^{INK4A}\) and p14/p19\(^{ARF}\), which are involved in growth arrest, cellular senescence and apoptosis. With regard to cell cycle checkpoints, p16\(^{INK4A}\) controls the G1-S transition by binding to CDK4/6, inhibiting its kinase activity and thereby preventing Rb phosphorylation. Conversely, Rb phosphorylation leads to increased p16\(^{INK4A}\) expression, creating a feedback loop between p16 and Rb. p16\(^{INK4A}\) expression is also associated with the cellular senescence process through a telomere-dependent or telomere-independent mechanism, and p16\(^{INK4A}\) expression gradually increases with aging in most mammalian tissues [17]. There is an inverse relationship between p16\(^{INK4A}\) and Bmi-1, a polycomb family transcriptional repressor, and reduced self-renewal in stem cells is due to p16\(^{INK4A}\) upregulation.

In MSCs, p16\(^{INK4A}\)-positive cells show growth retardation and increased activity of senescence-associated \(\beta\)-galactosidase. Furthermore, transfection of small interfering RNA targeting p16\(^{INK4A}\) in senescent MSCs results in a reduced number of senescent cells, with the cells maintaining the ability to proliferate, suggesting that p16\(^{INK4A}\) is an important regulator of MSC aging [18].

It has been reported that the epigenetic modification of p16\(^{INK4A}\) is associated with the spontaneous transformation (immortalization) of MSCs. During the senescence process, a highly proliferative MSC population that had lost its senescence-related properties emerged among senescent MSCs. Transformed MSCs exhibited Ezh2- and H3K27me-independent but H3K9me-dependent enhanced DNA methylation of the p16\(^{INK4A}\) gene [19]. With p16\(^{INK4A}\) suppression, MSCs cultured long term under hypoxic (1% pO\(_2\)) conditions have shown increased life spans compared to MSCs cultured under normoxic (20% pO\(_2\)) conditions. Under hypoxic conditions, MSCs also exhibited superior properties for differentiation into chondro- and adipogenic lineages, which are associated with inhibited activation of extracellular signal-regulated kinase. The MSCs derived from patients with systemic lupus erythematosus showed retarded proliferation, differentiation and immunosuppressive effects with upregulated p16\(^{INK4A}\) expression and downregulated CDK4, CDK6 and p-Rb expression. The knockdown of p16\(^{INK4A}\) expression reversed senescent features and impaired the properties of MSCs, suggesting that p16\(^{INK4A}\) plays an essential role in the aging process of patient-derived MSCs [20].

**High-Mobility Group A2**

HMGA2 is a nonhistone chromosomal HMGA family protein that alters chromatin structure through DNA binding. HMGA2 contains three separate DNA-binding domains that consist of 8 or 9 amino acids and show a high affinity for short AT-rich sequences [21]. Hmga2-knockout mice display a pigmy phenotype with impaired muscle development, whereas the overexpression of Hmg2a leads to gigantism and somatic overgrowth. As an architectural transcription factor, HMGA2 is involved in gene regulation. HMGA2 overexpression activates genes related to cell proliferation, such as cyclin A, cyclin F, cyclin E1 and CD25A [22]. HMGA2 has also been found to be highly expressed in various undifferentiated tissues during embryogenesis and both benign and malignant tumor samples. Although HMGA2 is not expressed in most adult tissues, it is expressed and plays important
roles in regulating stemness in ESCs and adult stem/progenitor cells, including hematopoietic stem cells and neural stem cells (NSCs).

Recently, several studies have reported that the transcriptional regulator Hmga2 is highly expressed in young and fetal NSCs, but decreases with age, suggesting that Hmga2 is dispensable for the formation of NSCs and plays roles in preventing the premature aging of NSCs. The elevated expression of let-7 miRNA, an inhibitor of Hmga2, contributes to the reduction of Hmga2 during aging. HMGA2 is able to promote fetal NSC self-renewal through its ability to repress the expression of p16INK4A and p19ARF [23]. Consistent with these data, Lee et al. [13] demonstrated that HMGA2 expression decreased during the aging process, an observation that was concomitant with the increased expression of p16INK4A, p21CIP1/WAF1 and p27KIP1 in MSCs. However, the mechanism by which Hmga2 downregulates p16INK4A and p19ARF expression has not yet been established.

It has recently been demonstrated that HMGA2 overexpression induces the phosphorylation of AKT and its downstream effectors in the mammalian target of rapamycin (mTOR)/p70S6K pathway [22]. The mTOR, which is activated by the PI3K/AKT pathway, is a serine/threonine protein kinase that regulates cell growth, cell proliferation and cell survival; however, paradoxically, the inhibition of mTOR activity is also associated with longevity in certain cells, such as epithelial stem cells [24]. In MSCs, the activation of the mTOR/p70S6K signaling pathway by HMGA2 was sufficient to increase the expression of cyclin E and CDC25A and to decrease the expression of p16INK4A, p19ARF and p21CIP1/WAF1. Furthermore, HMGA2 enhanced cell proliferation and reduced MSC aging in vitro, whereas HMGA2 inhibition compromised cell proliferation and adipogenic differentiation [22].

As mentioned above, the INK4a/ARF locus has been shown to oppose reprogramming and act as a reprogramming barrier; therefore, the role of HMGA2, with its ability to repress the INK4a/ARF locus, in the reprogramming process is of great interest for future research. Taken together, HMGA2 plays an important age-associated role in the stemness of adult stem cells.

**Lamin A/ZMPSTE24**

The nuclear lamina, a filamentous meshwork providing the regulation between the inner nuclear membrane and chromosome, is composed of the A- and B-type lamins. Two major products of A-type lamins, lamins A and C, are derived from the LMNA gene by alternative splicing. Lamins A and C are localized to the nuclear envelope and are also present in the nucleoplasm where their basic functions include DNA replication, transcriptional regulation and structural support [25]. The disturbance of lamin and lamin-interacting protein expression in *Caenorhabditis elegans*, *Drosophila melanogaster* and *Xenopus laevis* eggs is associated with aging, suggesting that nuclear lamins are closely involved in invertebrate aging. In vertebrates, the mutation of the LMNA gene is responsible for at least 12 distinct disorders, collectively known as ‘laminopathies’, which involve different tissues, including adipose, peripheral nerve, skin, muscle and bone. Hutchinson-Gilford progeria syndrome (HGPS) is a well-known laminopathy characterized by the features of premature aging. HGPS is caused by a G608M mutation within exon 11 of LMNA that leads to the truncation of prelamin A within the carboxyl-terminus. This 50-amino acid internal deletion produces progerin, which lacks the cleavage site for metalloproteinase ZMPSTE24/FACE-1, resulting in the accumulation of farnesylated, truncated prelamin A. Both progerin and prelamin A accumulate in the nuclear envelope, leading to premature senescence characterized by nuclear blebbing, heterochromatin disorganization and defects in DNA replication, transcription and repair [26].

Recently, several lines of evidence have shown that, in addition to premature aging syndrome, progerin may participate in the normal aging process. In samples from apparently healthy individuals, progerin protein accumulates with age in dermal fibroblasts and keratinocytes, both in vitro and in vivo. Extensive alternative splicing changes occur due to progressive telomere damage during the aging process and play an important role in activating progerin production [27]. However, it is unclear whether the very low expression of progerin in healthy individuals compared to HGPS patients (>160-fold lower) is significant enough to demonstrate physiological relevance.

Declines in both stem cell number and function have been observed in mouse models of accelerated aging. Similarly, human premature aging syndrome shows stem cell depletion, with the downregulation of p63, a member of the p53 family. Indeed, the hallmarks of HGPS are mesenchymal cell lineage alterations. In accordance with these studies, MSCs differentiated from HGPS patient-derived induced pluripotent stem cells exhibit high progerin levels, DNA damage and nuclear abnormalities. The introduction of progerin into MSCs interferes with cellular function by activating the major downstream ef-
factors of the Notch signaling pathway. The expression of progerin enhances osteogenic differentiation, reduces adipogenic differentiation, suggesting that progerin alters the molecular identity and differentiation potential of MSCs [28].

The prelamin A precursor (664 amino acids) undergoes a series of posttranslational modifications to become mature lamin A (646 amino acids). First, protein farnesyltransferase triggers the farnesylation of the cysteine in the carboxyl-terminus CAAX motif. Second, the last three amino acids (i.e. AAX) are cleaved from the protein, and the exposed farnesyl cysteine is methylated by Icmt methyltransferase. Third, the endoproteolytic release of the last 15 amino acids from the carboxyl-terminus is catalyzed by ZMPSTE24, a membrane zinc metalloproteinase [29]. ZMPSTE24 is indispensably required for the production of mature lamin A because it performs both the first cleavage step that releases the AAX tripeptide and the final endoproteolytic step [29]. A deficiency in ZMPSTE24 expression can cause restrictive dermopathy, with the features of severe progeroid syndrome, resulting in early neonatal death. Cells lacking ZMPSTE24 show the accumulation of a farnesylated, methylated prelamin A at the nuclear lamina that is toxic to cells through the induction of DNA double-strand breaks [26]. Although interest in prelamin A accumulation and ZMPSTE24 deficiency has increased, several fundamental issues related to the regulation of the ZMPSTE24 expression levels and activities have not been addressed. Some reports have demonstrated that the ZMPSTE24 expression level is downregulated in aged or senescent human vascular smooth muscle cells and fibroblasts [30]; however, the signaling pathway modulating the ZMPSTE24 expression level has not been clarified. Our recent unpublished data suggest that ZMPSTE24 expression is severely affected by replicative or HDAC inhibitor-mediated senescence in MSCs. Briefly, ZMPSTE24 downregulation by vitro aging in MSCs induces the upregulation of prelamin A, which leads to the induction of abnormal nuclear morphology and DNA damage. These results, together with the lamin A-dependent nuclear abnormalities that induce DNA damage, telomere shortening and genomic instability, suggest that the prelamin A maturation process has significant biological relevance in MSC aging.

Conclusion

Although the differentiation potential of MSCs is relatively restricted compared to such pluripotent stem cells as ESCs and induced pluripotent stem cells, MSCs are a much safer source for cell therapy with regard to the risk of transplanted stem cells forming tumors and becoming cancerous. However, unlike pluripotent stem cells, as MSCs age, their properties gradually become compromised, including the properties of multilineage differentiation, homing, immune modulation and wound healing. The proper use of MSCs for clinical applications requires a general understanding of the MSC aging process. For the use of MSCs in therapy, methods that allow the generation of large populations of MSCs without affecting their properties of differentiation or immunomodulation need to be established. The information described in this review might suggest a possible method to improve the therapeutic efficacy by regulating specific factors or the microenvironment associated with the MSC aging process.

For a full list of references, please see the online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000353857).

References


