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[Mini Review]

# Impact of mesenchymal stem cell senescence on inflammaging

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**Abstract**

Life expectancy has dramatically increased around the world over the last few decades, and staying healthier longer, without chronic disease, has become an important issue. Although understanding aging is a grand challenge, our understanding of the mechanisms underlying the degeneration of cell and tissue functions with age and its contribution to chronic disease has greatly advanced during the past decade. As our immune system alters with aging, abnormal activation of immune cells leads to imbalance of innate and adaptive immunity and develops a persistent and mild systemic inflammation, inflammaging. With their unique therapeutic properties, such as immunomodulation and tissue regeneration, mesenchymal stem cells (MSCs) have been considered to be a promising source for treating autoimmune disease or as anti-aging therapy. Although direct evidence of the role of MSCs in inflammaging has not been thoroughly studied, features reported in senescent MSCs or the aging process of MSCs are associated with inflammaging; MSC niche-driven skewing of hematopoiesis toward the myeloid lineage or oncogenesis, production of pro-inflammatory cytokines, and weakening their modulative property on macrophage polarization, which plays a central role on inflammaging development. This review explores the role of senescent MSCs as an important regulator for onset and progression of inflammaging and as an effective target for anti-aging strategies.

**Introduction**

Life expectancy has steadily increased for nearly 200 years, mainly because of the reduction in early and mid-life mortality. Furthermore, the decline in late-life mortality in recent years has resulted in the continuing increase in life expectancy. In fact, worldwide life expectancy will increase with a probability of at least 65% for women and 85% for men by 2030, it will reach over 90 years particularly in Korea, with a 57% probability (for women)

(1). As society gets aged, social burdens and costs for supporting individual longevity, such as health and medical care, are increased. To deal with public-health expenditure and social requirements more efficiently and economically, comprehensive and in-depth understandings of the aging process and physiology in the old are strongly needed.

As the immune system possibly records all the immunological experiences and stimuli it was exposed to, our immune system shows prominent changes during the aging process (2). An 'aging immune system' is often termed 'Immunosenescence', and it refers to both innate and adaptive immune changes. Immunosenescence is considered to have a clinical significance, because it might be the origin of diseases of the elderly, such as infections, cancer, autoimmune disorders, and chronic inflammatory diseases (3). Aging has been associated with changes in the hematopoietic system, including diminished long-term repopulation and lineage-biased hematopoiesis of immune cells (4, 5), leading to a subsequent impaired immune defense against various infections (3) and spontaneous proinflammatory activation (6). Several studies have reported that an age-associated chronic proinflammatory state would be responsible for detrimental degenerative diseases, such as rheumatoid arthritis (7), atherosclerosis (8), and neurodegenerative disease (9). Franceschi and colleagues termed the persistence of low-grade chronic inflammatory status as 'inflammaging' and have intensively investigated this pleiotropic phenomenon since 2000 (10). For two decades, the study of inflammaging has been widely developed by the efforts of researchers in the various fields, including microbiology and endocrinology, and has emerged as an important concept to provide a dynamic reassessment of immune changes with aging (11, 12).

Among the emerging cell therapy or anti-aging remedies, mesenchymal stem/stromal cell (MSC) therapy has attracted attention because of the cells' unique properties, stemness, and immunomodulatory ability. MSCs are considered as a 'safer source' for cell therapy with

minimal risk of transplanted stem cells forming tumors and becoming cancerous, however, it has a limited self-renewal ability similar to other adult stem cells. The aging of MSCs leads to an age-associated decline in their number and functions including multilineage differentiation, homing, immune modulation and wound healing (13). To accomplish a successful anti-aging therapy, it is important to figure out whether the therapeutic efficacy of MSCs could be affected by dysfunctions associated with the aging process. Our group and others showed relatively impaired therapeutic ability of aged MSCs in animal models such as colitis or chronic wounds (14, 15). However, present studies about aging and stem cell therapy have focused on *in vitro* stem cell aging itself, so called 'replicative senescence' (16). It has been highlighted for recent years that aging of MSC niche causes spontaneously inflammatory responses and interferes the effect of MSC therapy (17). Furthermore, although studies of inflammaging mainly target the prediction for disease susceptibility and successful anti-aging therapy, and uncovering the secret of the aging process and its related dysfunction, less is known about how MSCs are associated with the features of inflammaging. In this review, we investigated how aging of MSCs residing in the body and related microenvironment could exacerbate inflammaging and hamper the therapeutic potentials of emerging therapies including allo- and auto-transplantation of MSCs. Therefore, we sought to summarize previous results and propose possible hypotheses about the effect of MSCs on inflammaging.

**Inflammaging:**

Inflammaging refers to a persistent low-grade systemic pro-inflammatory status that appears in the normal aging process of mammals. Inflammation is a defense mechanism against life-threatening invasion by harmful agents and maintains homeostasis in child- and adulthood, but chronic inflammation can have a deleterious effect on the body and might be a

significant risk factor increasing morbidity and mortality for most if not all degenerative diseases and geriatric syndromes in elderly people (18).

The etiology of inflammaging would be divided into endo- and exo-genous reasons. An important feature of inflammaging is an accumulation of damaged macromolecules and cellular debris because of its increased production and chronically inhibited damage surveillance and repair functions in multiple tissues (19). Aging on the cellular level may be responsible for inflammaging. Secretion of proinflammatory cytokines from senescent cells accumulated in tissues with age, the so-called 'senescence-associated secretory phenotype' (SASP), contributes to the onset of inflammaging (20). Age-associated changes in the immune system (Immunosenescence), which refers to impaired adaptive immunity and compensatory activation of the innate immune system, are also included in the sources of inflammaging (21). In addition to these microbial products leaking into surrounding tissues, dysregulated activation of the coagulation system and inadequate regulation of the complement pathway would be another cause for inflammaging (19). Among exogenous reasons, viral and bacterial infections and the subsequent exposure to nucleic acids of the microorganisms, which facilitate activation of innate immune receptors such as TLR and NLR as PAMPs were categorized as exogenous reasons (22). In addition to this, infection-mediated pro-inflammatory cytokines (23) and disorder in phytochemical consumption (24, 25) also deteriorates modulation of oxidative stress and inflammatory signaling.

Inflammaging is a situation in which immunity exerts antagonistic pleiotropy programmed during evolution, and it has several distinctive features. The most prominent feature is chronic activation of innate immunity via dysregulated stimulation of pattern-recognition receptors by non-degraded waste in the body (Damage-Associated Molecular Pattern; DAMP), in which macrophages play a pivotal role. Innate immune cells subsequently secrete a robust amount of pro-inflammatory cytokines/chemokines, including

interleukin (IL)-6 (26). Accordingly, cell-surface receptors are stimulated by secreted pro-inflammatory cytokines, and intracellular signaling cascades are initiated to activate transcription factors, including NF-κB (nuclear factor kappa-light-chain enhancer of activated B cells) and STAT (signal transducer and activator of transcription) (27, 28). Furthermore, NF-κB-mediated activation of NLRP3 inflammasomes, with the release of pro-inflammatory cytokines such as IL-1β and IL-18, facilitates the activation of inflammaging (29). To avoid an inadequate accumulation of bodily waste, rescue machinery termed 'autophagy/mitophagy' exists in vertebrates, and the function becomes defective with the condition of inflammaging (30). Dysregulation of the ubiquitin-proteasome system, activation of the DNA damage response, and dysbiosis are also included in the category of features of inflammaging. In the following part of this review, we will discuss a possible role of aged MSCs residing in the body and exogenously introduced in the development or progression of inflammaging (Figure 1).

**How do senescent MSCs exacerbate inflammaging?**

***A. Senescent MSCs: Another source for DAMP***

Both innate and adaptive immune systems have continuously evolved to protect our bodies from harmful agents. Those life-threatening stressors can be divided into two different categories by their origins; external and internal. Microbes, including bacteria, viruses, fungi, and parasites, and even ingredients we've taken for energy, belong to external stressors. On the other hand, all types of materials created by tissues and cells during cell turnover and metabolism in living organisms, including cellular debris, metabolites, products of incomplete degradation or non-enzymatic processing, termed 'molecular garbage', are internal factors (2, 18). To deal with molecular garbage and maintain homeostasis in the body, several adaptive strategies, such as the recognition of PAMP (Pathogen-Associated

Molecular Pattern) or MAMP (Microbial-Associated Molecular Pattern), which can directly activate pattern-recognition receptors (PRRs) and downstream inflammatory cascades, have been developed. As individuals age, the physiological machinery becomes decrepit and functionally impaired; elderly people subsequently become vulnerable to infections like cytomegalovirus (CMV) or human immunodeficiency virus (HIV) (23, 31). In contrast, an age-mediated increase in the production of DAMPs or alarmins can reportedly fuel the progression of inflammaging.

MSC treatment has emerged as an anti-aging therapy because of these cells' unique immunomodulatory function and tissue regenerative capacity (32, 33). Especially, paracrine secretion of MSCs plays a crucial role in clinical feasibility, including anti-aging therapy, and with these secretory factors, the conditioned medium (CM) of MSCs can be administered to various diseases. MSC CM contains not only anti-inflammatory cytokines but also encapsulated vesicles of nucleic acids and proteins, called 'exosomes', which exert a therapeutic effect on various diseases, including cancer (34) and myocardial infarction (35). Furthermore, recent studies have demonstrated that debris from MSCs is one of the effectors to activate immunomodulation through the activation of an alternative type of macrophages (36). Nonetheless, various abilities of MSCs gradually diminish during cellular senescence. As MSCs become aged, the cells become enlarged and heterogeneous in shape, granules and cell inclusions are accumulated in the cytoplasm, and debris is formed in the culture medium (37). Moreover, aging exerts telomere shortening, impaired functions in DNA machineries, such as methylation or histone acetylation, and an increase in levels of ROS and nitric oxide (NO) (38). As described above, antigenic load, such as materials related to DAMP, is a major contributor to inflammaging. MSCs residing all over in our body presumably spout out much cell debris similar to that from other usual aged cells, and products derived from MSCs activate innate immune response through the activation of innate immune-cell receptors, such

as Toll-like receptors (TLRs), NOD-like receptors (NLRs), and receptors of advanced glycation end-products (RAGE) (39-41). Accordingly, aged MSCs would exacerbate the initiation and progression of inflammaging by producing lots of cellular wastes throughout the body.

MSCs are known to generate higher amounts of extracellular vesicles (EVs), including microvesicles (MVs; 0.1–1 mm in diameter) and exosomes (40–100 nm in diameter), which are important mediators of intercellular communications, than do other cell types (42). MVs derived from cell surfaces reflect the properties of their parental cells and take part in cellular physiologies with neighboring cells and their own parental cells by diffusing around the adjacent microenvironment. MVs shed from MSCs express markers CD29, CD44, CD73, CD90 and CD105, as expressed on the MSC cell surface (43) and contribute to tissue regeneration, immunomodulation, and hematopoietic support, which are known to be unique biological functions of MSCs. Importantly, as shown in MSC aging, MVs shed from senescent MSCs undergo several phenotypical changes, such as that the number of secreted MVs from aged MSCs considerably increased while the average size decreased (44). Furthermore, the release of MVs or exosomes increased in senescent human fibroblasts, tumor cells (45) and endothelical cells (46). Cellular stress such as telomere attrition or DNA damage induced by aging or disease may induce a p53-dependent increase in the biogenesis of MVs (45). Increase of MVs in senescent endothelial cells appears to be mediated Rho kinase activity as treatment with fasudil (Rho kinase inhibitor) blocked MV formation (46). Similar to the cellular products as described above, secretion of MVs in aged MSCs seems to facilitate more frequent stimulation of innate immune-cell receptors. According to the paper using multi-omics analyses, several molecules mediated DAMP reaction such as S100A6, S100A11, and HSPs were significantly increased in the aged MSCs (47). Moreover, miRNAs can be crucial factors mediated DAMP response (48). Expression of miR-146a (49), known

to have an important role for induction of age-associated pro-inflammatory response, significantly increases in senescent MVs. Last, the treatment with old MVs to normal MSCs causes a decrease in osteogenic genes, including ALP, RUNX2, and OCN, and pro-osteogenesis abilities, and consequently leads to impoverished bone formation in bone marrow (BM) niche (44). The situation may be akin to HSCs from old individuals, in which a high number of HSCs is found in aged human or mouse than young counterpart, but showed impaired functionality (50, 51).

### *B. Aged MSCs secrete a robust amount of pro-inflammatory cytokines*

Inflammation is dedicated to protecting our bodies from various infections for a lifetime, but it may become detrimental in the late period of life. It recruits large quantities of pro-inflammatory cytokines in the inflammaging process (52). Senescent cells generally present a distinctive property to secrete enormous amounts of cytokines and chemokines called SASP, which corrupts indispensable cellular processes, such as proliferation, migration, differentiation, and tissue remodeling, and consequently triggers the onset of fatal degenerative diseases, including cancers (53). In a similar context, senescent MSCs excessively release secretome, including IL-6, IL-8, interferon-gamma (IFN-γ), monocyte chemoattractant protein (MCP)-1, and matrix metalloproteinases (MMP2, TIMP2). A systemic inflammatory response caused by these secretory molecules reduces the immunomodulatory function of MSCs and promotes cancer progression (54, 55). As we have already discussed, secreted subcellular organelles, such as exosomes or MVs, play a crucial role in secretion of SASP in MSCs (43). During aging, MSCs show a persistent increase in the activation of TLR signaling, which mediates excessive production of pro-inflammatory cytokines (56). Furthermore, an age-dependent increase of adipogenesis might be a possible contributor for SASP through activations of peroxisome proliferator-activated receptor

gamma 2 and CCAAT/enhancer binding protein, and adipose tissue acts as a reservoir for cytokines (57, 58).

Among cytokines, a pleiotropic cytokine, IL-6, has been at the center of the stage of inflammaging research field from the very beginning. Although the IL-6 level in the plasma of young individuals remains low or undetectable, it prominently increases as individuals age. Even in the serum of healthy centenarians, IL-6 interestingly shows a higher level than in young people. Augmented IL-6 level in the serum of the elderly is associated with an impediment of physical ability, cognitive dysfunction, onset of cancers, and disease progression of general degenerative disorders (52). A growing body of evidence has proven that MSCs aggressively secrete IL-6 with advancing age, which is the result of accumulated genetic damage and activation by the other cytokines, such as TNF-α and IFN-γ (59). CM containing a high level of IL-6 has been reported to promote proliferation and migration of cancer cells (60). More importantly, upregulated IL-6 secretion could disrupt homeostasis of HSCs and HPCs (61). Moreover, pro-inflammatory cytokines accelerate the secretions of other cytokines by MSCs. It is well known that priming with cytokines, representatively IFN-γ and TNF-α, improves the production of nitric oxide synthase (NOS) or $PGE_2$ in MSCs (62). Furthermore, G-CSF downregulates the secretion of CXCL12 from BM MSCs and releases HSCs to populate (63).

### *C. Aged MSCs facilitate a shift in macrophage polarization*

Inflammaging is a macrophage-centered phenomenon (18). The cells residing in the tissue and circulation are responsible for chronic inflammation in the elderly. Moreover, SASP factors modulate M1/M2 fate decision by shifting M2 cells toward M1 (64). Conversely, MSCs can exert beneficial effects on the macrophage polarization from M1 to M2, so that the cells could be employed to treat various immune-related disorders (65). Given

that senescent MSCs augments myeloid cell generation and innate immune activation, aging of MSCs also affects macrophage polarization (66). Interestingly, macrophages co-cultured with young MSCs expressed M2 markers, Arg1 and IL-10, whereas the cells with aged MSCs increased M1-related TNF-α. In addition to this, macrophages co-cultured with aged MSCs increased the migratory ability, which is a typical property of classically activated M1 macrophages (67). As described above, aged MSCs altered their features to produce essential inducers for M1 differentiation, including IFN-γ, IL-1 and DAMP, therefore activating the NF-κB signal (54). Consequently, MSCs in the aged microenvironment lost their unique competence of M1 suppression.

### *D. Inflammaging of BM niche accelerates skewed blood-cell differentiation*

Hematopoiesis is an essential and life-long process, which unceasingly produces and eliminates mature blood cells for the constitution of circulating and residing cells in the body throughout an individual's lifetime. Blood cells, including red blood cells (RBCs), platelets, myeloid cells, and lymphoid cells, arise from a unique and small cellular population termed hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) in the BM. Cell fate decisions, such as self-renewal, differentiation, and cell death, are securely regulated by both cell-intrinsic and extrinsic factors (68). Interestingly, recent studies have revealed, using single-cell transplantation and lineage-tracing technology, that hematopoiesis of defined HSCs and HPCs proceeded heterogeneously, which indicates that cells have subtypes and preferences to differentiate into a particular type of blood cell ; lineage priming (69, 70).

Functionally impaired hematopoiesis is a hallmark of the aging process, as is a remarkably decreased self-renewal capacity of HSCs (71). Of note, hematopoietic changes with advancing age are intimately related to inflammaging, which includes depression in both the adaptive and the innate immune system, vulnerability to infections, spontaneous

development of autoimmune diseases, and hematopoietic malignancies (72). In addition, the pattern of hematopoiesis and subsequent composition of mature blood cells in systemic circulation are considerably affected by age-associated changes. Most of all, mounting evidence has supported that myeloid cells are increased in number, but their actual functionality is impeded (5). For example, phagocytic activity of macrophages reduces even in the presence of stimuli, and a dysregulated skewing of a pro-inflammatory (classical) type of macrophage is frequently detected (73, 74). In a similar context, the transplantation of aged HSCs showed biased clonal expansion toward myeloid lineage, indicating an age-associated autonomous HSC differentiation pattern (75). The number of naïve B cells is decreased and the diversity of the B cell repertoire is also reduced, accompanying impeded antibody affinity and even autoantibody production (76). In addition, the ratio of CD4 and 8 T cells is gradually increased with aging (77).

The HSC niche, including MSCs and endothelial cells (ECs), plays a crucial role in homeostasis of HSCs by maintaining the microenvironment in the BM (78). As the aging process progresses, MSCs cannot maintain their functional and regenerative capacities and sometimes promote inflammation and cancer progression. Recent studies uncovered that the ineffective hematopoiesis caused by aberrant crosstalk between HSCs and MSCs caused by the primary alteration of MSCs and it might lead to myelodysplastic syndrome (MDS) (79), which supports niche-driven oncogenesis in the hematopoietic system. It is obvious that cellular senescence helps MSCs to differentiate eccentrically toward adipocytes (80, 81). It is suggested that biased adipogenesis in aged BM aggravates hematopoietic reconstitution, extracellular matrix composition, bone formation, and subsequent fracture repair (82, 83). As differentiated adipocytes reportedly interrupt lymphopoiesis (84), age-associated adipogenesis might exert an increased propensity to differentiate toward the myeloid rather than the lymphoid lineage, which could be another reason for hematopoietic malignancies

including MDS. This hypothesis could be further supported by the results from previous studies that young HSCs transplanted toward aged BM showed myeloid-biased long-term repopulation (85).

### *E. Age-associated mutations impede MSC niche function*

Skewed differentiation of HSCs toward the myeloid lineage in elderly individuals coincides with several recurrent somatic mutations, especially *TET2* (Ten-Eleven Translocation 2) and it is associated alteration in DNA methylation (86). Notably, *TET2* mutation is frequently detected in patients with myeloid leukemia, myelomonocytic leukemia (CMML) (~50 %), myeloid proliferative neoplasm (MPN) (~13 %), MDS (~25 %), and acute myeloid leukemia (AML) (~23 %) (87). It is reported that 2-hydroxyglutarate (2-HG), an oncometabolite produced by mutated isocitrate dehydrogenase (IDH) 1 and 2, catalytically inactivates the *TET2* enzyme, and the mutation is also found in AML (~20 %), gliomas (60~80 %), and cholangiocarcinomas (7~28 %) (88). With advancing aging, a general decrease in $O_2$ supply and impaired $O_2$ diffusion to target tissues, caused by age-associated poor vascularization, leads to tissue hypoxia (89). Interestingly, a hypoxic environment helps mammalian cells increase the production of oncometabolite, 2-HG (88, 90). Although hypoxic preconditioning has frequently been employed as a strategy for improving the immunomodulation or angiogenesis ability of MSCs (32), a hypoxic environment conversely leads to decline in osteogenic differentiation, related to functional impairment in hematopoiesis (91). Oncometabolite 2-HG-induced mutation of *IDH1* and *2* impairs osteoblast differentiation, presumably suggesting a mechanism underlying hypoxia-mediated reduced osteogenic differentiation of MSCs (90). Moreover, systemic chronic hypoxia facilitates premature senescence of MSCs in BM by altering the diversity of gut microbial communities, particularly a decrease in *Lactobacilli* colonies and subsequent accumulation of

D-galactose, a well-known agent for senescence induction (92).

Somatic genetic mutations in the gene *additional sex comb-like 1* (*ASXL1*) are the other risk factors promoting dysregulated clonal myelopoiesis, which is frequently mutated in patients with MDS (15~25 %) and AML (10~15 %) (93). ASXL1 proteins control transcriptional repression by recruiting polycomb repressive complex 2 (PRC2) to promoters, with a subsequent increase in H3K27 methylation for gene inactivation (94). In fact, *Asxl1* mutant mice showed spontaneous development of peripheral leukopenia and dysplastic myeloid cells in BM, and *ex vivo* analyses revealed various pathogenic features of MDS, including aberrant myeloid differentiation with a significant increase of granulocytic and monocytic cell fraction, and considerable growth arrest of HSC pool mediated by apoptosis and cell-cycle arrest. In addition to hematopoiesis, the mice showed impaired development in body size and weight (95). It is well defined that deletion of the epigenetic modulator gene *ASXL1* in myeloid cells results in upregulated osteoclastogenesis in association with reduction of H3K27me3 through inhibition, suggesting diminished bone mass and subsequently impaired BM niche function (94). Moreover, mutations in *ASXL1* expression in the BM-MSCs could exert reduced hematopoietic supportive ability and biased myelopoiesis, which is proved by using genetically modified animals (96). Given the possible role of frequent age-related mutations in BM niche function, it may be that MSC aging facilitates inflammaging through exacerbated hematopoietic supportive function and biased hematopoiesis in the elderly, which could lead to the pathogenesis of myeloid malignancies.

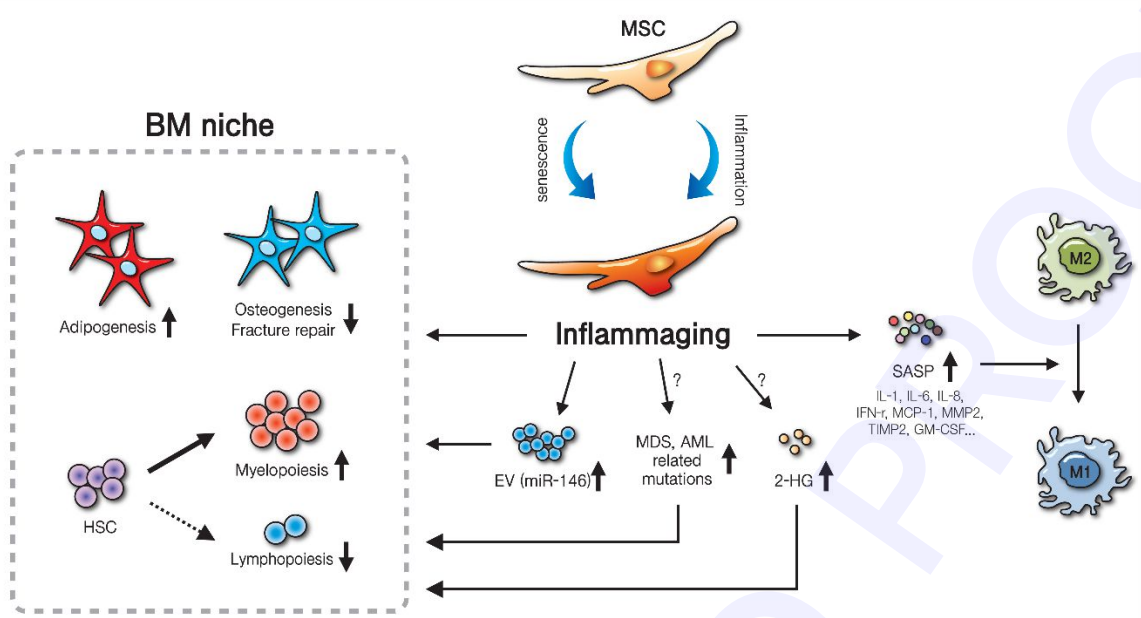


**Figure 1. MSCs as a key modulator of inflammaging.** The diagram illustrates how senescence and inflammation regulate MSC fate and lead to inflammaging. Cellular senescence facilitates MSCs to differentiate toward adipogenesis, and biased adipogenesis in the BM niche skews hematopoietic reconstitution, inhibits lymphopoiesis but increased myelopoiesis. Aged MSCs show increased secretion of extracellular vesicles (miR-146a) and SASP that stimulates innate immune cell receptors or macrophage polarization from M2 to M1. Age-related alterations may contribute to increased MDS or AML-related mutations or 2-HG production that possibly further exacerbated hematopoietic niche function or the pathogenesis of myeloid malignancies.

BM, bone marrow; EV, extracellular vesicle; MDS, myelodysplastic syndrome; AML, Acute myeloid leukemia; 2-HG, 2-hydroxyglutarate; SASP, Senescence-associated secretory phenotype

**Concluding Remarks and Future Perspectives**

Finding the determinants of the aging process that help us understand age-related diseases has long been a quest by gerontologists and practitioners in the geriatric field for decades. With emerging interests in microbiology and immunology, researchers have tried to connect and expand their knowledge to find the clues to the secrets of aging and its related dysfunction. Recently, a new paradigm of aging-related concepts termed 'immunosenescence' and 'inflammaging' emerged, which explains the aging phenomena in terms of immunology (6, 10, 11). Aging-dependent alterations of immune cells and the BM microenvironment lead to subclinical inflammatory states, and subsequent chronically sustained inflammatory stimuli contribute to the functional decline of the immune system and even increased risks for leukemogenesis, supporting the inflammaging concept (68). As a BM niche factor, MSCs play a key role in modulating the immune system in the body; however, their role in the aging-immune-inflammation axis remains to be elucidated. Thus, herein, we discussed the previous reports related to the senescent MSCs and their behaviors, and propose their potential role in the progression of inflammaging.

One crucial factor for induction and progression of inflammaging is sustained stimulation of DAMP originating from senescent MSCs as their secretion of damaged macromolecules and self-debris increased with age and could lead to a subsequent spontaneous innate immune response. Age-biased bone marrow adipogenic differentiation, causes not only weakened BM niche function and bone homeostasis but also dysregulates hematopoiesis, resulting in biased myelopoiesis. Inflammaging of MSCs might be involved in genomic instability of the hematopoietic system by accumulation of recurrent *ASXL1* mutations in a niche microenvironment or through mutacinogenic enzyme production. Probably the most prominent contribution of MSCs to inflammaging is mass production of pro-inflammatory cytokines during the aging process, which then further promote M1

polarization of macrophages.

Despite present achievements in investigating the effect of MSCs on inflammaging progression, there is still plenty of unexplored territory remaining to be elucidated, which includes other mechanisms that might contribute to inflammaging progression. For example, MSCs can suppress the inflammasome, which is important to their therapeutic potential against immune-related disorders (65, 97), but the effect of senescence on the regulation of inflammasomes is less investigated. Studies on centenarians show very different inflammaging phenotypes, such as pro-inflammatory cytokine levels in plasma, even in the individuals without chronic pathologies (18); so future study should be conducted to define features of individuals and cases (such as threshold on the stress or genotoxins), or how the inflammaging-related cytokines act in specific tissue-residing MSCs which could regulate the onset of specific chronic disease.

It also could be envisioned how inflammaging contributes to changing the therapeutic efficacy of transplanted MSCs. Although some studies have reported that *in vitro* senescent MSCs present reduced therapeutic potentials, including immunomodulation and engraftment (98), their specific mode of action in the elderly and influences by inflammaging are less investigated, regardless of whether the transplanted cells are early- or late-passaged MSCs. Inflammation is essential for survival and has beneficial effects on harmful signal neutralization in young individuals; however, inflammation in the elderly is detrimental, and this environment possibly stimulates transplanted MSCs for further hyperactive production of SASP, followed by inhibition of regenerative or immunomodulatory ability (18). Therefore, we also need to consider the antagonistic pleiotropy of pro-inflammatory cytokines on the therapeutic mechanism of transplanted MSCs. Last, most of the inflammaging studies have focused on which stimuli, such as molecules and cytokines, are important for induction and progression, but, to define the relation between inflammaging and MSCs, a systemic

approach to understanding the interaction of MSCs with each niche- or tissue-specific signaling cascade of receptors is needed. Our effort to understand the complex interplay between MSCs and the aged environment and to integrate MSC therapy in the concept of inflammaging will contribute to improving preventive and personalized medicine for elderly individuals.

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## References


1. Kontis V, Bennett JE, Mathers CD, Li G, Foreman K and Ezzati M (2017) Future life expectancy in 35 industrialised countries: projections with a Bayesian model ensemble. Lancet 389, 1323-1335
2. Franceschi C, Salvioli S, Garagnani P, de Eguileor M, Monti D and Capri M (2017) Immunobiography and the Heterogeneity of Immune Responses in the Elderly: A Focus on Inflammaging and Trained Immunity. Front Immunol 8, 982
3. Fulop T, Larbi A, Dupuis G et al (2017) Immunosenescence and Inflamm-Aging As Two Sides of the Same Coin: Friends or Foes? Front Immunol 8, 1960
4. Yu K-R, Espinoza DA, Wu C et al (2018) The impact of aging on primate hematopoiesis as interrogated by clonal tracking. Blood 131, 1195-1205
5. Pang WW, Price EA, Sahoo D et al (2011) Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proc Natl Acad Sci U S A 108, 20012-20017
6. Derhovanessian E, Maier AB, Beck R et al (2010) Hallmark features of immunosenescence are absent in familial longevity. J Immunol 185, 4618-4624
7. Smolen JS, Aletaha D and McInnes IB (2016) Rheumatoid arthritis. Lancet 388, 2023-2038
8. Libby P, Ridker PM and Maseri A (2002) Inflammation and atherosclerosis. Circulation 105, 1135-1143
9. Heppner FL, Ransohoff RM and Becher B (2015) Immune attack: the role of inflammation in Alzheimer disease. Nat Rev Neurosci 16, 358-372
10. Franceschi C, Bonafe M, Valensin S et al (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908, 244-254
11. Fransen F, van Beek AA, Borghuis T et al (2017) Aged Gut Microbiota Contributes to Systemical Inflammaging after Transfer to Germ-Free Mice. Front Immunol 8, 1385
12. Calcada D, Vianello D, Giampieri E et al (2014) The role of low-grade inflammation and metabolic flexibility in aging and nutritional modulation thereof: a systems biology approach. Mech Ageing Dev 136-137, 138-147
13. Yu KR and Kang KS (2013) Aging-related genes in mesenchymal stem cells: a mini-review. Gerontology 59, 557-563
14. Yu KR, Lee JY, Kim HS et al (2014) A p38 MAPK-mediated alteration of COX-2/PGE2 regulates immunomodulatory properties in human mesenchymal stem cell aging. PLoS One 9, e102426
15. Badiavas AR and Badiavas EV (2011) Potential benefits of allogeneic bone marrow mesenchymal stem cells for wound healing. Expert Opin Biol Ther 11, 1447-1454
16. Wagner W, Horn P, Castoldi M et al (2008) Replicative senescence of mesenchymal stem cells: a continuous and organized process. PloS one 3, e2213
17. Yang YM, Li P, Cui DC et al (2015) Effect of aged bone marrow microenvironment on mesenchymal stem cell migration. Age (Dordr) 37, 16

18. Franceschi C, Garagnani P, Vitale G, Capri M and Salvioli S (2017) Inflammaging and 'Garb-aging'. Trends Endocrinol Metab 28, 199-212
19. Franceschi C and Campisi J (2014) Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 69 Suppl 1, S4-9
20. Robbins PD (2017) Extracellular vesicles and aging. Stem Cell Investig 4, 98
21. Agarwal S and Busse PJ (2010) Innate and adaptive immunosenescence. Ann Allergy Asthma Immunol 104, 183-190; quiz 190-182, 210
22. Franceschi C, Garagnani P, Parini P, Giuliani C and Santoro A (2018) Inflammaging: a new immune–metabolic viewpoint for age-related diseases. Nature Reviews Endocrinology 14, 576
23. Frasca D and Blomberg BB (2016) Inflammaging decreases adaptive and innate immune responses in mice and humans. Biogerontology 17, 7-19
24. Martucci M, Ostan R, Biondi F et al (2017) Mediterranean diet and inflammaging within the hormesis paradigm. Nutr Rev 75, 442-455
25. Szarc vel Szic K, Declerck K, Vidakovic M and Vanden Berghe W (2015) From inflammaging to healthy aging by dietary lifestyle choices: is epigenetics the key to personalized nutrition? Clin Epigenetics 7, 33
26. Franceschi C, Garagnani P, Parini P, Giuliani C and Santoro A (2018) Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 14, 576-590
27. Salminen A, Huuskonen J, Ojala J, Kauppinen A, Kaarniranta K and Suuronen T (2008) Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging. Ageing Res Rev 7, 83-105
28. Chazaud B and Mouchiroud G (2014) Inflamm-aging: STAT3 signaling pushes muscle stem cells off balance. Cell Stem Cell 15, 401-402
29. Latz E and Duewell P (2018) NLRP3 inflammasome activation in inflammaging. Semin Immunol 40, 61-73
30. Salminen A, Kaarniranta K and Kauppinen A (2012) Inflammaging: disturbed interplay between autophagy and inflammasomes. Aging (Albany NY) 4, 166-175
31. Steele AK, Lee EJ, Vestal B et al (2014) Contribution of intestinal barrier damage, microbial translocation and HIV-1 infection status to an inflammaging signature. PLoS One 9, e97171
32. Kang I, Lee BC, Choi SW et al (2018) Donor-dependent variation of human umbilical cord blood mesenchymal stem cells in response to hypoxic preconditioning and amelioration of limb ischemia. Exp Mol Med 50, 35
33. Lee BC, Kim JJ, Lee JY et al (2019) Disease-specific primed human adult stem cells effectively ameliorate experimental atopic dermatitis in mice. Theranostics 9, 3608-3621
34. Katakowski M, Buller B, Zheng X et al (2013) Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. Cancer Lett 335, 201-204
35. Lai RC, Arslan F, Lee MM et al (2010) Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res 4, 214-222

36. de Witte SFH, Luk F, Sierra Parraga JM et al (2018) Immunomodulation By Therapeutic Mesenchymal Stromal Cells (MSC) Is Triggered Through Phagocytosis of MSC By Monocytic Cells. Stem Cells 36, 602-615
37. Bonab MM, Alimoghaddam K, Talebian F, Ghaffari SH, Ghavamzadeh A and Nikbin B (2006) Aging of mesenchymal stem cell in vitro. BMC Cell Biol 7, 14
38. Li Y, Wu Q, Wang Y, Li L, Bu H and Bao J (2017) Senescence of mesenchymal stem cells (Review). Int J Mol Med 39, 775-782
39. Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF and Schmidt AM (2005) Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology 15, 16R-28R
40. Pisetsky DS, Erlandsson-Harris H and Andersson U (2008) High-mobility group box protein 1 (HMGB1): an alarmin mediating the pathogenesis of rheumatic disease. Arthritis Res Ther 10, 209
41. Salminen A, Ojala J, Kaarniranta K and Kauppinen A (2012) Mitochondrial dysfunction and oxidative stress activate inflammasomes: impact on the aging process and age-related diseases. Cell Mol Life Sci 69, 2999-3013
42. Yeo RW, Lai RC, Zhang B et al (2013) Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. Adv Drug Deliv Rev 65, 336-341
43. Mokarizadeh A, Delirezh N, Morshedi A, Mosayebi G, Farshid AA and Mardani K (2012) Microvesicles derived from mesenchymal stem cells: potent organelles for induction of tolerogenic signaling. Immunol Lett 147, 47-54
44. Lei Q, Liu T, Gao F et al (2017) Microvesicles as Potential Biomarkers for the Identification of Senescence in Human Mesenchymal Stem Cells. Theranostics 7, 2673-2689
45. Lehmann BD, Paine MS, Brooks AM et al (2008) Senescence-associated exosome release from human prostate cancer cells. Cancer Res 68, 7864-7871
46. Burger D, Kwart DG, Montezano AC et al (2012) Microparticles induce cell cycle arrest through redox-sensitive processes in endothelial cells: implications in vascular senescence. J Am Heart Assoc 1, e001842
47. Peffers MJ, Collins J, Fang Y et al (2016) Age-related changes in mesenchymal stem cells identified using a multi-omics approach. Eur Cell Mater 31, 136-159
48. Fleshner M and Crane CR (2017) Exosomes, DAMPs and miRNA: Features of Stress Physiology and Immune Homeostasis. Trends Immunol 38, 768-776
49. Balasubramanyam M, Aravind S, Gokulakrishnan K et al (2011) Impaired miR-146a expression links subclinical inflammation and insulin resistance in Type 2 diabetes. Mol Cell Biochem 351, 197-205
50. de Haan G and Lazare SS (2018) Aging of hematopoietic stem cells. Blood 131, 479-487
51. Pang WW, Schrier SL and Weissman IL (2017) Age-associated changes in human hematopoietic stem cells. Semin Hematol 54, 39-42
52. Minciullo PL, Catalano A, Mandraffino G et al (2016) Inflammaging and Anti-Inflammaging:

The Role of Cytokines in Extreme Longevity. Arch Immunol Ther Exp (Warsz) 64, 111-126

53. Coppe JP, Desprez PY, Krtolica A and Campisi J (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol 5, 99-118
54. Mattiucci D, Maurizi G, Leoni P and Poloni A (2018) Aging- and Senescence-associated Changes of Mesenchymal Stromal Cells in Myelodysplastic Syndromes. Cell Transplant 27, 754-764
55. Bonafe M, Storci G and Franceschi C (2012) Inflamm-aging of the stem cell niche: breast cancer as a paradigmatic example: breakdown of the multi-shell cytokine network fuels cancer in aged people. Bioessays 34, 40-49
56. Lepperdinger G (2011) Inflammation and mesenchymal stem cell aging. Curr Opin Immunol 23, 518-524
57. Cartwright MJ, Tchkonia T and Kirkland JL (2007) Aging in adipocytes: potential impact of inherent, depot-specific mechanisms. Exp Gerontol 42, 463-471
58. Starr ME, Evers BM and Saito H (2009) Age-associated increase in cytokine production during systemic inflammation: adipose tissue as a major source of IL-6. J Gerontol A Biol Sci Med Sci 64, 723-730
59. Romieu-Mourez R, Francois M, Boivin MN, Bouchentouf M, Spaner DE and Galipeau J (2009) Cytokine modulation of TLR expression and activation in mesenchymal stromal cells leads to a proinflammatory phenotype. J Immunol 182, 7963-7973
60. Di GH, Liu Y, Lu Y, Liu J, Wu C and Duan HF (2014) IL-6 secreted from senescent mesenchymal stem cells promotes proliferation and migration of breast cancer cells. PLoS One 9, e113572
61. O'Hagan-Wong K, Nadeau S, Carrier-Leclerc A et al (2016) Increased IL-6 secretion by aged human mesenchymal stromal cells disrupts hematopoietic stem and progenitor cells' homeostasis. Oncotarget 7, 13285-13296
62. Carvalho JL, Braga VB, Melo MB et al (2013) Priming mesenchymal stem cells boosts stem cell therapy to treat myocardial infarction. J Cell Mol Med 17, 617-625
63. Balandran JC, Purizaca J, Enciso J et al (2016) Pro-inflammatory-Related Loss of CXCL12 Niche Promotes Acute Lymphoblastic Leukemic Progression at the Expense of Normal Lymphopoiesis. Front Immunol 7, 666
64. Oishi Y and Manabe I (2016) Macrophages in age-related chronic inflammatory diseases. NPJ Aging Mech Dis 2, 16018
65. Shin TH, Kim HS, Kang TW et al (2016) Human umbilical cord blood-stem cells direct macrophage polarization and block inflammasome activation to alleviate rheumatoid arthritis. Cell Death Dis 7, e2524
66. Pajarinen J, Lin T, Gibon E et al (2019) Mesenchymal stem cell-macrophage crosstalk and bone healing. Biomaterials 196, 80-89
67. Yin Y, Wu RX, He XT, Xu XY, Wang J and Chen FM (2017) Influences of age-related changes in mesenchymal stem cells on macrophages during in-vitro culture. Stem Cell Res Ther 8, 153

68. Kovtonyuk LV, Fritsch K, Feng X, Manz MG and Takizawa H (2016) Inflamm-Aging of Hematopoiesis, Hematopoietic Stem Cells, and the Bone Marrow Microenvironment. Front Immunol 7, 502
69. Shepherd MS and Kent DG (2019) Emerging single-cell tools are primed to reveal functional and molecular heterogeneity in malignant hematopoietic stem cells. Curr Opin Hematol 26, 214-221
70. Wilson NK, Kent DG, Buettner F et al (2015) Combined Single-Cell Functional and Gene Expression Analysis Resolves Heterogeneity within Stem Cell Populations. Cell Stem Cell 16, 712-724
71. Chen Z, Amro EM, Becker F et al (2019) Cohesin-mediated NF-kappaB signaling limits hematopoietic stem cell self-renewal in aging and inflammation. J Exp Med 216, 152-175
72. Geiger H, Denkinger M and Schirmbeck R (2014) Hematopoietic stem cell aging. Curr Opin Immunol 29, 86-92
73. Linehan E, Dombrowski Y, Snoddy R, Fallon PG, Kissenpfennig A and Fitzgerald DC (2014) Aging impairs peritoneal but not bone marrow-derived macrophage phagocytosis. Aging Cell 13, 699-708
74. Lloberas J and Celada A (2002) Effect of aging on macrophage function. Exp Gerontol 37, 1325-1331
75. Lim Z, Brand R, Martino R et al (2010) Allogeneic hematopoietic stem-cell transplantation for patients 50 years or older with myelodysplastic syndromes or secondary acute myeloid leukemia. J Clin Oncol 28, 405-411
76. Gibson KL, Wu YC, Barnett Y et al (2009) B-cell diversity decreases in old age and is correlated with poor health status. Aging Cell 8, 18-25
77. Castilho JL, Shepherd BE, Koethe J et al (2016) CD4+/CD8+ ratio, age, and risk of serious noncommunicable diseases in HIV-infected adults on antiretroviral therapy. AIDS 30, 899-908
78. Ghajar CM, Peinado H, Mori H et al (2013) The perivascular niche regulates breast tumour dormancy. Nat Cell Biol 15, 807-817
79. Zambetti NA, Ping Z, Chen S et al (2016) Mesenchymal Inflammation Drives Genotoxic Stress in Hematopoietic Stem Cells and Predicts Disease Evolution in Human Pre-leukemia. Cell Stem Cell 19, 613-627
80. Kim M, Kim C, Choi YS, Kim M, Park C and Suh Y (2012) 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. Mech Ageing Dev 133, 215-225
81. Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T and Kassem M (2001) Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. Biogerontology 2, 165-171
82. Ambrosi TH, Scialdone A, Graja A et al (2017) Adipocyte Accumulation in the Bone Marrow

during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration. Cell Stem Cell 20, 771-784 e776

83. Baker N, Boyette LB and Tuan RS (2015) Characterization of bone marrow-derived mesenchymal stem cells in aging. Bone 70, 37-47
84. Bilwani FA and Knight KL (2012) Adipocyte-derived soluble factor(s) inhibits early stages of B lymphopoiesis. J Immunol 189, 4379-4386
85. Ergen AV, Boles NC and Goodell MA (2012) Rantes/Ccl5 influences hematopoietic stem cell subtypes and causes myeloid skewing. Blood 119, 2500-2509
86. Busque L, Patel JP, Figueroa ME et al (2012) Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. Nat Genet 44, 1179-1181
87. Inoue S, Lemonnier F and Mak TW (2016) Roles of IDH1/2 and TET2 mutations in myeloid disorders. Int J Hematol 103, 627-633
88. Suijker J, Baelde HJ, Roelofs H, Cleton-Jansen AM and Bovee JV (2015) The oncometabolite D-2-hydroxyglutarate induced by mutant IDH1 or -2 blocks osteoblast differentiation in vitro and in vivo. Oncotarget 6, 14832-14842
89. Valli A, Harris AL and Kessler BM (2015) Hypoxia metabolism in ageing. Aging (Albany NY) 7, 465-466
90. Intlekofer AM, Dematteo RG, Venneti S et al (2015) Hypoxia Induces Production of L-2-Hydroxyglutarate. Cell Metab 22, 304-311
91. Cicione C, Muinos-Lopez E, Hermida-Gomez T, Fuentes-Boquete I, Diaz-Prado S and Blanco FJ (2013) Effects of severe hypoxia on bone marrow mesenchymal stem cells differentiation potential. Stem Cells Int 2013, 232896
92. Xing J, Ying Y, Mao C et al (2018) Hypoxia induces senescence of bone marrow mesenchymal stem cells via altered gut microbiota. Nat Commun 9, 2020
93. Abdel-Wahab O, Adli M, LaFave LM et al (2012) ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. Cancer Cell 22, 180-193
94. Rohatgi N, Zou W, Collins PL et al (2018) ASXL1 impairs osteoclast formation by epigenetic regulation of NFATc1. Blood Adv 2, 2467-2477
95. Uni M, Masamoto Y, Sato T et al (2019) Modeling ASXL1 mutation revealed impaired hematopoiesis caused by derepression of p16Ink4a through aberrant PRC1-mediated histone modification. Leukemia 33, 191-204
96. Zhang P, Chen Z, Li R et al (2018) Loss of ASXL1 in the bone marrow niche dysregulates hematopoietic stem and progenitor cell fates. Cell Discov 4, 4
97. Sun X, Hao H, Han Q et al (2017) Human umbilical cord-derived mesenchymal stem cells ameliorate insulin resistance by suppressing NLRP3 inflammasome-mediated inflammation in type 2 diabetes rats. Stem Cell Res Ther 8, 241
98. Turinetto V, Vitale E and Giachino C (2016) Senescence in Human Mesenchymal Stem Cells: Functional Changes and Implications in Stem Cell-Based Therapy. Int J Mol Sci 17