

Aging and Mesenchymal Stem Cells: Therapeutic Opportunities and Challenges in the Older Group

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Keywords

Cellular senescence · Mesenchymal stem cells · Mesenchymal stem cell therapy · Antiaging · Aged group

Abstract

With aging, a portion of cells, including mesenchymal stem cells (MSCs), become senescent, and these senescent cells accumulate and promote various age-related diseases. Therefore, the older age group has become a major population for MSC therapy, which is aimed at improving tissue regeneration and function of the aged body. However, the application of MSC therapy is often unsatisfying in the aged group. One reasonable conjecture for this correlation is that aging microenvironment reduces the number and function of MSCs. Cellular senescence also plays an important role in MSC function impairment. Thus, it is necessary to explore the relationship between senescence and MSCs for improving the application of MSCs in the elderly. Here, we present the influence of aging on MSCs and the characteristics and functional changes of senescent MSCs. Furthermore, current therapeutic strategies for improving MSC therapy in the elderly group are also discussed.

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Introduction

Aging has a significant impact on human health and increased risk of human diseases, such as cardiovascular disorders, diabetes, and neurodegenerative diseases. For example, axonal regeneration was less effective in aged mice than young mice when nerve conduit and autologous nerve were transplanted [1].

Mesenchymal stem cells (MSCs) have great potential for the treatment of degenerative diseases, such as osteoporosis, large bone defects, and nerve defects. It is reported that axonal regeneration using nerve conduits was improved when induced pluripotent stem cell derived neurospheres were transplanted in the aged mice [1]. Furthermore, MSC therapy has been widely and successfully applied in various diseases, including liver injury, multiple sclerosis, graft-versus-host disease, Crohn's disease, diabetes mellitus, and myocardial infarction. These achievements relied on the following characteristics of MSCs: (1) multiple differentiation potential: it was the first detailed description in 1999, including osteogenesis, chondrogenesis, and adipogenesis [2]; (2) replication capacity: upon stimulation by biological signals, MSCs are activated and recruited to injured sites to proliferate, regenerate, and re-

place damaged tissues [3, 4]; (3) low immunogenicity: MSCs have vague cell surface markers, which could cause less host immune response. Therefore, MSC therapies are likely candidates for use in aged individuals to restore the loss of tissue homeostasis caused by degenerative disease.

However, the application of MSC therapy is less effective in the elderly group than in the young group. For example, bone regeneration impaired in older mice when human muscle-derived stem cells were transplanted into the defect bone area [5].

This phenomenon indicated close relationship between aging and stem cells. On the one hand, with aging, a portion of stem cells become senescent, that is, characterized by permanent growth arrest, resistance to apoptosis, macromolecular damage, and altered metabolism [6]. These senescent cells (SNCs) promote various age-related diseases. On the other hand, aging microenvironment, including a senescence-associated secretory phenotype (SASP), impairs the function of transplanted MSCs [7–9], which is a major factor that contributes to the unsatisfactory therapeutic effect of MSC therapy.

In this review, we discuss the effect of aging microenvironment on the biological properties of MSCs and their therapeutic potential, as well as the characteristics and functional changes of senescent MSCs. Possible techniques to improve the therapeutic effect of MSCs and anti-senescence in the older population are discussed as well. The aim of this review is promoting the therapeutic effect of MSCs in the elderly group.

Senescence and Aging

It is generally recognized that a causal link has been established between senescence and aging [10]. With aging, SNCs accumulate in various tissues and organs which – once reaching a sufficient number – may drive age-related diseases. However, they are different. Generally, senescence is a cellular program mainly triggered by stressful stimuli, including DNA damage, oncogene activation, and telomere shortening [6], while aging is an individual condition caused by the passage of time. Therefore, SNCs can be detected at any physical age from embryogenesis to adulthood, regardless of organismal age. Besides, more than 80% of the cells in the older age group are not SNCs. However, young groups with premature phenotype might contain more SNCs even at a much younger age. For example, similar to Werner syndrome in human, mice with mutants in the Werner helicase and telomerase develop clinical symptoms of premature aging once they reach adulthood [11].

Cellular Senescence

Cellular senescence is a cell state characterized by cell cycle arrest, antiapoptosis, and special secretory features related to age-related diseases [6]. According to stimulating factors, cellular senescence can be divided into replicative senescence which occurs by replication and premature senescence which is caused by stressful stimuli [12].

Characteristics of SNCs

Cell Cycle Arrest

Different from quiescence and terminal differentiation, SNCs lost their ability to divide to some extent but remained active and metabolically active [13]. The senescence growth arrest is generally irreversible. However, SNCs can re-enter cell cycle in some contexts. Cyclin-dependent kinase inhibitors (CDKi) p16 and p21 are the 2 main signaling pathways that initiate and maintain cell cycle arrest by activating the transcriptional regulator retinoblastoma protein. Mitogen-activated protein kinases (MAPKs) [14] and P53 [15] are 2 upstream enzymes of CDKi and also promote growth arrest in SNCs by controlling CDKi.

Resistance to Apoptosis

SNCs are antiapoptotic and thus block programmed cell death [16, 17]. P53 signaling is a main pathway modulating the expression of a wide variety of molecules and genes related to senescence or apoptosis. It regulates extrinsic pathways and intrinsic pathways, 2 major apoptotic pathways. Extrinsic pathways involve particular “death” receptors from the tumor necrosis factor receptor family and death-inducing signaling complex, which lead to a cascade of activation of caspases and induce apoptosis. Intrinsic apoptotic pathways are dominated by the B-cell lymphoma-2 (BCL-2) family of proteins, which govern the release of cytochrome C from the mitochondria.

Secretory Features

SNCs secrete distinctive bioactive factors, including pro-inflammatory cytokines and chemokines, growth modulators, and proteases, collectively termed SASP [6]. SNCs do not exist as a single, discrete cell but are diverse: SNCs of different origins and stimuli secrete different SASP factors, which drive the pathogenesis of diseases and trigger cell apoptosis through different senescence mechanisms.

The mechanisms related to SASP regulation remain incompletely understood. Several transcription factors associated with regulation of SASP have been reported,

mainly through the action of transcription factor nuclear factor-kappa B (NF- κ B) and CCAAT/enhancer-binding proteins- β [18]. Besides, there are some factors involved in regulating SASP through the NF- κ B pathway, such as p38-MAPKs [14], mTOR [19], and transcription factor GATA4 [20]. Cyclic GMP-AMP synthase/stimulator of interferon genes triggers the secretion of SASP factors, thereby promoting paracrine senescence [21].

Methods for Detecting SNCs

Although some markers of identification of SNCs are more universal, such as senescence-associated β -galactosidase [22], currently, no single marker has been found to specifically identify SNCs [23] due to many factors affecting marker specificity, including cell type, tissue developmental stage, and stimulus. Remarkably, with the development of technology and concept, more and more novel methods have been used for identification of SNCs. Cell cycle arrest is a main feature of SNCs, suggesting high expression of cyclin kinase inhibitors, such as P15 [24–26], P16, P19 [27–29], P21, P27 [24, 30, 31], P53 [27, 32, 33], and P57 [26, 29, 30], and less expression of proliferation markers, such as bromodeoxyuridine [34, 35] and ki67 [28, 36, 37]. Furthermore, markers of apoptosis such as P53, decoy receptor 2, and BHLHB2 gene [37–39] are related to senescence.

Senescence triggers the production and accumulation of distinct heterochromatic structures and thus results in DNA damage. Senescence-associated distension of satellites [6, 35, 40, 41] assay and telomere-associated DNA damage response foci [42, 43] assay have also been recognized as specific markers of senescence *in vivo*. Other markers of DNA damage include γ -H2A.X [36, 41, 42], telomere dysfunction-induced foci [44–46], terminal restriction fragmentation [47], senescence-associated heterochromatic foci [48–50], and high-mobility group box 1 [36, 51, 52].

Therefore, it is necessary to combine various kinds of above methods, as well as a special SASP. There is still a long way before finding the gold standard for identifying SNCs.

Aging Microenvironment and MSCs

MSC therapy has shown great application prospects in cell and animal experiments. However, the effect of stem cell therapy is currently unsatisfactory in clinic. Some scholars suspect that host age may be responsible for it. In most cell and animal experiments, the hosts are young

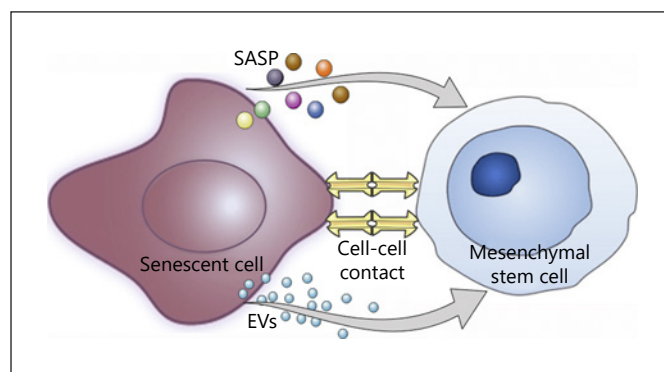


Fig. 1. Effect of senescent microenvironment on MSCs. Senescent microenvironment plays a role in MSCs through SASP, EVs derived from SNCs, and cell-cell contact. MSCs, mesenchymal stem cells; SNCs, senescent cells; SASP, senescence-associated secretory phenotype; EVs, extracellular vesicles.

individuals. While clinically, the elderly are the main recipients of MSC therapy. It is reported that bone regeneration impaired in older mice compared with young mice when human muscle-derived stem cells were transplanted into the defected bone area [5]. Similarly, after 6 months of repairing the skull defects by fetal bone marrow stromal cells (fetal BMSCs) with the decalcified bone matrix scaffolds, the repair effect of the skull defect in old goats is worse compared to young goats [53]. Furthermore, serum from old mice has a negative impact on muscle satellite cells (a kind of MSCs) and neurogenesis in young mice [7]. Serum from old rat reduces the proliferation and survival of bone marrow-derived MSCs and promotes its senescence [8]. What is more, exposure to young system environment can rejuvenate aging progenitor cells [54].

To sum up, it is reasonable to doubt that aging environment has adverse effects on the function of MSCs. However, mechanisms of aging microenvironment on MSCs are incompletely understood. It has been reported that the age-associated functional degeneration of stem cell is induced by the surrounding molecules to a significant extent, in addition to cell-intrinsic mechanisms [55]. In this part, we discuss how aging microenvironment affects MSCs (shown in Fig. 1), and what effects does it have on MSCs.

Senescence-Associated Secretory Phenotype

SASP contains various age-associated components; these components mediate paracrine senescence and affect the function of MSCs, including proliferation ability, clonal formation ability, differentiation potential, im-

immune characteristics, telomerase activity, cell migration ability, and adhesion. The SASP can create a chronic sterile inflammatory environment which is mainly mediated by NF- κ B signaling and thus drive stem cell dysfunction and aberrant remodeling [56]. Pro-inflammatory factors, such as IL-1 β and IL-6, induce the expression of p53 and p21, increase oxidative and DNA damage and thus cause senescence of IMR90 cells [57]. In addition, the early SASP regulates p15INK4b and p21CIP1 and induces senescence in nearby cells by transforming growth factor- β (TGF- β) family members [57–59].

Age-related insulin-like growth factor-binding protein 4 and 7, a kind of chemokines, could directly induce a senescent phenotype in P1 MSCs [60] and inhibit osteogenic differentiation of rat MSCs [61]. SASP is not always harmful. For example, intestinal stem cells after heavy ion radiation in mice and cells undergoing OIS show an increased expression of vascular endothelial growth factor (VEGF, one of the SASP components) [57, 62]. However, after pretreating with VEGF, the stem cells show a remodeling ability and attenuate age-related decay of adult hippocampal neurogenesis. In addition, MSCs release VEGF, activate the Jagged-1/Notch-1 signaling pathway, inhibit secretion of TGF- β 1, and thus rescue rat embryonic myoblasts from doxorubicin-induced senescence [63]. Furthermore, TNF- α -exposed MSCs exhibit upregulation of SASP molecules, including IL-6 and IL-8 and MCP-1 [64]. However, IL-8 exerts a chemotactic effect on human bone marrow MSC [65] and enhances the angiogenic and proliferation potential of MSCs [66, 67]. However, no positive influence of SASP on MSCs has been reported so far.

Extracellular Vesicles

Acting in a similar manner as SASP, extracellular vesicles (EVs), including exosomes from the endosomal compartment, and endosomes, microvesicles, and apoptotic bodies shredded from the plasma membrane, have recently been described as a new mechanism of cell-cell communication. These lipid-based carriers shuttle between cells, delivering their microRNAs, messenger RNAs, DNA, and proteins to target cells via endocytosis and membrane fusion.

A senescence-associated increase in EV secretion was first described by Lehmann et al. [68] and was reported to induce senescence of adjacent cells subsequently [69–73] and to promote the proliferation and inflammation of cancer cells [74]. With aging, SNC-derived microRNA-183-5p suppresses MSC proliferation and induces its senescence [70]. Moreover, microvesicular miR-31 secret-

ed by SNCs inhibits the osteogenic ability of MSCs [69]. Very long-chain C24:1 ceramide, which is associated with cellular senescence and apoptosis, can induce senescence of MSCs [71]. In addition, SNC-derived EVs exhibit antiapoptotic activity by targeting 5 common proapoptotic mediators (PTEN, P53, APAF-1, CDKN1B, and MYC) [75]. However, it is still unknown which of these miRNAs exert this effect.

EVs are actually a heterogeneous population that can be classified based on their origin [76, 77]. EVs from different cells or tissue have different RNAs or proteins; therefore, it is very important to study EVs from different origins and its pathway to promote senescence for exploring the mechanism of senescence and alleviating age-related diseases. Terlecki-Zaniewicz et al. [75] identified senescence-specific differences in miRNA composition of EVs in senescent human dermal fibroblasts, with an increase in miR-23a-5p and miR-137 and a decrease in miR-625-3p, miR-766-3p, miR-199b-5p, miR-381-3p, and miR-17-3p. EVs from senescent human umbilical vein endothelial cells were enriched in miR-21-5p and miR-217, which target 2 key enzymes in methylation pattern maintenance DNMT1 and SIRT1. Thus, it promotes senescence signal transmission, affects DNA methylation, and modulates senescence features [73].

Given that the function of EVs secreted by SNCs seems to be similar to SASP, increasing with aging, inducing senescence of adjacent cells, and resisting apoptosis, some researchers support that EVs are part of the SASP [75]. However, it is not clear whether EVs play a beneficial role in tissue homeostasis in vivo, such as wound healing or tissue regeneration [78].

Cell Junction

For SNCs, secretory factors are the main mechanism of information transmission between cells, but not the only mechanism. Cell junction is an important way of cell communication between adjacent cells. It has been proved that gap junction-mediated cell-cell contact increases the formation rate of DNA damage foci and steady-state foci levels in bystander cells [79] and induces DNA damage of neighboring cells, thus resulting in its senescence. Anat Biran et al. found that intercellular protein transfer is dependent on cell-cell contact mediated by actin polymerization and regulated primarily by CDC42 [80]. On all these counts; aging microenvironment exacerbates the inflammatory response and decreases the number, biological function, and immune modulation activity of MSCs.

Characteristics and Functional Changes of Senescent MSCs

Except that an aging microenvironment hinders the function of transplanted MSCs, senescence of autologous and transplanted MSCs contribute to an unsatisfied therapeutic effect [81]. For example, transplanting adipose-derived MSCs from aged mice, not from young mice, induces physical dysfunction in older recipients [82]. In addition, the adverse effect caused by senescent MSCs is also proved by *in vitro* experiments. Muscle-derived stem/progenitor cells came from old mice or premature aging models of mice show a decreased regeneration function [83]. It implied the importance of evaluating the impact of senescence on biological properties of MSCs and monitoring activities of MSCs preparations before application.

Senescent MSCs are usually characterized by an enlarged, more granular, and flat fried egg morphology, with constrained nuclei and a granular cytoplasm. During aging, the quantity of BM-MSCs with osteogenic potential decreases [84, 85], which may contribute to age-related diseases. Also, self-senescence will affect the quality of MSCs, including proliferation ability, clonal formation ability, differentiation potential, immune characteristics, telomerase activity, cell migration ability, and adhesion [86]. The aforementioned reasons may explain the dissatisfied therapeutic effect of stem cells.

Specifically, aged MSCs exhibit a reduced cell proliferation and osteogenic ability (alkaline phosphatase activity, extracellular matrix mineralization, and osteogenesis-related genes), while an increased adipogenic differentiation ability (adipocyte protein 2, resistin, and lipid accumulation) [87], which contributes to decreased bone formation [88] and results in osteoporosis [89–92] and dissatisfied ability of osseointegration [87]. What is more, senescent MSCs impair basal and calcium-regulated functions of the CXCR4/SDF-1 axis in bone marrow-derived cells and thus exhibit decreased vasculogenesis ability [93]. Senescent MSCs have been proved to have metabolic inflexibility with impairment of proteasome activity and the autophagic flux [94]. They also exhibit a significant decline in the cell colony number, one of the most convenient and common predictive indicators of MSC senescence [95].

Current Strategies for Improving MSC Therapy in the Elderly

Autologous MSC therapies are preferable due to tissue regeneration ability and biosafety concerns (immune rejection) in recent years. Besides, increasing evidence suggests that MSCs may not be immune-privileged [96, 97]. Unfortunately, the appliance of MSCs usually requires *in vitro* expansion, which exposes cells to replicative senescence. Therapies based on autologous MSCs have been hindered due to decreased quantity and impaired quality of MSCs with aging, as described previously [84]. How to rejuvenate senescent MSCs and improve the function of MSCs is a focus of scholars in regenerative medicine.

Targeting MSCs or Its Derivatives Improving the Quality of MSCs

Several lines of evidence support the feasibility of extracting high-quality autologous MSCs from older donors. (1) It has been proved that it is the clonal composition of stem cell populations, instead of individual stem cells, that changed with aging. (2) MSCs derived from the elderly population consist of mixtures of cells which contain not only SNCs but also healthy cells [98–100]. (3) With aging and some pathological conditions, SNCs accumulates in various tissues and organs, and the proportion is between 1 and 20% [101]. Once reached a sufficient number, SNCs may do harm to adjacent cells and tissues and drive age-related diseases. Injecting a small number of aging cells (less than 0.03% of all cells in the whole body) into mice can shorten the life span of mice and cause dysfunction of the body [101]. Travis J. Block first isolated a subset of “youthful” cells from elderly donors and then expanded these cells on a “young microenvironment” and thus harvested high-quality MSCs from elderly populations, which suggests that it may be able to store masses of high-quality autologous MSCs from the elderly population for treatment of age-related diseases [102].

Bioactivator-Treated MSCs

Due to long-term culture *in vitro*, human-derived MSCs were induced cellular senescence, which called replicative senescence. Treated by FGF-2, these cells show a suppressed expression level of p21, p53, and p16 mRNA, as well as decreased percentages of G1 cell growth arrest [103].

Macrophage migration inhibitory factor is an endocrine molecule used to restrict abnormal movement of macrophages. Pretreatment with migration inhibitory factor improved the proliferation, cell viability, trophic

effects, telomere length, and telomerase activity of senescent MSCs, which may be due to oxidative stress inhibition and phosphatidylinositol 3-kinase-RAC- α serine/threonine-protein kinase (Akt) signaling pathway activation [104].

Melatonin clears free radicals and reactive species and stimulates a number of antioxidant enzymes and thus protects macromolecules from oxidative damage, possibly by acting on membrane-bound receptors MT1 and MT2 and activating RK/MAPK and phosphatidylinositol 3-kinase/Akt signaling pathways [105].

Resveratrol-treated adult stem cells rescue the quantitative decline of BMSCs, improves bone structural organization, increases mineral density, and significantly alleviates progeroid features and prolongs the life span in *Zmpste24^{-/-}* mice (a kind of premature aging mice, responsible for prelamin A maturation) [106]. Liu et al. reported that it is associated with enhanced binding between SIRT1 and prelamin A by resveratrol.

Interestingly, IL-6 maintains MSC “stemness,” which may act through the ERK1/2 pathway [107]. The IL-6 and the IL-6 receptor complex activates the downstream signal transducer and activator of transcription (STAT) 3 pathway, thereby promoting osteogenic differentiation of BM-MSCs [108]. However, IL-6 is a pro-inflammatory factor, which can enhance senescence. Therefore, the effects and underlying mechanisms of IL-6 in MSCs and the interaction between these mechanisms require further investigation.

Cell-Free Therapy

Despite some success, many concerns have gained attention regarding the curative effect of aged MSCs because of their poor survival, low retention [109–111], replicative aging, and instability in clinical outcomes [112]. Emerging evidence suggests that much of the therapeutic benefit related to MSC therapy may owe to the biological activities of factors and molecules secreted by these cells. Growth factors, cytokines, and different components of the extracellular matrix have been widely studied as a cell-free therapeutic.

Bioactivator

Growth factors and cytokines can be used to alleviate inflammation, enhance angiogenesis, reduce fibrosis, and promote tissue generation. For example, TGF- β stimulates both Smad3-dependent and independent activation of macrophages, promotes secretion of VEGF and TGF- β 1, and protects against adverse cardiac tissue remodeling [113]. These bioactivators usually cooperate

with scaffold materials for delivery and sustained release, which contributes to prolonging the impact on surrounding tissues. SDF-1 facilitates cell homing and thus promotes defected cartilage repair when using radially oriented scaffolds to deliver SDF-1 locally [114]. Compared to conventional patch material, VEGF- and bFGF-embedded nanofiber matrices were confirmed to promote vascularization and thus restore cardiac function after ischemia [115].

However, these bioactivators fail to exert an abiding impact on clinical application due to their short half-lives and instability. It is reported that most of these limitations could be overcome by directly delivering gene, instead of the protein [116].

EVs of MSCs

Although several different target genes controlled by some specific signaling pathways and mechanisms were found in MSCs and EVs, MSCs and their EVs shared similar miRNA components [117, 118]. It seems that EVs have a long-term effect on surrounding tissues when used in vitro.

EVs are powerful biological entities released by cells and exhibit anti-inflammatory, antiapoptotic effects, and pro-angiogenic ability, thus promoting changes in their targets. It has been reported that MSC-EVs play a positive role in various diseases, such as acute kidney injury [119], lung injury [120–123], liver injury [124], neuroinflammation [125], allergic airway inflammation [126], acute graft-versus-host disease [127], autoimmune diseases [128], hair follicle growth [129], bone formation [130], and osseointegration between titanium surfaces and bone [131].

The current EV-based therapy is limited by the purity of EV preparations [132, 133]. The 2 commonly used methods are ultracentrifugation and differential centrifugation [109]. However, repeated ultracentrifugation not only reduces particle yield and recovery but also damages isolated vesicles, reducing their quality [133, 134]. Protein precipitation protocols are frequently used as well. Richard J. Lobb confirmed that ultrafiltration was far more time-efficient than ultracentrifugation [133]. Ultrafiltration combined with ultracentrifugation is a highly purified method because it removes non-EV-associated proteins from EVs [135]. Besides, some scholars have integrated many methods that separated free secreted proteins from EVs [136] (e.g., immunoaffinity isolation [137], polymer-based precipitation, density gradient, anion exchange [138], and microfluidics [139]). It is essential to standardize and optimize EV purification protocols.

Apoptotic body is a member of EVs. It has been reported that apoptotic cells may stimulate progenitor or stem cell proliferation, thus replacing damaged cells and promoting tissue regeneration [140, 141]. Subsequently, Liu et al. [142] showed that coculture of apoptotic bodies from MSCs from normal mouse bone marrow with MRL/lpr and cas3^{-/-} mouse MSCs in vitro improves proliferation, osteogenic differentiation, new bone formation, and adipogenic differentiation. In addition, apoptotic bodies are capable of maintaining MSC homeostasis by directly regulating the WNT/ β -catenin pathway [142, 143]. In this sense, apoptotic bodies from MSCs are promising and potential tools in tissue engineering. How to accelerate its clinical application still needs efforts.

Targeting Senescence

Another way to reinforce MSC therapy in aging is preventing senescence or reducing SNC burden. Senescence does harm to the quantity and quality of MSCs and the etiology of numerous tissues; therefore, disrupting or preventing senescence and its deleterious effects may be a promising way to delay health decline during aging. There are 4 strategies to alleviating these effects: suppressing the formation of SNCs, eliminating already formed SNCs, inhibiting the secretion of SASP, and cleaning up secreted SASP. Here, we discuss each of aforementioned therapeutic strategies of senescence, summarize the latest progress of these strategies, and emphasize their relative advantages and side effects.

Inhibit the Formation Mechanism of SNCs

Arguably, one of the most effective ways to target senescence is to start from the origin – preventing the production of SNCs, which is mainly based on epigenetics currently. However, interfering with epigenetics of senescence may lead to cancer [144]. Interventions targeting the formation of SNCs, such as knocking down the expression of p16Ink4a or p53 or short-term cyclic expression of Oct4, Sox2, Klf4, and c-Myc in mice, ameliorate aging features but promote the development of cancer [144, 145]. Based on Ocampo [144], Y. Lu et al. transplant, 3 of above genes that had nothing to do with cancer cells were used to devise a drug which controls the expression of these genes. This research regenerated nerve from injured eye cells and alleviated age-related vision loss in mice [146], but it needs safety assessment and considerable refinement before it can be deployed safely in humans.

To date, no study targeting the epigenetic of senescence has been conducted in human. One pivotal prob-

lem concerning inhibiting generation signaling of SNCs is how to prevent the formation of SNCs without increasing the risk of cancer and other side effects.

Eliminating SNCs

Eliminating SNCs is the most widely studied method to target senescence, which delays several pathologies and increases healthy life span [147, 148]. Some agents have been put into clinical practice. Among them, most products are mainly by inducing apoptosis and activating the immune system to eliminate SNCs.

The BCL-2 gene family plays a central role in modulating programmed cell death by the intrinsic apoptosis pathway [149–152]. The multiregional (BH1-4) anti-apoptotic proteins, such as BCL-2, BCL-XL, BCL-W, myeloid cell leukemia 1, and A1, antagonize proapoptotic BH3-only proteins and suppress the essential apoptosis effectors such as BCL-2 antagonist killer 1 and BCL-2-associated X protein, which leads to the release of cytochrome C, thus driving programmed cell death [153, 154].

ABT-737, the first discovered BCL-2 and BCL-XL inhibitor [155], has been widely used to study and define apoptosis biology. ABT-737 efficiently removes DNA damage-induced SNCs in the lungs as well as P53 activation-induced SNCs in the epidermis [156], enhancing liver regeneration [157]. However, the poor oral absorption of ABT-737 contributed to the emerging of its second-generation orally available analog, navitoclax (also known as ABT-263). Navitoclax, a BCL-2 and BCL-xl dual inhibitor, is one of the most effective and broad-spectrum BCL-2 family senolytic discovered so far [158–161]. Unfortunately, because platelets solely depend on BCL-xl for survival [149, 162–164], BCL-xl inhibition, including ABT263 and other small molecular inhibitors, induces platelet apoptosis and leads to severe thrombocytopenia [149, 162, 163]. Yonghan He et al. modified navitoclax using proteolysis-targeting chimera technology, improved senolytic activity, and, importantly, reduced platelet toxicity [161, 165, 166]. Unlike BCL-xl, BCL-2 is dispensable for generation and survival of platelet in mice and humans [166, 167]. Inhibition of BCL-2 does not induce thrombocytopenia, such as ABT199 (also known as venetoclax) [164]. How to decrease clinical side effects or improve ability to eliminate BCL inhibitor deserves more exploration.

Dasatinib (D) is a synthetic small-molecule inhibitor of Src family tyrosine kinase (TYK), which is used to treat chronic myelogenous leukemia that is positive for the Philadelphia chromosome. D promotes apoptosis caused

by dependence receptors, such as the ephrins, in part by inhibiting Src family TYK [168, 169]. Quercetin (Q) is a natural bioflavonoid and antioxidant, which exists in many plants. SNCs with a wide range of cell types are selectively eliminated by treatment with the D plus Q (D + Q) [42]. Interestingly, D nor Q alone can only kill certain cell types of SNCs, while the combination of D and Q can achieve additive benefits [170]. Furthermore, D + Q has been proved to remove naturally occurring SNCs in human adipose tissue, accompanied by an increase in cleaved caspase-3 [101], confirming that D + Q is effective to kill human SNCs partly by inducing apoptosis. However, whether other pathways have such effects still needs further study. D + Q has made significant progress in clinical trials, such as age-related osteoporosis, diabetic chronic kidney disease [171], and idiopathic pulmonary fibrosis [172], indicating D + Q has great prospects as a senolytic.

Fisetin, a natural flavonoid, is another senolytic, which acts in part by inhibiting BCL-2 family members, such as BCL-xL and HIF-1 α . Fisetin has been shown to eliminate SNCs in vivo [159, 173], while not hurting non-SNCs [174]. Importantly, no adverse effects of fisetin have been reported, even when administrated at high doses [175].

Furthermore, other senolytics, such as UBX0101, A1331852, or A1155463, are being studied [176, 177]. However, research results are less impressive. Unity, a pioneer biotechnology company in the research of senolytic drugs in the USA announced that its flagship product UBX0101 failed to beat placebo in a phase II clinical trial of osteoarthritis involving 183 people. Several major concerns remain to be solved, including possible side effects and optimal drug delivery mode before we can apply these treatments in clinic.

The immune system clear SNCs is an emerging therapeutic opportunity [178]. Natural killer cells, macrophages, B cells, T cells, neutrophils, and mast cells are involved. Some agents have been proposed to clear SNCs, such as DC vaccines and development of Abs against MDA-vimentin [179, 180]. Besides, SNCs derived from cell types outside the formal immune system could essentially be considered as an integral component of the immune system. For example, senescent stellate cells stimulate T cells, activate potentially natural killer cell proliferation in vivo, which in turn kill senescent stellate cells and appear to contribute to limitation of liver fibrosis [181, 182].

Inhibition of SASP Secretion Pathway

The Janus kinase (JAK)/STAT pathway is a common pathway of many cytokine signal transduction, which is widely involved in cell proliferation, differentiation,

apoptosis, and inflammation. It is highly activated in SNCs than non-SNCs. The JAK family consists of 4 members, including JAK1, JAK2, JAK3, and TYK2. These 4 members act on downstream STAT proteins to regulate a series of biological reactions. Among them, JAK1 and 2 play important roles in inflammatory signaling and other endocrine signaling.

Inhibition of the JAK pathway downregulates the secretion of SASP, including IL-6, MCP-1, and TNF- α , and alleviates age-related dysfunction [182–185]. For example, JAK inhibitors have been found to alleviate senescence of BMSCs, enhance osteogenic differentiation [186], enhance muscle stem cell function, promote muscle regeneration [187], improve antitumor responses by reprogramming the SASP in SNCs [188], and promote hair growth. Furthermore, some agents have been put into clinical implementation. Ruxolitinib, a specific JAK1/2 inhibitor, has been approved by the Food and Drug Administration (FDA) for the treatment of myelofibrosis.

In addition to JAK inhibitors, other compounds have been proved to inhibit inflammation-related SASP factors targeting the NF- κ B pathway. For example, metformin, a commonly prescribed drug for treating type 2 diabetes, has decreased the secretion of SASP factors of oncogene-induced SNCs by inhibiting NF- κ B activation. It has been proved to inhibit inflammatory reaction, increase insulin sensitivity, reduce lipotoxicity, and alleviate physical dysfunction in aged mice. Glucocorticoids and rapamycin (an MTOR inhibitor) have decreased IL1A production, which establishes and maintains the SASP by activating NF- κ B, thus decreased the secretion of inflammation-related SASP factors, postpone aging, and extend life span.

SNCs derived from different cell types have overlapping but distinct molecular signatures. Different senolytics target specific SASP molecules. How to select senolytics in disease and how to alleviate side effects of senolytics must be considered.

Inhibiting Individual Components of the SASP

Blocking of individual components of SASP components or their receptors, such as TGF- β or its receptor, has the advantage of a clear target and therefore potentially decreases the risk of off-target effects. However, a drawback of this method is that only one of many SASP factors can be targeted at a time. Up to now, no research of such agents in aging or senescence has been implemented.

Although therapies targeted at SNCs and their secretions have gained some achievements, these methods are

limited to eliminate SNCs or suppress their SASP, without reversing them to non-SNCs. The possibility of turning SNCs into normal and functional cells is worth exploring.

Conclusions and Prospects

As mentioned previously, aging microenvironment impairs the function of MSCs, induces cellular senescence, undermines various tissue and organ, and promotes age-associated diseases. However, senescence is not always harmful but offers a variety of benefits, such as tumor suppression [189–191], embryonic development [192, 193], and wound healing [194–196]. One thing to be considered is whether suppressing or removing SNCs can promote tumors or delay wound healing. It is highly valuable to investigate how to minimize the harmful effects of eliminating SNCs.

Advanced age is associated with dysfunction of different tissues and organs and pharmacokinetic changes, most of which are converted into drug accumulation in the body, leads to a higher possibility of drug toxicity [197]. Furthermore, the elderly are more susceptible to the adverse effects of polypharmacy. The increase in the number of medications increases the possibility of interaction between clinically important drugs and adverse drug effects [198]. Interaction between senolytics and MSC therapies and its influence on body needs further research.

In addition, systemic or local administration, intermittent or continuous administration, and how to minimize the side effect must be addressed for the optimization

of senolytics usage in the aged people. It is reported that intermittent administration to eliminate SNCs (such as once every 2 weeks or even once every month) has been proved to effectively delay the aging process and has undetectable side effects [40, 42, 101].

In conclusion, MSC therapy has achieved great success and entered clinical trials. The potential of therapeutically targeting SNCs and their secretions is beginning to emerge. However, senescence mechanism and regulatory genes of aging still need further research. It remains unknown whether there is any gene or molecule that can reverse SNCs to normal and functional cells without causing cancer. In this case, it will make milestone progress in the alleviation of aging-related diseases and extend the life with high quality.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

Funding Sources

Key R&D projects in Hunan Province: mechanism of miR-287 on osteogenic differentiation of bone marrow mesenchymal stem cells from the alveolar cleft and its early clinical diagnosis and treatment.

Author Contributions

Yueying Zhou conceptualized and revised the review; Huan Chen wrote the review; Ousheng Liu and Sijia Chen contributed to the revision of the review.

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