



# Senotherapeutics and Their Molecular Mechanism for Improving Aging

JooHo Park<sup>1</sup> and Dong Wook Shin<sup>2,\*</sup>

<sup>1</sup>Department of Applied Life Science, Graduate School, BK21 Program, Konkuk University, Chungju 27478,

<sup>2</sup>College of Biomedical and Health Science, Konkuk University, Chungju 27478, Republic of Korea

## Abstract

Aging is defined as physiological dysfunction of the body and a key risk factor for human diseases. During the aging process, cellular senescence occurs in response to various extrinsic and intrinsic factors such as radiation-induced DNA damage, the activation of oncogenes, and oxidative stress. These senescent cells accumulate in many tissues and exhibit diverse phenotypes, such as resistance to apoptosis, production of senescence-associated secretory phenotype, cellular flattening, and cellular hypertrophy. They also induce abnormal dysfunction of the microenvironment and damage neighboring cells, eventually causing harmful effects in the development of various chronic diseases such as diabetes, cancer, and neurodegenerative diseases. Thus, pharmacological interventions targeting senescent cells, called senotherapeutics, have been extensively studied. These senotherapeutics provide a novel strategy for extending the health span and improving age-related diseases. In this review, we discuss the current progress in understanding the molecular mechanisms of senotherapeutics and provide insights for developing senotherapeutics.

**Key Words:** Senotherapeutics, Senescence, Aging, Molecular mechanism

## INTRODUCTION

Aging is defined as a physiological dysfunction in an organism and is closely related to many chronic human diseases, including metabolic syndrome, cancer, and neurodegenerative diseases (Campisi, 2013; Munoz-Espin and Serrano, 2014; Hoang *et al.*, 2021).

Senescent cells generally accumulate in various tissues during aging. Cellular senescence is a heterogeneous process characterized by genetic, epigenetic, and environmental factors (van Deursen, 2014; Lagoumtzi and Chondrogianni, 2021). Senescence occurs in response to various stimuli, such as telomere shortening, DNA damage by radiation, oncogene activation, mitochondrial dysfunction, and endoplasmic reticulum stress. Senescence is classified as replicative senescence and stress-induced premature senescence. Replicative senescence, known as the Hayflick limit, was first found in human primary fibroblasts to stop dividing after many divisions. This type of cellular senescence is involved in the shortening of telomeric DNA. Stress-induced premature senescence is caused by various intrinsic and extrinsic factors such as oxidative stress, ionizing radiation, genotoxic stress, oncogenic

transformation, and various pathogens (Coppe *et al.*, 2010; Lopez-Otin *et al.*, 2013; Kennedy *et al.*, 2014; Munoz-Espin and Serrano, 2014; He and Sharpless, 2017; Borghesan *et al.*, 2020).

Cellular senescence has both beneficial and harmful effects on neighboring cells. The senescence process is fundamental for avoiding uncontrolled cellular replication. The accumulation of senescent cells during the aging process is critical for the tumor suppressor mechanism, which is a state of cell cycle arrest that suppresses abnormal proliferation of damaged cells and oncogenic development (Coppe *et al.*, 2010; Munoz-Espin and Serrano, 2014). However, many recent studies have also demonstrated that cellular senescence can cause various deleterious processes, such as chronic inflammation, immune surveillance, and organismal aging (Lopez-Otin *et al.*, 2013; Munoz-Espin and Serrano, 2014; He and Sharpless, 2017).

Despite growth inhibition, senescent cells are metabolically active and produce a senescence-associated secretory phenotype (SASP) that contains pro-inflammatory cytokines, bioactive lipids, and other factors that mediate the autocrine/paracrine effects of senescent cells (Childs *et al.*, 2017; Birch

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**\*Corresponding Author**

E-mail: biocosmed@kku.ac.kr

Tel: +82-43-840-3693, Fax: +82-43-840-3929

and Gil, 2020). In addition, these factors can induce chronic inflammation and age-associated disorders (Childs *et al.*, 2017; He and Sharpless, 2017). These factors stimulate senescence-associated signaling pathways and result in cell cycle arrest, increase SA- $\beta$ -galactosidase activity, and affect neighboring young cells in a paracrine manner (Bang *et al.*, 2019). Senescent cells respond less to external signals, such as growth factors, abnormal structures, and mitochondrial dysfunction. Senescent cells are enlarged, vacuolated, and flattened. In addition, the lack of lamin A/B and accumulation of lipofuscin have been observed in cellular senescence (Kirkland and Tchkonja, 2017; Hernandez-Segura *et al.*, 2018; Gorgoulis *et al.*, 2019).

Resistance to apoptosis is another hallmark of cellular senescence (Baar *et al.*, 2017; Childs *et al.*, 2017; Demaria *et al.*, 2017). Apoptosis plays a key role in the clearance of both damaged and cancerous cells. Apoptosis is regulated by pro-apoptotic and anti-apoptotic proteins, such as the B-cell lymphoma 2 (Bcl-2) family, caspase, and death receptors. Enhanced reactive oxygen species (ROS) levels, another characteristic of senescent cells, accelerate chronic inflammation and autoimmunity, which are highly related to age-related diseases, such as metabolic syndrome, cognitive decline, and frailty (Munoz-Espin and Serrano, 2014; Hernandez-Segura *et al.*, 2018).

Much research effort has recently been made to therapeutically target the harmful effects of cellular senescence (Baker *et al.*, 2011, 2016; Childs *et al.*, 2017; Krimpenfort and Berns, 2017). The targeting of senescent cells by several pharmacological interventions, known as senotherapeutics, has been reported to ameliorate many senescence-associated diseases and delay the development of age-related disorders. Senotherapeutics can be classified into three development strategies. First, senolytics selectively eliminate senescent cells. Second, senomorphics induce senescent cells to obtain the functions and morphology of young cells or delay the progression of young cells to senescent cells. Third, senescence-targeting immunotherapeutics mediate the clearance of senescent cells. Some senolytics and senomorphics have been reported to prevent or treat age-related diseases in animal models (Baar *et al.*, 2017; Childs *et al.*, 2017; Kirkland and Tchkonja, 2017; Borghesan *et al.*, 2020).

Therefore, this review provides insights into the development of various senotherapeutics for improving age-related diseases and eventually increasing the health span.

## THE IMPLICATION OF SENESCENCE IN AGE AND AGE-RELATED DISEASES

Senescent cells accumulate because of either continuous stress stimuli or a reduction in immune function during the aging process. This phenomenon causes chronic inflammation through uncontrolled secretion of SASP (He and Sharpless, 2017; Hernandez-Segura *et al.*, 2018; Gorgoulis *et al.*, 2019). There are increased levels of interleukin-1A (IL-1A) and interleukin-6 (IL-6), one of several SASP factors, during aging. Suppression of the inflammatory key protein, nuclear factor-kappa B (NF- $\kappa$ B), can prevent DNA damage-induced senescence in mice (Salminen *et al.*, 2012). SASP leads to pathological angiogenesis in a retinopathy mouse model and contributes to atherosclerosis progression (Oubaha *et al.*,

2016; Ferrucci and Fabbri, 2018). Conversely, selective ablation of senescent cells ameliorates some age-associated symptoms and extends the health span of mice (Baker *et al.*, 2011; Krimpenfort and Berns, 2017; van Deursen, 2019).

Baker *et al.* (2011) first revealed a direct relationship between cellular senescence and age-related symptoms. They designed a novel transgene using a senescence biomarker,  $p16^{Ink4a}$  and generated an INK-ATTAC (apoptosis through targeted activation of caspase) transgenic mouse model in the BubR1 progeroid mouse background for inducible elimination of  $p16^{Ink4a}$ -positive senescent cells upon the administration of AP20187. AP20187, an inducer of dimerization of the FK506 binding protein-caspase 8 fusion protein, was used to trigger senolysis. This approach delayed the progression of  $p16^{Ink4a}$ -mediated age-related symptoms in adipose tissue and muscle, implying that cellular senescence is implicated in the generation of age-related disorders and that the elimination of senescent cells can prevent tissue dysfunction and extend the health span. Baker *et al.* (2016) further demonstrated a direct association between cellular senescence and age-related disorders. They revealed that the treatment of AP20187 eliminated  $p16^{Ink4a}$ -positive senescent cells in INK-ATTAC transgenic mice with two different genetic backgrounds (C57BL/6 and mixed) and led to enhanced median health span in both mice. These data implied that AP20187 diminished age-related abnormal function and structural destruction of various organs, such as the adipose tissue, kidney, and heart. Several studies have demonstrated that the genetic clearance of  $p16^{Ink4a}$ -positive senescent cells in INK-ATTAC transgenic mice ameliorated the symptoms of lipodystrophy (Xu *et al.*, 2015a), hepatic steatosis (Ogrodnik *et al.*, 2017), cardiac dysfunction (Lewis-McDougall *et al.*, 2019), and cerebral disorders (Ogrodnik *et al.*, 2021).

In previous studies,  $p16^{Ink4a}$ -positive senescent macrophages in the  $p16^{Ink4a}$ -trimodality reporter ( $p16$ -3MR) transgenic mice were cleared by treatment with ganciclovir, which contributed to diminishing the formation of atherosclerotic plaques in low-density lipoprotein receptor-deficient ( $LDLR^{-/-}$ ) mice (Childs *et al.*, 2016) and osteoarthritis model mice (Jeon *et al.*, 2017). Conversely, transplantation of senescent ear fibroblasts into the knee caused osteoarthritis in a mouse model (Xu *et al.*, 2017). Transplanting a small number of senescent cells into young mice was also sufficient to induce physical dysfunction, eventually leading to reduced survival (Xu *et al.*, 2018).

These results suggest that senotherapeutics can either prevent tissue dysfunction or contribute to extending the health span of aged models.

## THREE STRATEGIES TO TARGET SENESCENT CELLS

### Senolytics

Chronic/periodic administration of senolytics eliminates senescent cells present in aged tissues, and the immune response contributes to discarding apoptotic bodies for subsequent regeneration. Senolytics target signaling pathways that are activated in senescent cells and specifically kill chronically senescent cells (Baar *et al.*, 2017; Kirkland and Tchkonja, 2017). These senolytic compounds are extensive and are continuously found (Table 1, Fig. 1). Various senolytics, such as Bcl-2 family inhibitors, histone deacetylase (HDAC) inhibitors,

**Table 1.** Each senolytic compound for improving age and age-related disorders

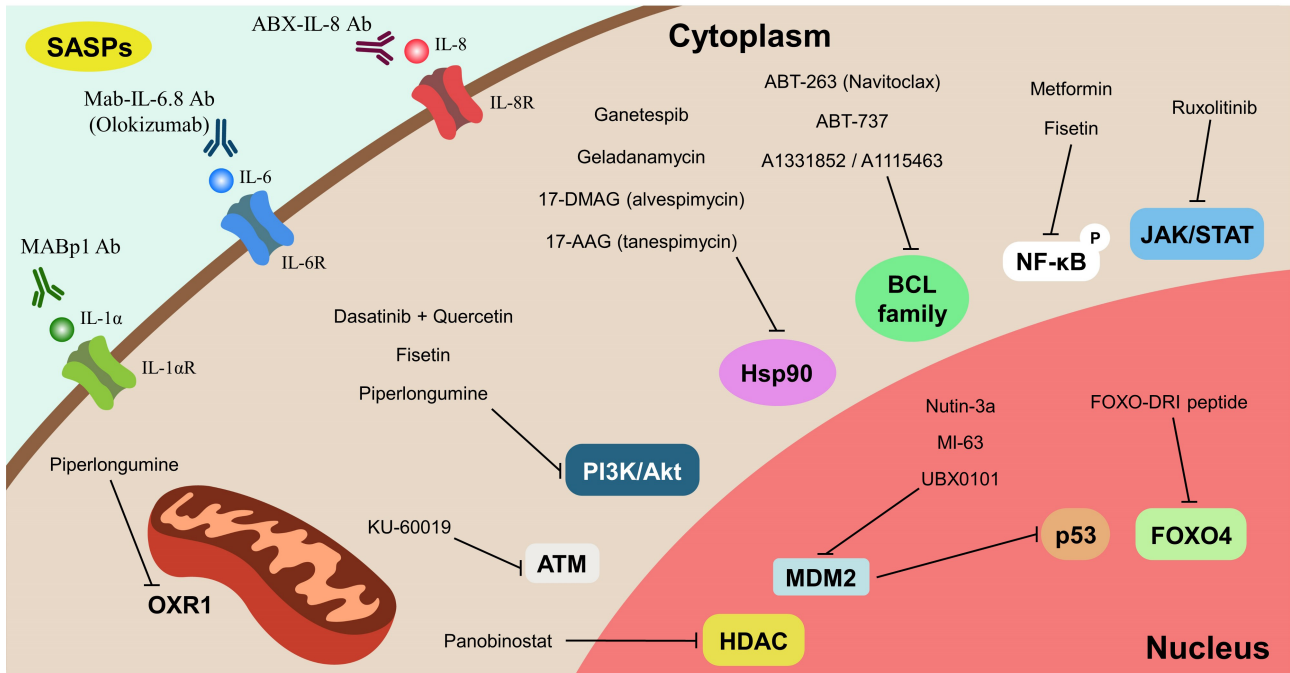
Chemical name (active material)	Cell or animal type	Working concentration	Molecular targets	References
Dasatinib+Quercetin	Aged C57B/L6 mice/Ercc <sup>-1Δ</sup> mice	Dasatinib (5mg/kg/day) Quercetin (50 mg/kg)	The PI3K/Akt pathway	Zhu <i>et al.</i> , 2015 Xu <i>et al.</i> , 2018
	Preadipocytes/HUVECs	Dasatinib (0-300 nM) Quercetin (0-30 μM)		
ABT-263 (Navitoclax)	Male C57B/6J, p16-3MR transgenic mice	50 mg/kg/day	↓ Bcl 2, Bcl-w, Bcl-xL	Chang <i>et al.</i> , 2016 Zhu <i>et al.</i> , 2016 Miura <i>et al.</i> , 2022 Sharma <i>et al.</i> , 2020
	Preadipocytes/HUVECs	0-20 μM		
	IMR-90 fibroblasts			
	Ercc <sup>-/-</sup> MEFs			
ABT-737	Osteoarthritic chondrocytes	0-10 μM	↓ Bcl 2, Bcl-w, Bcl-xL	Yosef <i>et al.</i> , 2016
	IMR-90 fibroblasts			
A-1331852/A-1155463	Epidermal cells from aged mice	0-10 μM	↓ Bcl-xL	Zhu <i>et al.</i> , 2017
	DNA damaged lung cells			
Panobinostat	HUVECs	0-25 nM	↓ Bcl	Samaraweera <i>et al.</i> , 2017
	IMR-90 fibroblasts			
17-DMAG (Alvespimycin)	A549 cell lines*	10 mg/kg	↓ HDAC	Fuhrmann-Stroissnigg <i>et al.</i> , 2017
	FaDU cell line*			
	Ercc <sup>-1Δ</sup> mice			
	Ercc <sup>-/-</sup> MEFs			
Ganetespi	MSCs (Ercc1-deficient mice)	0.1-1 μM	↓ Hsp90	Fuhrmann-Stroissnigg <i>et al.</i> , 2017
	IMR-90/WI-38			
Ganetespi	HUVECs	0-800 nM	↓ Hsp90	Fuhrmann-Stroissnigg <i>et al.</i> , 2017
Geladanamycin	Ercc <sup>-/-</sup> MEFs	0.1-1 μM	↓ Hsp90	Fuhrmann-Stroissnigg <i>et al.</i> , 2017
17-AAG (Tanespimycin)	Ercc <sup>-/-</sup> MEFs	0.1-1 μM	↓ Hsp90	Fuhrmann-Stroissnigg <i>et al.</i> , 2017 Trendowski, 2015
Fisetin	Wild C57BL/6: FVB mice	100-500 mg/kg	The PI3K/Akt pathway	Yousefzadeh <i>et al.</i> , 2018 Zhu <i>et al.</i> , 2017
	Ercc <sup>-1Δ</sup> mice			
	INK-ATTAC male mice			
Piperlongumine	HUVECs	0-60 μM	↓ OXR-1	Wang <i>et al.</i> , 2016 Zhang <i>et al.</i> , 2018
	WI-38 fibroblasts	0-10 μM		
FOXO4-DRI	Xpd <sup>TFD/TTD</sup> mice	5 mg/kg	↓ p53-FOXO4 interaction	Baar <i>et al.</i> , 2017 Zhang <i>et al.</i> , 2020
	p16::3MR mice			
	Naturally aged mice			
	IMR-90 fibroblasts			
UBX0101	IMR-90 fibroblasts	0-25 μM	↓ MDM2	Jeon <i>et al.</i> , 2017
	Leydig cells	0-25 mM		
UBX0101	p16::3MR mice	1-5 mM injection	↓ MDM2	Jeon <i>et al.</i> , 2017
	Osteoarthritis mouse model			

\*A549 cell line, Non-Small Cell Lung Cancer (NSCLC); FaDu cells, Head and Neck Squamous Cell Carcinoma (HNSCC) cell lines. MEFs, mouse embryonic fibroblasts.

forkhead box protein O4 (FOXO4), p53 binding inhibitor, and heat shock protein 90 (HSP90) inhibitors, have been identified (Zhu *et al.*, 2015, 2016; Baar *et al.*, 2017; Jeon *et al.*, 2017;

Samaraweera *et al.*, 2017; Xu *et al.*, 2018).

Senolytics were first identified as combinations of quercetin and dasatinib (Zhu *et al.*, 2015). Zhu *et al.* (2015) examined



**Fig. 1.** Molecular targets of senolytics and senomorphics. Each senotherapeutic and its target are shown at the cellular level.

the gene expression profiles of young and senescent cells. They found that senescent cells increased the expression levels of anti-apoptotic biomarkers such as BCL-2 family members and the phosphoinositide 3-kinase (PI3K)/Akt pathway. In addition, they screened 46 candidate drugs having the ability to preferentially kill senescent cells *in vitro* (Zhu *et al.*, 2015). They found that the combination of dasatinib and quercetin efficiently accelerated apoptosis of senescent cells in naturally aged, irradiated, and even *Ercc1*<sup>-/-</sup>-progeroid mice and led to an extended lifespan and decreased age-associated symptoms. Dasatinib, a protein tyrosine kinase inhibitor used clinically for cancer therapy, effectively eliminated senescent preadipocytes. Quercetin, a natural plant flavonoid that targets the BCL-2 and PI3K/AKT pathways, ablated senescent endothelial cells and mouse bone marrow-derived mesenchymal stem cells (Zhu *et al.*, 2015). In addition, transplantation of senescent cells into healthy mice causes physical abnormalities, which could be antagonized by an oral combination of dasatinib and quercetin (Xu *et al.*, 2018). These senolytic effects of dasatinib and quercetin have also been demonstrated in various *in vivo* models, where intervention treatment ameliorated the symptoms of several age-related diseases, such as physical dysfunction, hepatic steatosis, insulin resistance, neurodegeneration, and skeletal muscle dysfunction (Xu *et al.*, 2018; Wissler Gerdes *et al.*, 2020). The combination of dasatinib and quercetin is currently in progress in human clinical trials for idiopathic pulmonary fibrosis (NCT02874989), Alzheimer's disease (NCT04685590), frailty (NCT04733534), chronic kidney disease (NCT02848131), hematopoietic stem cell transplant survivors (NCT02652052), skeletal health (NCT04313634), and adult cancer survivors (NCT04733534).

Upregulation of Bcl-2 and Bcl-xL contributes to the resistance of senescent cells to apoptosis (Chang *et al.*, 2016; Zhu *et al.*, 2016), implying that inhibitors of these anti-apoptotic

proteins can be effective candidates for senolytics. ABT-263 (known as Navitoclax), ABT-737, A1331852, and A1155463, which inhibit BCL-2 family members, were identified as candidate senolytics *in vitro* and *in vivo* animal models (Chang *et al.*, 2016; Yosef *et al.*, 2016; Zhu *et al.*, 2016, 2017). ABT-263 eliminated senescent IMR-90 human lung fibroblasts and human umbilical vein epithelial cells (HUVECs) (Zhu *et al.*, 2016). ABT-263 also rejuvenated aged hematopoietic stem cells and muscle stem cells by clearing senescent cells (Chang *et al.*, 2016). By binding the inhibitory domain of anti-apoptotic Bcl-2 and Bcl-xL, ABT-263 specifically eliminated both senescent muscle stem cells (MuSCs) and senescent bone marrow hematopoietic stem cells (HSCs) and eventually contributed to the amelioration or rejuvenation of MuSCs and HSCs in aged mice (Miura *et al.*, 2022). In addition, ABT-263 cleared senescent foam cell macrophages in atherosclerotic lesions, preventing atherosclerosis progression in *LDLR*<sup>-/-</sup> mice (Garrido *et al.*, 2022). However, this compound caused trabecular bone loss and damages the function of osteoprogenitors in aged mice (Sharma *et al.*, 2020). ABT-737 specifically eliminated etoposide-induced senescence in IMR-90 fibroblasts. ABT-737 treatment also efficiently eliminated senescent lung epithelial cells in irradiated mice and senescent epidermal cells in *p14ARF* transgenic mice. Furthermore, it stimulated hair follicle stem cell proliferation (Yosef *et al.*, 2016). Both A1331852 and A1155463, selective Bcl-xL inhibitors, preferentially stimulated the clearance of irradiation-induced senescent HUVECs and IMR-90 fibroblasts (Zhu *et al.*, 2017).

Panobinostat, an HDAC inhibitor approved by the FDA, was found to possess senolytic activity in the chemotherapy-induced senescence of squamous cell carcinoma cell lines and non-small cell lung cancer (Samaraweera *et al.*, 2017). The senolytic effect of panobinostat was related to the upregulation of histone H3 acetylation and downregulation of BCL-XL

expression. The potential application of senolytics targeting these senescent cancer cells can be a novel strategy for improving cancer metastasis and stemness.

FOXO4 is an important protein that is involved with the viability of senescent cells (Baar *et al.*, 2017; Krimpenfort and Berns, 2017; Zhang *et al.*, 2020). A previous study demonstrated that FOXO4-induced nuclear localization of p53 repressed its association with the mitochondrial apoptotic pathway (Baar *et al.*, 2017). They designed a FOXO4 inhibitor peptide (FOXO4-DRI peptide) to mimic the binding surface of both FOXO4 and p53 for disrupting their interaction. This disruption induced the translocation of p53 to the cytosol and caused caspase-3/7-dependent apoptosis in senescent IMR-90 fibroblasts and HUVECs. When administered *in vivo*, FOXO4-DRI improved hair density and renal dysfunction in both naturally aged mice and fast-aging *Xpd<sup>TTD/TTD</sup>*-progeroid (Baar *et al.*, 2017). In addition, another study demonstrated that FOXO4-DRI ameliorated age-related progression of hypogonadism in aged mice by inducing apoptotic cell death of senescent Leydig cells, known as interstitial cells, in the testes (Zhang *et al.*, 2020). These findings suggest that it is possible to regulate cellular senescence by targeting the mutual interaction between FOXO4-p53.

Murine double minute 2 (MDM2) E3 ligase, a major negative regulator of p53, promotes proteasome-dependent degradation of p53 (Fu *et al.*, 2009). UBX0101, an inhibitor of the MDM2/p53 protein interaction, was known to be a senolytic candidate (Jeon *et al.*, 2017). Intra-articular injection of UBX0101 ablated senescent cells in the articular cartilage and synovium. This administration led to attenuation of the onset of post-traumatic osteoarthritis in an osteoarthritis mouse model (Jeon *et al.*, 2017). UBX0101 also eliminated senescent cells by inducing apoptosis and contributed to the ability of chondrocytes to form cartilage in osteoarthritis tissues (Jeon *et al.*, 2017). UBX0101 was the first senolytic drug in phase 1 clinical trial to treat patients suffering from osteoarthritis (<https://clinicaltrials.gov/ct2/show/NCT03513016>). However, intra-articular injection of UBX0101 for treating osteoarthritis failed to meet its clinical requirements in phase 2 clinical trials (<https://clinicaltrials.gov/ct2/show/NCT04129944>).

HSP90 is an ATP-dependent chaperone that is involved in the folding, stabilization, and degradation of various proteins. Most of these proteins are important for various cellular processes, including cell survival and responses to cellular stress (Taipale *et al.*, 2010). Ganetespib, geldanamycin, 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG; alvespimycin), and 17-N-allylamino-17-demethoxygeldanamycin (17-AAG; tanespimycin), which are inhibitors of HSP90, were selected as new senolytic candidates by screening a library of autophagy regulators in senescent *Ercc1<sup>-/-</sup>* mouse embryonic fibroblasts (MEFs) (Trendowski, 2015; Fuhrmann-Stroissnigg *et al.*, 2017). These HSP90 inhibitors showed senolytic activity in a senescent cell-type-specific manner. Ganetespib exhibited senolytic activity in senescent HUVECs, whereas 17-DMAG exhibited senolytic activity in senescent human fibroblasts (IMR-90 and WI-38) (Fuhrmann-Stroissnigg *et al.*, 2017). In addition, 17-DMAG accelerated apoptosis by blocking the HSP90-AKT interaction to destabilize the active state of AKT in senescent MEFs. Treatment of 17-DMAG in the *Ercc1<sup>-Δ</sup>* mouse model significantly decreased tissue senescence and attenuated the progression of several age-related pathologies (Fuhrmann-Stroissnigg *et al.*, 2017). In addition,

previous studies demonstrated that other natural and synthetic HSP90 inhibitors have senolytic activities (Fuhrmann-Stroissnigg *et al.*, 2018; Dutta Gupta and Pan, 2020).

Fisetin is a natural flavonoid present in many plants, including fruits, vegetables, and flowers. It also has various pharmacological effects, including anti-inflammatory, antioxidant, antidiabetic, anticarcinogenic, and neuroprotective properties (Khan *et al.*, 2013; Sundarraj *et al.*, 2018). The biological activities of fisetin are involved with various molecular targets and signaling pathways, including the PI3K/Akt, NF- $\kappa$ B, and NRF2 pathways (Chiang *et al.*, 2015; Pal *et al.*, 2015). Fisetin preferentially eliminated ionizing radiation (IR)-induced senescent HUVECs but not preadipocytes or senescent IMR-90 (Zhu *et al.*, 2017). Furthermore, it preferentially eliminated genotoxin-induced senescence in human fibroblasts and oxidative stress-induced senescence in MEFs. Fisetin significantly decreased the characteristics of senescence in human adipose tissue (Zhu *et al.*, 2017; Yousefzadeh *et al.*, 2018). Administration of fisetin significantly downregulated the accumulation of senescent cells and senescence markers in several organs of accelerated aged *Ercc1<sup>-Δ</sup>* mice. Fisetin intervention in progeroid and old mice significantly decreased cellular senescence in several tissues, recovered tissue homeostasis, ameliorated frailty, and extended the lifespan (Yousefzadeh *et al.*, 2018). Fisetin is currently being tested in clinical trials for chronic kidney disease (NCT03325322), skeletal health (NCT04313634), osteoarthritis (NCT04210986), frailty (NCT03675724), and adult cancer survivors (NCT04733534). Fisetin exhibits a chemical structure similar to that of quercetin, which is only different in the 5-hydroxy group, implying that more effective analogs can be developed by the structural optimization process.

Piperlongumine, a natural amide alkaloid isolated from long peppers, was known to have senolytic activity (Wang *et al.*, 2016; Zhang *et al.*, 2018). Piperlongumine specifically caused apoptosis in ionizing radiation-induced or senescent human WI-38 fibroblasts by increasing ROS production and inhibiting the PI3K/Akt/mTOR pathway (Wang *et al.*, 2016). In addition, piperlongumine could bind to oxidation resistance 1 (OXR1), a protein that regulates the expression levels of antioxidant enzymes and leads to their degradation (Zhang *et al.*, 2018).

## SENMORPHICS

Senomorphics is an agent that transforms the characteristics of senescent cells into those of young cells by intervening with senescence-associated signaling pathways and SASP without causing apoptosis of senescent cells (Kirkland and Tchkonja, 2017; Lagoumtzi and Chondrogianni, 2021). Senomorphics suppress the function of the SASP by targeting senescence-related signaling pathways, such as MAPKs, NF- $\kappa$ B, mTOR, and IL-1 $\alpha$  (Yun *et al.*, 2018; Birch and Gil, 2020; Lagoumtzi and Chondrogianni, 2021). Senomorphics are also related to well-known anti-aging compounds (Table 2, Fig. 1).

Ataxia-telangiectasia mutated (ATM) kinase, a serine/threonine protein kinase stimulated by DNA double-strand breaks, modulates cellular senescence (Fausti *et al.*, 2013). The ATM kinase inhibitor KU-60019 exhibited a possible senomorphic activity by recovering the lysosome/autophagy system and metabolic reprogramming (Kang *et al.*, 2017).

NF- $\kappa$ B is a transcription factor that mediates senoinflamma-

**Table 2.** Each senomorphic compound for improving age and age-related disorders

Chemical name (active material)	Cell or animal type	Working concentration	Molecular targets	References
Ruxolitinib	Aged C57BL/6 mice Preadipocytes	60 mg/kg 0-1 $\mu$ M	$\downarrow$ JAK 1/2 family	Xu <i>et al.</i> , 2015b
8K-NBD peptide	Ercc <sup>-1<math>\Delta</math></sup> mice Ercc <sup>-</sup> MEFs	10 mg/kg 0-100 $\mu$ M	$\downarrow$ NF- $\kappa$ B	Tilstra <i>et al.</i> , 2012
Metformin	Lens epithelial cells from aged C57BL/6 mice IMR-90 fibroblasts Raw 264.7 cells	0.1% 0-5 mM	$\uparrow$ AMPK $\downarrow$ NF- $\kappa$ B	Chen <i>et al.</i> , 2022 Moiseeva <i>et al.</i> , 2013
KU-60019	ATM-deficient fibroblasts	0-3 $\mu$ M	$\downarrow$ ATM kinase	Kang <i>et al.</i> , 2017
Nutlin-3a (MI-63)	IMR-90/WI-38 fibroblasts HCA2 foreskin	0-10 $\mu$ M	$\downarrow$ MDM2	Wiley <i>et al.</i> , 2018
MABp1 Ab	HCA2 foreskin	240 ng/mL	$\downarrow$ IL-1 $\alpha$	Orjalo <i>et al.</i> , 2009
Mab-IL-6.8 Ab (Olokizumab)	HepG2 cells IMR-90 fibroblasts Cynomolgus monkey arthritis model Human hepatocytes HUVECs	1.25 ng/mL 20 mg/kg 10 $\mu$ g/mL 12.5 ng/mL	$\downarrow$ STAT3 $\downarrow$ IL-6	Kuilman <i>et al.</i> , 2008 Shaw <i>et al.</i> , 2014
ABX-IL-8 Ab	BALB/c nude mice A375SM/TXM-13 melanoma cells HUVECs	1 mg/3 time/week 0-100 $\mu$ M	$\downarrow$ IL-8	Huang <i>et al.</i> , 2002

tion or inflammaging, and its activation is related to the aging process. Thus, suppression of NF- $\kappa$ B is considered a possible target of senomorphics (Mato-Basalo *et al.*, 2021). The 8K-NBD peptide, an inhibitor of NF- $\kappa$ B, decreased cellular senescence and attenuated age-related pathologies in Ercc1<sup>-4</sup>-progeroid mice (Tilstra *et al.*, 2012).

Janus family tyrosine kinase (JAK)-signal transducers and activators of transcription (STAT) modulate the expression levels of some pro-inflammatory cytokines (Novakova *et al.*, 2010). The phosphorylation of JAK tyrosine kinase leads to the phosphorylation of STAT3, which is related to the expression levels of the Bcl-2 family and increases levels of IL-6 and IL-8 receptors (Yu *et al.*, 2009; Banerjee and Resat, 2016). STAT3 persistently activates NF- $\kappa$ B during chronic inflammation (Liang *et al.*, 2013). Thus, JAK inhibitors were applied to improve the anti-tumor response by reprogramming the SASP (Toso *et al.*, 2014). Ruxolitinib is a selective JAK1/2 inhibitor approved by the FDA for improving myelofibrosis (Harrison *et al.*, 2016). Ruxolitinib prevented fat loss, decreased lipotoxicity, and increased insulin sensitivity in middle-aged mice (22-month-old) (Xu *et al.*, 2015a). Another study revealed that ruxolitinib repressed the expression level of SASP in IR- and replicative-induced senescent preadipocytes and IR-induced senescent HUVECs. Ruxolitinib treatment also significantly reduced both systemic and adipose tissue inflammation and improved physical function in aged mice, implying its senomorphic characteristics (Xu *et al.*, 2015b).

Metformin is a well-known drug derived from French lilac for treating type 2 diabetes (Sanchez-Rangel and Inzucchi, 2017). Senescent IMR-90 fibroblasts treated with metformin exhibited the inhibition of SASP by decreasing the expression levels of IL-1 $\beta$ , IL-6, and C-X-C motif chemokine 5 (CXCL5) (Moiseeva *et al.*, 2013). Metformin exhibited senomorphic ac-

tion by suppressing the phosphorylation of I $\kappa$ B, the cytoplasmic inhibitor of NF- $\kappa$ B, and I $\kappa$ B kinase, which inhibits NF- $\kappa$ B (Moiseeva *et al.*, 2013; Barzilai *et al.*, 2016). Currently, metformin is being tested in clinical trials as a next-generation drug for improving the aging process and will be approved by the FDA (Kulkarni *et al.*, 2020). Recently, metformin was shown to ameliorate senescent lens epithelial cells by activating adenosine 5'-monophosphate protein kinase (AMPK) in aged mice (Chen *et al.*, 2022).

Administration of nutlin-3a or MI-63, MDM2 inhibitors, causes growth arrest, significantly reducing the expression levels of IL-6, IL-1 $\alpha$ , and SASP factors in genotoxic-induced senescent cells, eventually repressing the ability of senescent fibroblasts to stimulate the excessive proliferation of breast cancer cells (Wiley *et al.*, 2018).

Another repression strategy may be accomplished using specific neutralizing antibodies against each SASP factor, including IL-1 $\alpha$ , IL-6, and IL-8. IL-1 $\alpha$  plays an important role in the modulation of SASP. Thus, targeting either the IL-1 $\alpha$  receptor or IL-1 $\alpha$  diminished the expression of SASP during senescence (Orjalo *et al.*, 2009). Several studies demonstrated that the neutralizing anti-human IL-1 $\alpha$  monoclonal MABp1 antibody was efficient against type 2 diabetes, inflammation, and colorectal cancer in clinical trials (Dinarello *et al.*, 2012; Timper *et al.*, 2015; O'Sullivan Coyne and Burotto, 2017).

IL-6 is a representative pro-inflammatory cytokine in SASP and is associated with tumor proliferation and immunosuppression. A neutralizing Mab-IL-6.8 monoclonal antibody against IL-6, named olokizumab, significantly blocked STAT signaling (Kuilman *et al.*, 2008) and ameliorated pathological symptoms of arthritis, which is associated with senescent cells, in a primate animal model (Shaw *et al.*, 2014). Silencing of the SASP or a decrease in senescent cells must be de-

**Table 3.** Each candidate senescence-targeting immunotherapeutics for improving age and age-related disorders

Candidate target as surface protein	Expression	Molecular action of therapeutic strategies	References
CD28	human CD8 <sup>+</sup> T cell	Increase of CD28 by ectopic expression or other receptors associated with CD8 <sup>+</sup> T cell activation	Vicente <i>et al.</i> , 2016
CD44	Senescent cells	Antibodies against CD44 directly induce the recruitment of monocytes to a senescent lesion site in senescent endothelial cells	Mun and Boo, 2010
CD58/ICAM1 receptor	Senescent cells	Elimination of senescent cells by NK cells	Vicente <i>et al.</i> , 2016
CD26/dipeptidyl peptidase 4 (DPP4)	Replicative senescent fibroblasts	Elimination of senescent cells by NK cells	Kim <i>et al.</i> , 2017
Glycans or lipids	Senescent cells	Recognized by CD36, IgM on the surface of macrophages	Burton and Stolzing, 2018

tected by the immune system for subsequent elimination and regeneration.

IL-8 is a representative CXC motif chemokine in SASP and is associated with some types of cancer (Coppe *et al.*, 2010; Birch and Gil, 2020). The administration of ABX-IL-8, a humanized monoclonal antibody against IL-8, diminished the growth rate of several cancer xenograft models (Huang *et al.*, 2002; Waugh and Wilson, 2008).

The composites of SASP are complex, and future studies are required to classify SASP in a senescence-dependent manner.

### SENESCENCE-TARGETING IMMUNOTHERAPEUTICS

Another strategy for targeting senescent cells is to strengthen the function of the immune system, a process termed senescence-targeting immunotherapeutics (Table 3). During aging, a decline in immunological function is strongly related to the accumulation of senescent cells. Thus, the physiological role of the immune system in the elimination of senescent cells is crucial (van Deursen, 2014; Ovadya *et al.*, 2018). A previous study demonstrated that perforin knockout mice, which were impaired with the cytotoxic function of natural killer (NK) and T cells, accelerated the accumulation of senescent cells and chronic inflammation (Ovadya *et al.*, 2018). The reduction in the CD28 receptor is a characteristic of human CD8<sup>+</sup> T cell senescence. Senescent T cells have been observed in aged individuals and patients with cancer and arthritis (Vicente *et al.*, 2016), implying that senescent immune cells play a central role in aging-related diseases.

Immune cell systems, such as NK cells, macrophages, and CD8<sup>+</sup> T cells recognize and eliminate senescent cells (Birch and Gil, 2020; Borghesan *et al.*, 2020; Salminen, 2021). It is possible to diminish the number of senescent immune cells using specific antibodies that recognize senescence surface markers. Therefore, it is important to identify senescent cell surface markers (Kim *et al.*, 2017; Burton and Stolzing, 2018). The expression of CD44, a senescence-induced cell adhesion gene, was increased during the aging process in rat aorta endothelium. Antibodies against CD44 could significantly reduce the recruitment of monocytes to senescent lesion sites in senescent endothelial cells (Mun and Boo, 2010). NK cells

recognized the CD58/ICAM1 receptor that exists in senescent cells (Vicente *et al.*, 2016). Another study found that CD26/dipeptidyl peptidase 4 (DPP4) was expressed on the membrane surface of replicative senescent fibroblasts (Kim *et al.*, 2017). Kim *et al.* (2017) utilized DPP4 as a membrane target to facilitate antibody-dependent cell-mediated cytotoxicity, a mechanism of cell-mediated immune defense in NK cells, to eliminate senescent cells. They revealed that DPP4 helped NK cells recognize and kill senescent cells, implying a possible immunotherapeutic approach for clearing senescent cells. In the case of macrophages, these modified membrane receptors, including glycans and lipids, in senescent cells were recognized by receptors such as CD36 and IgM, which are present in macrophages (Burton and Stolzing, 2018). In addition, it is possible to increase the binding affinity of associated receptors. The use of chimeric antigen receptor T cell therapy to target specific senescent-associated molecules can be a meaningful strategy. This strategy has been currently applied in anticancer therapy (Amor *et al.*, 2020; Marofi *et al.*, 2021).

Another strategy is to rejuvenate senescent immune cells by reversing their abnormal functions to acquire immune functions. The functions of NK and T cells significantly decrease during aging. Stimulation of the nutrient-sensing component AMPK seemed to play a key role in the aging process (Akbar, 2017). Thus, inhibition of AMPK activity enhanced the functions of senescent immune cells (Di Mitri *et al.*, 2011). Similarly, suppression of p38 signaling, which is characteristic of senescent CD8<sup>+</sup> T cells, recovered proliferation and mitochondrial biogenesis (Henson *et al.*, 2014). In addition, the decline in the CD28 receptor was a representative characteristic of CD8<sup>+</sup> T cell senescence (Vicente *et al.*, 2016). Thus, the increase in CD28 by ectopic expression or other receptors associated with T cell activation could improve the senescence of CD8<sup>+</sup> T cells.

These results suggest that various immune cells play pivotal roles in delaying the onset of diseases caused by the accumulation of senescent cells.

### DISCUSSION

Recent evidence has suggested a strong relationship between cellular senescence, aging, and age-related diseases. Recent rapid progress in senotherapeutics has accelerated

the research field of aging and has contributed to the development of therapeutic strategies for delaying the human aging process (Burton and Stolzing, 2018; van Deursen, 2019; Birch and Gil, 2020). In this review, we provided insights into the current progress in the understanding of the molecular mechanisms of senotherapeutics.

Even though there has been much progress in the development of senotherapeutics, some crucial issues must be overcome in future clinical trials. Although no side effects of some senolytics have been observed in preclinical *in vivo* animal studies, the potential adverse effects of long-term clinical treatments with senotherapeutics should be carefully investigated for their successful application in aging and age-related diseases. Some senolytic drugs have been repositioned to improve aging and age-related diseases, but they may have unwanted side effects during long-term use. For example, ABT-263 (navitoclax) caused severe thrombocytopenia and neutropenic disorders and could not be used for targeting aging (Rudin *et al.*, 2012; Cleary *et al.*, 2014; Sharma *et al.*, 2020). Thus, the safety of long-term senotherapeutics must be considered.

Tissue atrophy is another problem that can occur because of the large-scale elimination of senescent cells by senotherapeutics (He and Sharpless, 2017). Accumulation of apoptotic senescent cells in specific tissues may have undesirable side effects.

Thus, treatments with senotherapeutics must be clinically applied without affecting efficacy or causing side effects. To accomplish this objective, future intensive studies need to be carried out to present a framework encompassing the molecular mechanisms of senescence and to understand how to improve these novel interventions for aging treatment.

## CONFLICT OF INTEREST

The authors have no conflicting interests.

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