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JB Review

Therapeutic strategies targeting cellular senescence for cancer and other diseases

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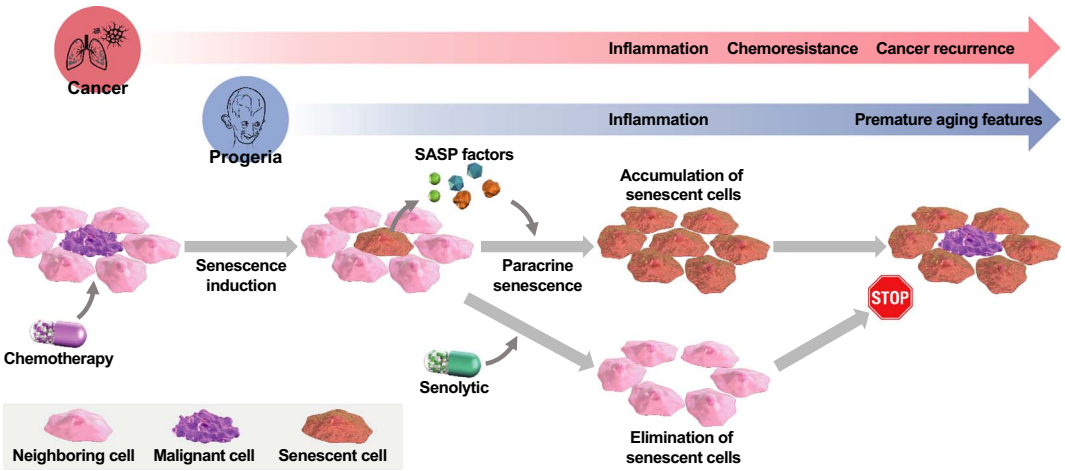
Cellular senescence occurs in response to endogenous or exogenous stresses and is characterized by stable cell cycle arrest, alterations in nuclear morphology and secretion of proinflammatory factors, referred to as the senescence-associated secretory phenotype (SASP). An increase of senescent cells is associated with the development of several types of cancer and aging-related diseases. Therefore, senolytic agents that selectively remove senescent cells may offer opportunities for developing new therapeutic strategies against such cancers and aging-related diseases. This review outlines senescence inducers and the general characteristics of senescent cells. We also discuss the involvement of senescent cells in certain cancers and diseases. Finally, we describe a series of senolytic agents and their utilization in therapeutic strategies.

Keywords: aging, cancer, cellular senescence, premature aging (progeria) syndromes, senescence- and aging-related diseases, senolytic agents.

Graphical Abstract



Inducers of Cellular Senescence

Cellular senescence is a state of stable cell cycle arrest triggered by various stimuli *(1, 2)*. In this review, we first outline how telomere shortening, oncogene activation and anticancer treatments elicit replicative senescence (RS), oncogene-induced senescence (OIS) and therapy-induced senescence (TIS), respectively.

Replicative senescence

Replicative senescence (RS) is the first type of cellular senescence discovered, and it was first described in 1961, by scientists at the Wistar Institute *(3)*. In this pioneering study, primary human fibroblasts were observed to reach a state of irreversible cell cycle arrest after exhaustion of their finite replication capacity. Subsequently, it was shown that RS is caused by telomere shortening *(3, 4)*. Mechanistically, short telomeres induce a DNA damage response (DDR); consequently, the p53 tumor suppressor is phosphorylated and stabilized, leading to p21-dependent cell cycle arrest *(2, 5, 6)*.

Oncogene-induced senescence

The activation of oncogenes can trigger cellular senescence, known as oncogene-induced senescence (OIS)

(1, 6). In 1997, the induction of oncogenic RAS was demonstrated to cause senescence in human fibroblasts *(7)*. Additionally, loss of tumor suppressors, such as phosphatase and tensin homolog (PTEN), has been found to induce senescence *(8)*. Genes that induce OIS are often involved in RAS signaling, PI3K/AKT and ARF/p53 pathways and dysregulation of these pathways triggers excessive cell proliferation and subsequently causes senescence through DDR *(6, 9)*.

Therapy-induced senescence

Anticancer treatments, such as chemotherapy and radiotherapy, can induce therapy-induced senescence (TIS) *(10)*. Anticancer treatments cause DNA damage and trigger DDR mediated by the ATM/CHK2 and ATR/CHK1 pathways, leading to the activation of p53 *(11)*. The degree of DNA damage determines whether cells become senescent or apoptotic after treatment. Low-dose chemotherapy has a senescence-promoting effect, whereas high-dose chemotherapy tends to cause apoptosis *(12)*. Senescent cancer cells exert an intricate influence on oncogenic processes, as discussed in a later section *(13)*.

Features of Senescent Cells

Cellular senescence is triggered by a variety of cellular stressors. Senescent cells have unique characteristics associated with stable cell cycle arrest, chromatin changes, epigenetic regulation and senescence-associated secretory phenotype (SASP), as described in this section.

Cell-cycle arrest

The activation of p21 and p16 contributes to cell cycle arrest in senescent cells *(2, 6)*. Specifically, p21 inhibits CDK1/2, and its activation is part of the DDR and is mediated by p53 *(14)*. Additionally, p21 can be activated in a p53-independent manner [e.g. the pathway associated with transforming growth factor-β (TGF-β)] *(14, 15)*. On the other hand, p16 inhibits CDK4/6, and its expression is regulated by multiple factors, such as the peroxisome proliferator-activated receptor γ (PPARγ) and the Ets family transcription factor *(16–18)*. Moreover, p21 is activated in the early stage of senescence and is essential for senescence entry and the initiation of cell cycle arrest, whereas p16 is activated in later stages and is responsible for the maintenance of cell cycle arrest in senescent cells *(19–21)*.

Chromatin changes

Senescence-associated heterochromatic foci (SAHF) are DNA foci that accumulate in senescent cells *(22)*. This important study showed that the p16/Rb pathway is required for SAHF formation and E2F-target gene repression *(22)*. SAHF are enriched in repressive epigenetic modifications, including histone H3K9me3 and H3K9me2 *(22–24)*. Moreover, SASP genes are excluded from SAHF, allowing for their expression in senescent cells *(25)*.

Senescence-associated distension of satellites (SADS) is another specific DNA structure detected in senescent cells *(26)*. SADS is defined as decondensed pericentromeric satellites and is commonly observed in various types of senescent cells, and they can be formed in the absence of either the p21 or p16 pathway.

Lamin B1, a structural component of nuclear lamina, is downregulated in senescent cells *(27)*. The degradation of lamin B1 by autophagy promotes the formation of cytoplasmic chromatin fragments (CCF) and SAHF *(28–30)*. CCF enriched in DNA damage markers, such as γH2AX, activate the cGAS/STING pathway and trigger proinflammatory responses *(28, 31)*.

Epigenetic regulation

Epigenetic reprogramming occurs during senescence *(32, 33)*. For instance, senescent cells show a reduction in overall DNA methylation levels, mainly resulting from DNA hypomethylation of heterochromatin domains *(34)*. Moreover, DNA methylation can be moderately enhanced in euchromatin domains and contributes to the repression of some cell cycle genes *(34, 35)*. Notably, alterations in genome-wide DNA methylation patterns in senescent cells resemble those observed in some cancers, implying that these cancers may originate from senescent cells. In addition, the histone acetyltransferase p300 induces the formation of super-enhancers in senescent cells and promotes the expression of senescence-related genes *(36)*. Moreover, the histone deacetylase SIRT1, which represses the expression of some SASP genes, is downregulated in senescent cells *(37)*. These findings illuminate the possibility that pharmacological modulation of epigenetic regulators can alter the expression of SASP genes or their subsets.

Senescence-associated secretory phenotype

Senescence-associated secretory phenotype (SASP) factors include chemokines, cytokines and extracellular matrix proteases. SASP factors are secreted from senescent cells and contribute to chronic inflammation and senescence-related diseases *(1, 38)*. The expression of SASP genes is highly heterogeneous and dependent on senescence inducers and cell type *(39, 40)*. Nuclear factor-κB (NF-κB) is considered one of the main regulators of SASP genes *(41)*. Additionally, other transcription factors, including CCAAT/enhancer-binding protein-β (C/EBPβ) and GATA4, are also involved in the activation of SASP genes *(42, 43)*. Moreover, the NOTCH1 and JAK2/STAT3 pathways are associated with the regulation of SASP genes *(44, 45)*.

Roles of Senescence in Cancer

As described above, the SASP is a significant feature of senescent cells, and SASP factors have contradictory anti- and pro-tumorigenic effects *(13, 46)*. The anti-tumorigenic functions of senescent cells are primarily associated with stable cell cycle arrest and immune surveillance *(12, 47)*. Oncogene activation triggers OIS and causes stable cell cycle arrest in premalignant cells via the p53/p21 and p16/Rb pathways, thus preventing immortalization of these cells *(48, 49)*. For instance, senescent cells accumulate in some benign and premalignant tumors (e.g. skin moles and premalignant prostatic intraepithelial neoplasia), suggesting that cellular senescence may prevent malignant transformation of these lesions. It has also been shown that the inactivation of cell cycle inhibitors, such as p21 and p16, is prone to develop tumors *(50, 51)*. It is worth noting that 14% to 80% of human tumors have inactive

p21 or p16, while oncogene mutations such as oncogenic RAS are observed in around 19% of tumors *(52–54)*. These studies demonstrate the crucial role of senescence-related cell cycle arrest in tumor suppression. Moreover, activation of the p53/p21 pathway facilitates the production of p21-activated secretory phenotype (PASP) factors, including CXCL14 chemokine, which recruits macrophages to remove premalignant senescent cells *(55, 56)*.

In contrast, the long-term persistence of senescent cells shows SASP-mediated pro-tumorigenic effects, such as chronic inflammation, angiogenesis and immune evasion *(12)*. For instance, the secretion of interleukin (IL)-6 SASP factor is involved in tumorigenesis through promoting angiogenesis *(13, 57)*. Similarly, senescent cells secrete vascular endothelial growth factors (VEGFs) that facilitate angiogenesis and tumorigenesis *(58)*. In addition, matrix metalloproteinase (MMP) factors secreted by senescent cells promote tumor growth by disrupting the extracellular matrix *(59)*. Moreover, some SASP factors promote immune evasion; IL-6 secreted by senescent stroma cells contributes to the recruitment of myeloid-derived suppressor cells (MDSCs) to the tumor microenvironment (TME) and compromises antitumor immune surveillance *(60)*.

In addition to paracrine effects, senescent cancer cells can escape cell cycle arrest and reproliferate, thereby contributing to tumor recurrence *(61–63)*. Senescence-escaping cancer cells exhibit the acquisition of chemotherapy resistance, stemness and increased aggression *(61, 62)*.

Involvement of Senescence in Human Diseases Other than Cancer

In addition to cancer, cellular senescence has both beneficial and detrimental effects in many aging-related diseases *(6, 64)*. Some diseases that present with fibrosis in organs such as the liver, skin, kidneys and heart, and conditions, such as atherosclerosis and pulmonary hypertension, may benefit from senescence *(6)*. In noncancerous hepatic fibrosis, SASP factors produced by senescent cells contribute to extracellular matrix degradation, and immune cells are attracted to the microenvironment to remove senescent hepatic stellate cells, thereby mitigating disease progression *(6, 65)*.

Certain diseases are promoted by detrimental effects of physiological and pathological senescence *(6, 64)*. Physiological senescence-associated diseases include some lifestyle-related diseases such as type 2 diabetes, aging-related diseases such as sarcopenia and osteoporosis, and neurodegenerative diseases such as Alzheimer's disease *(6, 64)*. For instance, senescent cells tend to accumulate in the adipose tissues of diabetic mice, contributing to insulin resistance *(66)*. Additionally, human geriatric satellite cells in the muscles exhibit senescence tendencies, which accounts for the loss of muscle regenerative potential in sarcopenia *(67)*.

Pathological senescence-associated diseases are closely associated with premature aging syndromes caused by genetic variations or mutations in genes encoding nuclear envelope proteins, DNA repair enzymes and telomere maintenance factors *(64)*. For instance, Hutchinson–Gilford progeria syndrome (HGPS) is caused by a heterozygous point mutation in lamin A *(68)*. Patients with HGPS have several aging-associated characteristics shortly after birth, including alopecia and loss of subcutaneous fat and skeletal muscles *(69)*. Preclinical evidence shows that the loss of lamin A causes the accumulation of senescent cells and accelerates aging phenotypes in mice *(70)*. Furthermore, other pathological senescence-associated diseases, such as Nestor–Guillermo progeria syndrome and atypical Werner syndrome, are caused by mutations in genes encoding the nuclear envelope architecture *(64)*. With the exception of nuclear envelope architectural proteins, mutations in genes encoding DNA repair enzymes and telomere maintenance factors cause pathological senescence-associated diseases *(64)*. Xeroderma pigmentosum and Cockayne syndrome are caused by mutations in genes involved in the nucleotide excision repair (NER) pathway *(71, 72)*. Bloom syndrome, classical Werner syndrome and ataxia telangiectasia are caused by mutations in genes involved in double-strand DNA damage repair *(73–75)*.

Development of Senolytic Agents

As cellular senescence is implicated in various types of cancer and other diseases, the potential use of senolytic agents, which remove senescent cells, is a promising approach for treating these conditions. Pharmacological removal of senescent cells was initially difficult, and the transgenic mouse models, such as INK-ATTAC and p16-3MR, were employed to provide early evidence that the elimination of senescent cells is beneficial for treating human diseases *(76, 77)*. INK-ATTAC and p16-3MR transgenic mice can selectively eliminate p16-expressing cells, reduce SASP expression and attenuate age-related pathologies *(76, 77)*. The first study of senolytics revealed that the combination of dasatinib and quercetin effectively eliminates senescent cells *(78)*. Since then, many senolytic agents have been developed to target different kinds of senescent cells and are predicted to have beneficial effects on senescence-related diseases *(2, 6)*. Before discussing their medical applications, we introduce senolytic agents categorized by their targets (Fig. 1 and Table 1).

BH3 mimetics targeting BCL-2-like proteins

Senescent cells are resistant to apoptosis via the upregulation of BCL-2-like proteins (e.g. BCL-2, BCL-W and BCL-XL), which inhibit the pro-apoptotic proteins BAX and BAK *(85, 104, 105)*. BH3-only proteins (e.g. BAD, BID and NOXA) interact with and inhibit the anti-apoptotic BCL-2-like proteins; therefore, BH3 mimetics that specifically inhibit BCL-2-like proteins can promote apoptosis *(106)*. For instance, the BH3 mimetics ABT-263 and ABT-737 act as senolytics, removing senescent cells via the apoptotic pathway *(82, 83, 85)*. Treatment with ABT-263, an inhibitor of BCL-2, BCL-W and BCL-XL, reduces the viability of various human and mouse primary senescent cells and removes senescent cells in aged mice *(82, 83)*. ABT-737 inhibits BCL-W and BCL-XL and removes senescent cells in mouse lungs and skin *(85)*. Other BH3 mimetics, A1331852 and A1155463, selectively inhibit BCL-XL and remove senescent human fibroblasts *(89)*. Moreover, the curcumin analog EF24, which is not a BH3 mimetic, facilitates the degradation of BCL-2-like proteins

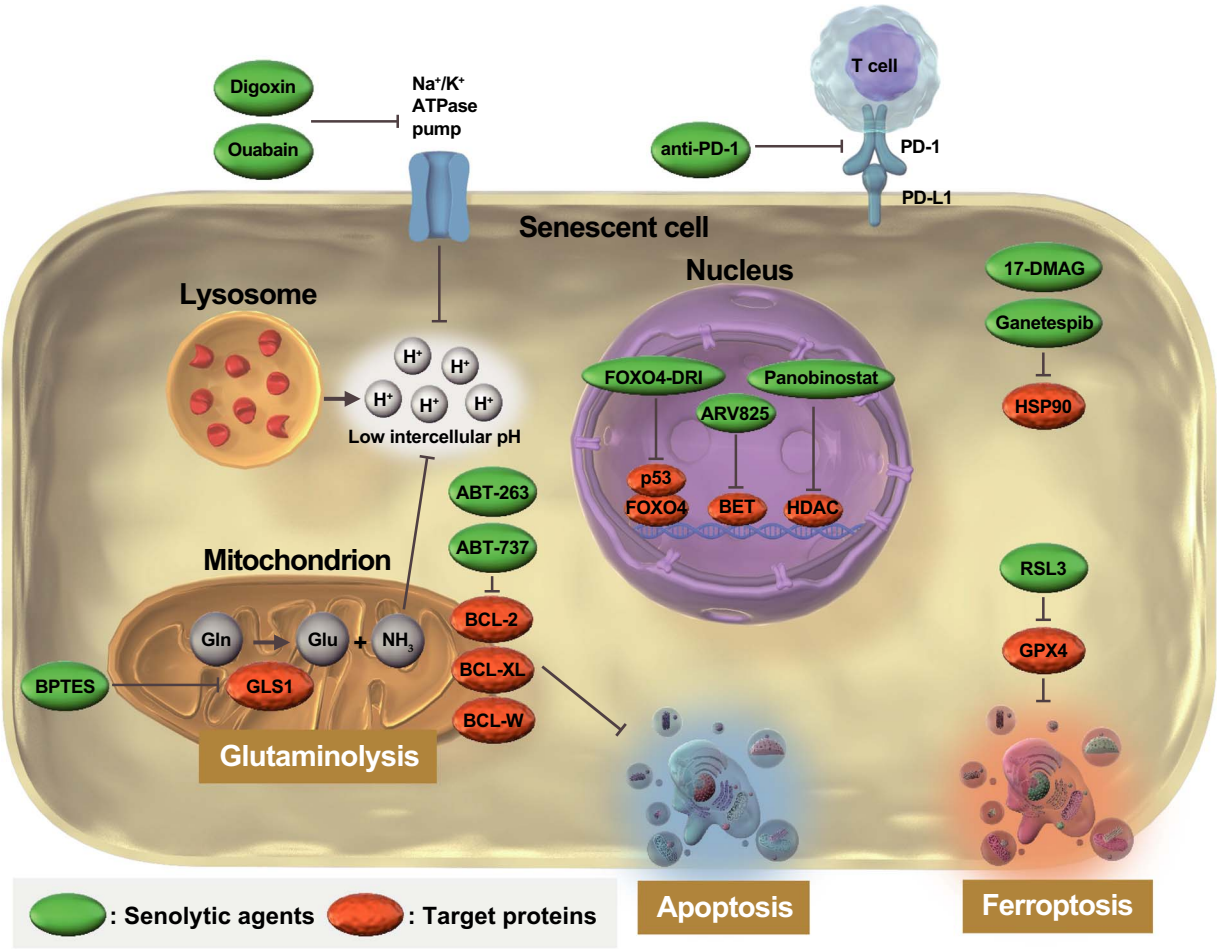


Fig. 1. Mode of action of senolytic agents. Certain senolytic agents are summarized schematically in the figure. Please see the text for further details on other senolytic agents. Abbreviations: FOXO4, forkhead box protein O4; BET, bromo- and extra-terminal domain protein; HDAC, histone deacetylase; Gln, glutamine; Glu, glutamate; NH_3, ammonia; GLS1, glutaminase-1; BCL-2, B-cell lymphoma 2; BCL-XL, B-cell lymphoma-extra-large; BCL-W, BCL-2-like protein 2; GPX4, glutathione peroxidase 4.

(BCL-2, BCL-XL and MCL-1), thereby promoting apoptosis in senescent cells *(90)*.

Efforts to overcome problems with ABT-263

ABT-263 is one of the most potent senolytic agents *(105)* that exhibits platelet toxicity *(107, 108)*. To reduce the toxicity of ABT-263 (navitoclax), it is either chemically conjugated with a cleavable galactose (Nav-Gal) or encapsulated with galacto-oligosaccharides [GalNP(nav)], preferentially inducing its activation in senescent cells *(86, 87)*. Moreover, 753B, a BCL-2/BCL-X proteolysis-targeting chimera (PROTAC), was developed to remove chemotherapy-induced senescent cells derived from acute myeloid leukemia *(88, 109)*. 753B exhibits less platelet toxicity than that of ABT-263 because platelets lack the expression of Von Hippel–Lindau (VHL) E3 ligase. In addition, recent studies revealed that MCL-1 plays a vital role in the survival of senescent cells tolerant to ABT-263 treatment *(84, 96)*. Therefore, combination treatment with the MCL-1 inhibitor S63845 and ABT-263 effectively removes various chemotherapy-induced senescent cancer cells that are not responsive to ABT-263 treatment alone *(84)*. Moreover, S63845 removes senescent prostate cancer cells in mouse models *(96)*.

Agents targeting autophagy and metabolic pathways

Senescent cells are metabolically active and manifest the upregulation and secretion of SASP factors *(1)*. Senescent cells producing SASP factors tend to accumulate misfolded proteins, which are degraded by autophagy in an energy-consuming manner *(80)*. Therefore, senescent cancer cells derived from chemotherapeutic drug treatment are vulnerable to inhibition of the autophagy and energy-generating catabolic pathways. For instance, treatment of chemotherapy-induced senescent cells with a bafilomycin A1 autophagy inhibitor or glucose metabolism inhibitors, such as phloretin, cytochalasin B or sodium oxamate, causes apoptosis. Other autophagy-related senolytics, such as azithromycin and roxithromycin, remove senescent fibroblasts by activating autophagy *(81)*. Notably, either promotion or inhibition of the autophagy pathway can lead to apoptosis in a context-dependent manner *(110)*.

Agents targeting intracellular pH regulation

Another feature of senescent cells is the accumulation of lysosomes, which are prone to damage *(1)*. Damage to the lysosomal membrane lowers the pH of senescent cells by causing leakage of lysosomal protons *(93)*. Senolytics exploiting the low pH of senescent cells have been

Table 1. Senolytic agents and their applications

Agent	Target	Application	Cell type[†]	Clinical trial[††]	Reference
Panobinostat	Histone deacetylase	TIS	A549, FaDu, H460, UMSCC47		*(79)*
Bafilomycin A1	Autophagy	TIS	Murine lymphoma cells		*(80)*
Azithromycin	Autophagy	TIS	MRC-5, BJ		*(81)*
Roxithromycin	Autophagy	TIS	MRC-5, BJ		*(81)*
ABT-263	BCL-2 BCL-X	TIS, OIS, RS, aged mice	HUVECs, IMR90, MEFs HSCs, MuSCs, SKBR7, Cal51, 4226, A549, U2OS	Phase 1 (NCT00878449, NCT00887757, NCT00888108, NCT00891605, NCT01009073, NCT05358639)	*(82–84)*
ABT-737	BCL-2 BCL-X	TIS, OIS, RS	IMR90, murine lung and epidermal cells		*(85)*
GalNP(nav)	BCL-2 BCL-X	TIS, pulmonary fibrosis	SK-MEL-103, murine lung cells		*(86)*
Nav-Gal	BCL-2 BCL-X	TIS, OIS	A549, L1475, SK-MEL-103, 4T1, HCT116, IMR90, MLg		*(87)*
753B	BCL-2 BCL-X	TIS	MOLM-14		*(88)*
A-1331852	BCL-X	TIS	HUVECs, IMR90,		*(89)*
A-155463	BCL-X	TIS	HUVECs, IMR90,		*(89)*
EF24	BCL-X Mcl-1	TIS, OIS, RS	WI-38, IMR-90, HUVECs, HRECs		*(90)*
ARV825	BET family	TIS, OIS, RS	IMR-90, TIG-3, HSCs, HCT116		*(91)*
FOXO4-DRI	FOXO4 p53	TIS, aged mice	IMR-90, murine kidney cells		*(92)*
BPTES	GLS1	OIS, aged mice	HCA2, IMR-90, murine liver kidney, lung and adipose cells		*(93)*
C968	GLS1	OIS, aged mice	HCA2, IMR-90, murine liver, kidney, lung and adipose cells		*(93)*
CB-839	GLS1	OIS, aged mice	HCA2, IMR-90, murine liver, kidney, lung and adipose cells		*(93)*
Phloretin	Glucose metabolism	TIS	Murine lymphoma cells		*(80)*
Cytochalasin B	Glucose metabolism	TIS	Murine lymphoma cells		*(80)*
Sodium oxamate	Glucose metabolism	TIS	Murine lymphoma cells		*(80)*
RSL3	GPX4	TIS, aged mice, kidney mice model	PTECs, murine kidney cells		*(94)*
17-DMAG	HSP90	TIS, RS	IMR-90, WI-38, MEFs		*(95)*
Ganetespib	HSP90	TIS, RS	IMR-90, WI-38, MEFs		*(95)*
S63845	MCL-1	TIS	HCC712, MDA-MB-175, MCF-7, HCC1428, ZR75–30, MPE600, T47D, PC3, LNCaP, TrampC1, RapidCap		*(84 , 96)*
Digoxin	Na^+/K^+ ATPase pump	TIS, OIS, RS	IMR-90, PBECs, SK-MEL-5, MCF-7, HCT116, A549, BJ, PDX of breast cancer		*(97 , 98)*
Ouabain	Na^+/K^+ ATPase pump	TIS, OIS, RS	IMR-90, PBECs, SK-MEL-5, MCF-7, HCT116, A549, BJ, PDX of breast cancer		*(97 , 98)*
Piperlongumine	OXR1	TIS, OIS, RS	WI-38		*(99)*
Anti-PD-1	PD-1/PD-L1	TIS, OIS, aged mice	HCA2, murine pancreatic cancer cells	Phase 1 (NCT04360941)	*(100 , 101)*
Quercetin	PI3K	TIS	HUVECs, human preadipocytes	Phase 2 (NCT02848131, NCT04733534)	*(78)*
Fisetin	PI3K	TIS	HUVECs	Phase 2 (NCT04733534)	*(89)*
GalNP doxorubicin	TOP2	TIS, pulmonary fibrosis	SK-MEL-103, murine lung cells		*(86)*
Dasatinib	Tyrosine kinase	TIS	HUVECs, human preadipocytes	Phase 2 (NCT02848131, NCT04733534)	*(78)*
uPAR-specific CAR T cells	uPAR	TIS, CCl_4 and NASH-induced liver fibrosis	PDX of lung cancer, murine cells		*(102)*
NKG2D-specific CAR T cells	NKG2D	TIS, OIS, RS, aged mice	IMR-90, WI-38, HEL1, murine cells		*(103)*

[†]Cell lines were used in the indicated references.

[††]Clinical trials aim to reduce senescent cells or combine senolytic agents with chemotherapeutic drugs in cancer treatment. Abbreviations: TIS, therapy-induced senescence; OIS, oncogene-induced senescence; RS, replicative senescence; HUVECs, human umbilical vein epithelial cells; MEFs, murine embryonic fibroblasts; HSCs, hematopoietic stem cells; MuSCs, muscle stem cells; GalNP(nav), 6-mer galacto-oligosaccharide encapsulated ABT-263 (navitoclax); Nav-Gal, ABT-263 with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide conjugation; HRECs, human renal epithelial cells; PDX, patient-derived xenograft; PBECs, primary bronchial epithelial cells; PTECs, primary tubular epithelial cells; NASH, non-alcoholic steatohepatitis.

developed *(93, 97, 98)*. Glutaminolysis promotes the survival of senescent cells by neutralizing intracellular pH *(93)*. Thus, treatment with the glutaminase 1 (GLS1) inhibitor BPTES removes senescent cells from various tissues (e.g. liver, kidney, lung and adipose) in mice and ameliorates aging-associated organ dysfunction *(93)*. In addition, cardiac glycosides, such as digoxin and ouabain, inhibit the Na^+/K^+ ATPase pump, which is coupled to the normal function of the Na^+/H^+ exchanger, leading to increased intracellular H^+ concentrations *(98)*. Because senescent cells already have high intracellular H^+ concentrations, they are vulnerable to cardiac glycoside treatment.

Agents targeting the immune system

Senescent cells can be removed through immune surveillance. Senescent cells heterogeneously express PD-L1, and PD-L1-positive senescent cells are resistant to T cell surveillance. Therefore, anti-PD-1 treatment, which blocks PD-L1/PD-1 immune checkpoint, removes senescent cells and ameliorates aging-associated dysfunction *(100)*. Furthermore, urokinase-type plasminogen activator receptor (uPAR) serves as a specific cell-surface marker of senescent cells, and uPAR-specific CAR-T (chimeric antigen receptor T) cells selectively remove senescent cells *(102)*. This system, involving uPAR-specific CAR-T cells, extends the survival of mice with lung adenocarcinoma treated with senescence-inducing drugs. Moreover, a recent study shows that natural killer group 2 member D (NKG2D) ligands are upregulated in senescent cells, and treatment with NKG2D-specific CAR-T cells reduces senescent cells and alleviates senescence-associated pathologies *(103)*.

Agents targeting kinases

Treatment with dasatinib, an inhibitor of tyrosine kinases, effectively removes senescent human preadipocytes. The combination treatment with dasatinib and quercetin [phosphatidylinositol-3 kinase (PI3K) inhibitor] removes senescent cells derived from mouse embryonic fibroblasts as well as human umbilical vein endothelial cells (HUVECs) and preadipocytes *(78)*. A clinical trial shows that combination treatment with dasatinib and quercetin reduces senescent cells in individuals with diabetic kidney disease. This is the first report describing that the senolytic treatment reduces senescent cells in human individuals *(111)*.

Natural products

Quercetin, a plant flavonol, has a senolytic effect on senescent HUVECs, and combined treatment with quercetin and dasatinib successfully reduces senescent cells in adipose tissues of humans with diabetic kidney disease *(78, 111)*. Piperlongumine (an alkaloid found in pepper) and fisetin (a plant flavonol) are natural products with senolytic effects *(89, 99)*. Piperlongumine removes senescent human cells (WI-38 fibroblasts) derived from replicative exhaustion, ionizing radiation, or oncogene expression *(99)*. Fisetin selectively induces apoptosis in senescent HUVECs but not in other cell lines, including IMR-90 lung fibroblasts *(89)*.

Other senolytics

RSL3 inhibits a GPX4 ferroptosis regulator and triggers ferroptosis, thus removing senescent cells from aged mouse kidneys *(94)*. ARV825, which promotes the degradation of BET family proteins, removes senescent stellate cells from obese mouse livers and chemotherapy-induced senescent HCT116 cells (a human colon cancer cell line) *(91)*. Panobinostat, a histone deacetylase inhibitor, removes chemotherapy-induced senescent cancer cells by reducing the expression of anti-apoptotic BCL-XL and increasing histone H3 acetylation *(79)*. Treatment with FOXO4-DRI, a peptide inhibitor of the interaction between p53 and FOXO4, removes ionizing radiation-induced and chemotherapy-induced senescent IMR-90 cells and restores fitness, fur density and renal function in aged mice *(92)*. Treatment with HSP90 inhibitors, including 17-DMAG and ganetespib, has shown senolytic effects on senescent primary cells *(95)*.

Senomorphic agents

Apart from senolytic agents that directly target and eliminate senescent cells, senomorphic agents target SASP-regulating pathways and inhibit SASP *(2)*. It is shown the first-line antidiabetic drug metformin inhibits SASP gene expression in oncogenic RAS-induced senescent IMR-90 cells by blocking the activity of the key SASP regulator NF-κB *(112)*. The mTOR inhibitor rapamycin is another senomorphic agent, and its treatment extends the mouse lifespan *(113)*. Moreover, JAK/STAT pathway inhibitors have senomorphic effects by suppressing SASP gene expression in senescent preadipocytes and reduce inflammation in old mice *(114)*. These senormorphic agents are expected to have therapeutic effects on senescence-related diseases by inhibiting SASP.

Chemo-Senolytic Approach for Cancer

Here, we describe how these senolytic agents have been considered for cancer therapy. Preclinical studies show that the 'one-two punch' sequential treatment with chemotherapeutic drugs and senolytic agents, referred to as the chemo-senolytic approach in this review, effectively removes various types of cancer cells *(12)*. Using cancer cell lines and xenograft mouse models, combination treatment with chemotherapeutic drugs, alisertib, etoposide or doxorubicin, and subsequently with an ABT-263 senolytic agent demonstrates efficient removal of lung, colon, breast and liver cancers *(84, 115, 116)*. Clinical trials for various cancers have begun to evaluate the efficacy of chemo-senolytic approaches in combination with chemotherapeutic drugs (etoposide, cisplatin, paclitaxel and olaparib) and ABT-263 senolytic agents (see clinical trials in Table 1). In this context, we have also demonstrated that the chemo-senolytic approach is promising for rare refractory tumors; combinational treatment with chemotherapeutic drugs (cisplatin or paclitaxel) and ABT-263 effectively removes angiosarcoma cells *(117)*. As described in the aforementioned section, ABT-263 has a platelet toxicity *(107, 108)*, and improved ABT-263, Nav-Gal and GalNP(nav) can selectively remove senescent lung cancer and melanoma cells, which are derived from treatment with cisplatin and palbociclib, respectively

(86, 87). BCL-2/BCL-X PROTAC, 753B, can remove chemotherapy (Ara-C)-induced senescent cells derived from acute myeloid leukemia (AML) *(88)*. Nav-Gal, GalNP(nav) and 753B are expected to have less toxicity in clinical trials compared to the original ABT-263.

In addition to ABT-263, other senolytic agents have been tested for chemo-senolytic approach in preclinical models. Treatment with a histone deacetylase inhibitor (panobinostat), MCL-1 inhibitor (S63845), degrader of BET family proteins (ARV825), or Na^+/K^+ ATPase pump inhibitors (digoxin and ouabain) removes various senescent cancer cells derived from treatment with chemotherapeutic drugs, including doxorubicin, etoposide and palbociclib *(79, 84, 91, 96–98)*. In addition, the HSP90 inhibitor (ganetespib) removes senescent prostate cancer cells derived from treatment with the R1881 androgen receptor agonist *(118)*. Peptides that disrupt the interaction between FOXO4 and TP53 can also remove senescent breast and colon cancer cells derived from doxorubicin treatment *(119)*.

Senescent cancer cells can also be removed by the immune system. In a mouse pancreatic cancer model, senescent cancer cells promote vascular remodeling, making them vulnerable to PD-1 blockade immunotherapy *(101)*. In addition, uPAR-specific CAR-T cells selectively remove senescent lung cancer cells, delaying tumor growth in a xenograft model *(102)*.

Senolytic treatment affects not only senescent cancer cells but also the tumor microenvironment (TME) *(12, 47)*. For instance, radiation or chemotherapy with cisplatin and docetaxel causes cellular senescence of cancer-associated fibroblasts (CAFs) in patients with rectal and ovarian cancer, and senescent CAFs mediate chemoresistance via the secretion of SASP factors. Therefore, the removal of senescent CAFs using ABT-263 enhances the vulnerability of cancer cells to chemotherapy *(120, 121)*. Another study shows that senescent neutrophils are increased in the TME of prostate cancer and that immunosuppressive neutrophils promote tumor growth. Thus, removal of senescent neutrophils by treatment with the histone deacetylase inhibitor romidepsin improves the efficacy of chemotherapy *(122)*.

Senolytic Approaches for Diseases Other than Cancer

Senolytic agents are used to treat several diseases other than cancer. Dasatinib and quercetin were the first senolytics reported to remove senescent cells in humans *(111)*. Other senolytics have been shown to remove disease-associated senescent cells in preclinical models *(123)*. For example, ABT-263 removes senescent foamy macrophages from atherosclerotic lesions and alleviates the detrimental effects of senescent cells *(124)*. Renal epithelial cells become senescent after injury and release SASP factors, promoting fibrosis and inhibiting tubular proliferative activity *(125)*. ABT-263 treatment removes senescent renal epithelial cells, restores proliferative ability and reduces fibrosis in injured kidneys. Moreover, ABT-263 treatment induces apoptosis in senescent human pulmonary endothelial cells and promotes the reversal of the hemodynamic and structural changes associated with pulmonary arterial hypertension *(126)*.

In addition to ABT-263, other senolytic agents have been tested in preclinical senescence models *(123)*. Digoxin, a cardiac glycoside, effectively removes senescent cells and reduces lung fibrosis in a mouse model *(98)*. FOXO4-DRI, a peptide inhibitor of the interaction between p53 and FOXO4, removes senescent cells and restores fitness, hair density and renal function in premature and naturally aging mouse models *(92)*. BPTES removes senescent cells and ameliorates senescence-associated organ dysfunction in aging mice *(93)*. Moreover, combination treatment with dasatinib and quercetin attenuates the transcriptional and cellular dysfunction of Down syndrome neural progenitor cells *(127)*.

Prospects

Researchers have recently found that the loss of epigenetic information contributes to the senescence of mammalian cells, and overexpression of the Yamanaka factors OCT4, SOX2 and KLF4 can reverse the epigenetic changes associated with aging *(128)*. A recent study also shows that overexpression of SOX5 alone can promote rejuvenation *(129)*. Mechanistically, SOX5 promotes the expression of HMGB2, which contributes to the rejuvenation of cartilage and alleviation of osteoarthritis in aged mice *(129)*. Moreover, the speed of transcription elongation is increased in senescent cells, and histone overexpression slows down RNA polymerase II elongation and delays the senescence of IMR-90 cells, demonstrating that the maintenance of chromatin structure plays an important role in longevity *(130)*. Because epigenetic alterations are reversible, chemical interventions can potentially be developed to promote rejuvenation by reversing the epigenetic changes that occur during aging. Moreover, mitochondrial outer membrane permeabilization (MOMP) releases mitochondrial DNA (mtDNA) into the cytoplasm, which drives SASP via activation of the cGAS-STING pathway *(131)*. MOMP inhibition suppresses inflammation and extends the lifespan of aging mice *(131)*. These mechanistic studies reveal the potential for developing new senolytic agents and lifespan-extending interventions.

Based on the studies partly introduced in this article, targeting cellular senescence may provide new potential therapeutic strategies for various types of cancers and premature aging syndromes. As many senolytic agents have been and continue to be developed using preclinical models, further efforts focusing on each disease will be fruitful in acquiring optimal anti-senescence therapies with maximal efficacy and minimal toxicity for patients.

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Conflict of interests

The authors declare no competing interests.

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