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Review

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Multiple characteristic alterations and available therapeutic strategies of cellular senescence

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Abstract: Given its state of stable proliferative inhibition, cellular senescence is primarily depicted as a critical mechanism by which organisms delay the progression of carcinogenesis. Cells undergoing senescence are often associated with the alteration of a series of specific features and functions, such as metabolic shifts, stemness induction, and microenvironment remodeling. However, recent research has revealed more complexity associated with senescence, including adverse effects on both physiological and pathological processes. How organisms evade these harmful consequences and survive has become an urgent research issue. Several therapeutic strategies targeting senescence, including senolytics, senomorphics, immunotherapy, and function restoration, have achieved initial success in certain scenarios. In this review, we describe in detail the characteristic changes associated with cellular senescence and summarize currently available countermeasures.

Key words: Senescence; Metabolism; Stemness; Microenvironment; Senolytic; Immune surveillance

1 Introduction

Cellular senescence is both a cell-autonomous and non-autonomous biological process. On one side, it is an evolutionarily conserved process to regulate organism growth and development, resembling the function of programmed apoptosis (Storer et al., 2013; Ritschka et al., 2017). However, it can also be induced by distinct stimuli and engage in a broad range of physiological and pathological processes (Manfredi, 2004; Pawelec, 2019). There are already well-established senescence inducers, including DNA damage, telomere shortening, oncogene activation, and chemotherapeutic agents (Kuilman et al., 2010). Moreover, novel stimuli like phosphatase and tensin homologue (PTEN) insufficiency (Toso et al., 2014; Sharma and Almasan, 2021), mitochondrial stress (di Mitri and Alimonti, 2016), unfolded protein response (UPR), and autophagy deficiency are increasingly implicated (Bai et al., 2022). Also, variable degrees of activation of signaling pathways sensing stress or damage have been observed in senescent cells (SnCs), such as p53, p38/mitogen-activated protein kinase (MAPK), nuclear factor-κB (NF-κB), mammalian target of rapamycin (mTOR), transforming growth factor-β (TGF-β), and Wnt, jointly endowing SnCs with context-specific properties (Hernandez-Segura et al., 2018). While cellular senescence is not equivalent to human aging, it has long been considered a major contributor to biological rather than physiological age due to its shared characteristics. Given its significance in biological activities, cellular senescence is now emerging as a promising druggable target for several age-related diseases.

SnCs are stably growth-arrested cells with a diverse range of alterations in characteristics from morphology to gene expression, which usually develop metabolic shift and apoptosis resistance. Classic senescence biomarkers include enhanced senescence-associated β-galactosidase activity (SA-β-Gal), accumulation of lipofuscin, loss of lamin B1, upregulation of cell cycle-related regulators like $p16^{INK4A}$ and $p21^{CIP1}$, formation of senescence-associated heterochromatin foci (SAHF) and senescence-associated secretory phenotype (SASP) (Kuilman et al., 2010). As they age, cells also lose the

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ability to control DNA methylation and show a DNA methylation landscape like that of cancer cells, providing another prognostic marker for senescence identification (Cruickshanks et al., 2013; Oltra et al., 2019; Shah et al., 2021). There are already successful examples of the use of epigenetic clocks, especially "meta-clocks," to precisely predict chronological age (Liu et al., 2020; Sugrue et al., 2021). However, no SnCs will display all these features and their occurrence depends greatly on the organism, cell type, microenvironment, and stimulus.

Given the stable cell cycle-arrested state, cellular senescence was primarily known for its ability to arrest tumor growth. However, increasing evidence has revealed an opposite role in multiple processes which may pose serious threats to health, making it necessary to introduce countermeasures. In this review, we discuss cellular senescence from several aspects and summarize the currently accessible therapeutic strategies (Fig. 1).

2 Senescence-associated alterations endowing cells with specific features and functions

A broad range of changes, such as cell cycle arrest, secretory phenotype, endoplasmic reticulum stress, apoptosis resistance, metabolic shift, stemness induction, and microenvironment remodeling, may accompany senescence and endow SnCs with context-specific survival advantages during the senescence process (Hernandez-Segura et al., 2018). In the following sections, particular attention is paid to the last three areas, since these alterations are often closely linked to the negative impacts of senescence and may hold great therapeutical potential in age-related diseases.

2.1 Metabolic shift

Metabolic shift is a well-documented senescence-associated event occurring in a variety of circumstances, such as atherosclerosis, bone disease, neurodegeneration, type 2 diabetes, and cancers (Hernandez-Segura et al., 2018). There is a reciprocal link between senescence and metabolism: senescence and SASP are susceptible to cellular and organismal metabolic states, which in turn may induce a senescent phenotype associated with metabolic dysfunction. Metabolic dysfunctions involving mitochondrial dysfunction, oxygen, and disrupted nicotinamide adenine dinucleotide (NAD^+) metabolism are major inducers of cellular senescence (Wiley and Campisi, 2021). Most SnCs undergoing metabolic reprogramming usually also undergo gain- or loss-of-function that endows them with either survival predominance or disease vulnerability (Lizardo et al., 2017; Wiley and Campisi, 2021). Senescence-associated metabolic shifts are primarily concerned with metabolites, material and energy supply, lysosomal integrity, and energy use and storage

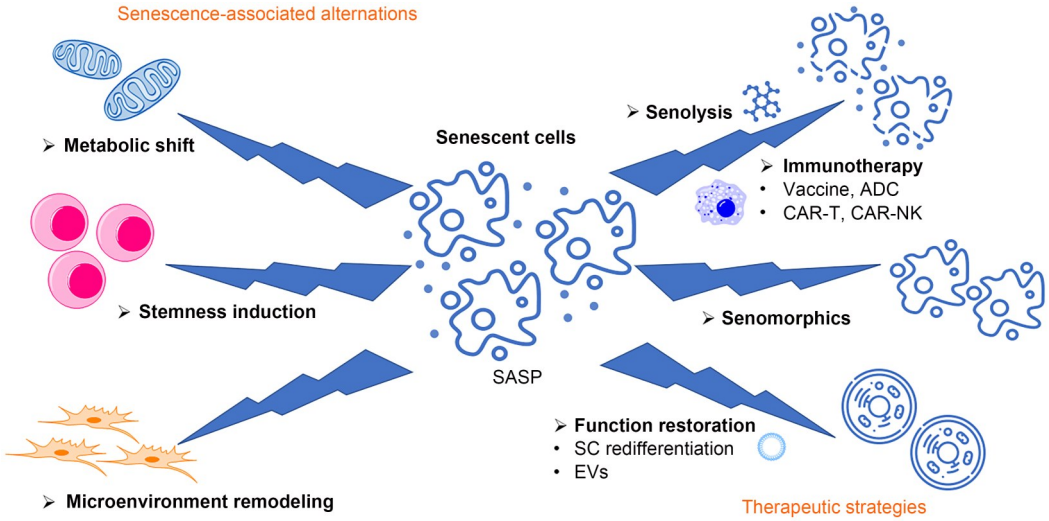

Fig. 1 Senescence-associated alterations and therapeutic strategies. Senescent cells (in the middle) with SASP undergo a series of specific property and function changes (on the left). Currently available therapeutic strategies are shown on the right. SASP: senescence-associated secretory phenotype; ADC: antibody-drug conjugate; CAR-T: chimeric antigen receptor (CAR)-T cell therapy; CAR-NK: CAR-natural killer cell therapy; SC: stem cell; EVs: extracellular vesicles.

(Table 1). Although these shifts may be negative for cell growth, they sometimes have significance as a reference in senescence-associated therapies.

2.2 Stemness induction

Stem cells (SCs) are undifferentiated cells of multicellular organisms with the capacity for multipotent differentiation and infinite self-renewal. Unlike conventional proliferative cells, they divide asymmetrically to produce daughter SCs and functional cells as needed (Venkei and Yamashita, 2018). Loss of cell regenerative capacity is inevitable as humans age, but sometimes can be reversed if treated appropriately, for example, via autophagy manipulation (García-Prat et al., 2016). Although not compatible cellular states per se, stemness and senescence are closely linked with overlapping signaling networks (Milanovic et al., 2018b). Key regulators in senescence, such as $p16^{INK4a}$, $p21^{CIP1}$, p53, and trimethylation of lysine 9 at histone H3 (H3K9me3), also play critical roles in the maintenance of SCs by preventing them from premature exhaustion. Also, some senescence-associated gene-encoded products can inhibit the conversion of normal cells to induced pluripotent stem cells (iPSCs), further revealing the complicated connection between senescence and stemness (Cheng et al., 2000; Liu et al., 2009; Milanovic et al., 2018a).

Typically, senescence-associated stemness is reflected in two main aspects. First, SnCs themselves have the potential to redevelop stemness under certain circumstances (Milanovic et al., 2018a). Moreover, SnCs can also endow adjacent normal cells with stemness, primarily through paracrine mediation (Nacarelli et al., 2020). Though positive for tissue regeneration and repair in some cases, this stemness acquisition is more closely linked to unfavorable health impacts, among which carcinogenesis may be the most representative. Indeed, oncogene-induced senescence (OIS) and therapy-induced senescence (TIS) have been widely studied in the induction of SC markers in recent years, and TIS-associated stemness is often associated with a poorer prognosis (Perrigue et al., 2020; Otero-Albiol and Carnero, 2021). This is supported by the cancer stem cell (CSC) theory, which has long been proposed as an explanation for tumor growth and in which CSCs are regarded as perpetrators of tumor initiation and progression (Batlle and Clevers, 2017). In this hypothesis, cellular senescence serves as a well-established cytoprotective mechanism that promotes CSC induction and tumor progression. Cancer cells can effectively evade cytotoxicity through appearing to be incompetent by having a senescent phenotype, but then restoring proliferation and contributing to cancer relapse once the stressors disappear.

Notably, senescence-associated stemness may in turn have profound implications for tumor aggressiveness and clinical therapeutic outcomes, thus offering opportunities for new cancer strategies. Currently, chemotherapy remains the mainstream therapy for cancers, but the associated TIS greatly contributes to the

Table 1 Therapeutic challenges and opportunities of senescence-associated metabolic shifts

Metabolic shift	Therapeutic challenge	Therapeutic opportunity	References
Metabolites	All three major nutrient substances go through metabolite change in SnCs that may cause senescence-related side effects.	The altered types or levels of metabolites sometimes can be candidate markers for human aging or relevant diseases.	Aird et al., 2013; Chaleckis et al., 2016; Gomes et al., 2020
Material and energy supply (autophagy)	Basic level of autophagy is necessary for cell growth, and autophagy deficiency is closely linked to increasing senescence incidents.	Autophagy manipulation can serve as an effective method for cellular stemness and senescence mediation.	García-Prat et al., 2016; Ho et al., 2017; Salazar et al., 2020
Lysosomal integrity	SnCs undergoing loss of lysosomal integrity are exposed to the risk of intracellular acidosis.	The biology of lysosomal metabolic profiles offers a promising strategy for inducing senolysis through glutaminolysis inhibition.	Johmura et al., 2021; Zhu et al., 2021
Energy use and storage	SnCs with mitochondrial dysfunction and increased ROS levels often have impaired energy metabolism.	The alteration of energy use and storage, including elevated intracellular glucose use and ATP production, may sometimes enable metabolic vulnerability to be overcome.	Dörr et al., 2013; Henson et al., 2014; Rajendran et al., 2019

SnCs: senescent cells; ROS: reactive oxygen species; ATP: adenosine triphosphate.

development of intrinsic or acquired resistance. Cisplatin, a standard chemotherapeutic agent in epithelial ovarian cancer (EOC), inevitably induces cellular senescence after treatment. These SnCs undergo upregulation of nicotinamide phosphoribosyl transferase (NAMPT, the rate-limiting enzyme for the NAD^+ biosynthesis), often giving rise to EOC CSCs and ensuing cisplatin resistance through mediation by SASP factors. Though negative for prognosis, this also demonstrates the therapeutic value of NAMPT inhibitors in a platinum-based standard of care (Nacarelli et al., 2020). Similar implications were obtained from a comparison of senescent and non-senescent B-cell lymphomas in Eμ-Myc transgenic mice: growing stem-cell signatures with activation of Wnt signaling were found only in senescent lymphomas (Milanovic et al., 2018a). In this research, the authors also confirmed that cells released from senescence and which had re-entered the cell cycle (manipulated by genetically targeting H3K9me3 or p53) had additive stemness features and a higher capacity for tumor initiation, compared to non-SnCs equally exposed to chemotherapy.

2.3 Microenvironment remodeling

The microenvironment is defined as the immediate small-scale medium for cell activities, whose integrity and composition are highly plastic. It consists mainly of stromal cells (involving fibroblast, epithelial, endothelial, immune cells, and others) and extracellular matrix (ECM), endowing cells with niche-dependent growth properties. Microenvironment remodeling occurring with age usually creates a breeding ground for diseases. In this section, we discuss senescence-associated microenvironment remodeling in relation to the composition of immune and non-immune cells.

2.3.1 Immunosurveillance and immunosenescence

Immune cells are important constituents of senescence-associated microenvironments since they construct a defense line against noxious stimuli to help maintain homeostasis. Compared to their younger counterparts, SnCs with their recognizable characteristics are more often subjected to immune surveillance. Macrophages and natural killer (NK) cells, two kinds of classic innate immune cells showing intense surveillance of abnormal cells, can eliminate SnCs effectively after recognition, thereby protecting the body from potential damage (Sturmlechner et al., 2021; Wang et al., 2021). However, there are also unanticipated consequences following senescence-associated immune activation that calls for particular attention. Predictably, corresponding self-protective mechanisms can evolve in SnCs to help them evade the latent immune surveillance (Table 2).

However, immune cells also have limited proliferative capacity and undergo senescence either naturally or non-naturally. Immunosenescence refers to the functional loss of both the innate and acquired

Table 2 Unanticipated consequences of immunosurveillance and the potential immune escape mechanisms of SnCs

Immune cells	Unanticipated consequence	Immune escape mechanisms of SnCs	References
Macrophages	VIS could promote macrophage paracrine senescence, jointly causing a cytokine escalation storm and ensuing tissue damage.	The overlapping characteristics between macrophages and SnCs often allow SnCs to escape immune surveillance.	Kowald et al., 2020; Lee et al., 2021
NK cells	Neuroblast senescence in the aged brain augments NK cell cytotoxicity leading to impaired neurogenesis and cognition.	Senescent dermal fibroblasts express HLA-E (a non-classic number of MHC) to interact with inhibitory receptor NKG2A on the surface of NK and highly differentiated $CD8^+$ T cells, greatly weakening their cytotoxicity.	Pereira et al., 2019; Jin et al., 2021
MDSCs	MDSCs suppress the functions of T and NK cells, usually indicating a poor clinical outcome for patients.	MDSCs accumulated in chronic inflammatory conditions often cause an immunosuppressive environment.	Weber et al., 2021
T cells	Tumor-induced senescent $CD4^+$ and $CD8^+$ T cells release pro-inflammatory factors that would suppress the proliferation of responder T cells, eliciting pro-tumoral effects.	Senescent T cells are incompetent in CAR-T-associated therapies targeting SnCs.	Montes et al., 2008; Amor et al., 2020

SnCs: senescent cells; VIS: virus-induced senescence; NK: natural killer; HLA-E: human leukocyte antigen-E; MHC: major histocompatibility complex; NKG2A: NK cell lectin-like receptor subfamily C member 1; $CD8^+$: cluster of differentiation 8-positive; MDSCs: myeloid-derived suppressor cells; CAR-T: chimeric antigen receptor-T cell therapy.

immune systems with age, along with remodeling of the immunologic microenvironment. For the acquired immune system, immunosenescence is typically characterized by decreases in naive T/B cells, increases in terminally differentiated T/B cells, and reduced T/B cell repertoire diversity (Haynes et al., 2003; Yager et al., 2008). Moreover, senescent immune cells are found to experience progressive loss of cytotoxicity, partly evidenced by the downregulation of functional molecules, such as interferon-γ (IFN-γ), granzyme B, and perforin (Crespo et al., 2013). Finally, altered signaling delivery is often found in these SnCs, which poses big therapeutic challenges to current immunotherapy (Erbe et al., 2021). Immunosenescence provides a canonical explanation of the different therapeutic responses of the old and young to immune checkpoint blockade (ICB) therapy (Lian et al., 2020; Shah et al., 2021). Besides, since functionally active T cells are necessary in adoptive cellular immunotherapy (ACT), involving chimeric antigen receptor (CAR)-T cell therapy (CAR-T) and T cell receptor (TCR)-engineered T cell therapy, immunosenescence leads to many more failures in ACT. Thus, undoubtedly, immunosenescence is the most life-threatening among the various types of senescence, and these senescence-associated immune function changes highlight the significance of personalized therapy (Oltra et al., 2019; Liu et al., 2020; Shah et al., 2021).

2.3.2 Fibroblasts and the extracellular matrix

Besides immune cells, special attention should be paid to other stromal cells, particularly fibroblasts, another type of well characterized cells involved in microenvironment remodeling. The predominant regulation model of fibroblasts is through the secretion of soluble factors, including cytokines, chemokines, growth factors, and matrix metalloproteinases (MMPs). These factors establish an intricate intra- and extracellular signaling network that usually contributes to disease occurrence and prognosis (Chen et al., 2018; Zhang et al., 2018; Faget et al., 2019; Muñoz-Galván et al., 2019; Chambers et al., 2021). There is already plenty of evidence to show that senescent fibroblasts and most SASP factors can promote cancer cell proliferation and invasion in vitro (Lawrenson et al., 2010; Kaur et al., 2016; Li et al., 2020). Furthermore, co-injection of senescent fibroblasts was found to stimulate tumor growth and progression in various mice tumor models, which was not established with normal epithelial cells (Krtolica et al., 2001). Senescent fibroblasts also contribute to reduced drug absorption since they can enhance drug efflux by upregulating the adenosine triphosphate (ATP)-binding cassette subfamily B member 4 (ABCB4, a member of the P-glycoprotein family), ultimately reducing drug availability and causing cancer resistance (Xu et al., 2019; Han et al., 2020; Hwang et al., 2020).

In addition to cell components, the ECM acts equally as a key contributor to microenvironment plasticity, and its composition and integrity vary widely with age (Blokland et al., 2020; Levi et al., 2020). The ECM is responsible for regulating the integrity of most tissues and mediates cargo trafficking across the body, exerting different effects simultaneously on the invasiveness of tumor cells, and infiltrating capacity of immune cells (Kaur et al., 2019). The crosstalk between senescence and the ECM is complicated and often interactive. On the one hand, the ECM can restrict the entry of cells into senescence, thus limiting SnC numbers. On the other hand, most SASP-composing factors, especially MMPs, can promote variability of the ECM, thus exacerbating pathological progress (Blokland et al., 2020). The general idea is that the expression and accumulation of most ECM molecules, such as collagen, elastin, and proteoglycan in SnCs decrease, and SnCs in vitro generally exhibit a catabolic phenotype that greatly affects adjacent ECM contractility (Mavrogonatou et al., 2019). It has also been reported that aging leads to degradation of the perilymphatic stroma, and that this could alter lymph node permeability and dictate the route of metastasis (Ecker et al., 2019; Kaur et al., 2019). Besides, senescence-associated ECM stiffness could also be a predictor of tumor progression since tumors of different origins have variable susceptibility to the matrix crosslinking and stiffening, further demonstrating the complexity of the ECM (Mavrogonatou et al., 2019).

In conclusion, sufficient attention should be paid to all these types of senescence-associated microenvironment remodeling. In view of the alterations and ensuing effects of cellular senescence summarized above from multiple angles, we strongly recommend that senescence-associated niche reshaping should be taken into account when considering clinical treatment options. In the following section, we focus on the current available senescence-associated therapeutics.

3 Senescence-associated therapeutic strategies

Both physiological aging and enforced cellular senescence are potential threats to life expectancy. Escaping from the possible side-effects is extremely urgent in clinical practice. The removal of SnCs has long been a hotspot in gero-protective research and initial success has been achieved in post-traumatic osteoarthritis, fibrotic pulmonary disease, and tissue homeostasis (Baar et al., 2017; Jeon et al., 2017; Schafer et al., 2017). However, therapeutics towards senescence are limited by our insufficient knowledge, making it an enormous challenge to overcome. Currently, four primary approaches targeting senescence are available for consideration, including selective elimination of SnCs, referred to as senolysis (Kirkland and Tchkonia, 2020; L'Hôte et al., 2021), immune-mediated SnC clearance, senomorphics, and function restoration. Each strategy has its pros and cons.

3.1 Senolytics

Firstly, SnCs have similar biological characteristics regardless of their tissue of origin. They can serve as therapeutic targets. Secondly, SnCs can be induced to enhance susceptibility to another well-organized program, a process that could be described as a "one-two punch" (Wang et al., 2019; Qing et al., 2021). Based on their functional characteristics, senolytic drugs, which target mainly the upregulated anti-apoptosis system in SnCs, are designed to induce lysis of SnCs. Since first being described by Zhu et al. (2015), senolytics have undergone rapid development. Classical senolytics are listed in Table 3. All these drugs have unique advantages in SnC elimination, but show potential side effects in certain circumstances which limit their clinical application. Given the innate crosslink between metabolism and senescence, targeting the metabolic shift in SnCs may also have therapeutical potential. Examples of these synthetic lethal metabolic effects are listed in Table 4.

3.2 Immunotherapy

As noted above, SnCs are more susceptible to immune surveillance, thus making immunotherapy an effective and feasible option in senescence-targeted strategic planning. Presently, senescence-associated immunotherapies are diverse. For example, senolytic vaccination, the combination of senolysis and immune targetability, can exclusively guide SnCs towards elimination. A recently reported successful case involved research conducted in a progeroid mice model (Suda et al., 2021). The approach adopted the ubiquitously expressed glycoprotein nonmetastatic melanoma protein B (GPNMB) in SnCs as the prey. Therapeutic results were encouraging, showing an improvement in normal and pathological age-related phenotypes and lifespan extension in male mice. As for immunosenescence itself, the CD153-cytosine phosphoguanine (CpG) vaccine was one such case strongly recommended to prevent the accumulation of senescent T cells (Yoshida et al., 2020). Furthermore, for the first time, an antibody-drug conjugate (ADC) that delivers cytotoxic duocarmycin into SnCs through specific β-2-microglobulin (B2M) recognition has been designed to specifically eliminate SnCs (Poblocka et al., 2021). The concept under these strategies relates to cytotoxicity after the antigen–antibody binding reaction.

Table 3 Existing senolytics and their potential side effects

Senolytics	Targets	Potential side effects	References
Navitoclax and ABT-737	BCL-2, BCL-X_L, BCL-W	Transient thrombocytopenia and lymphopenia	del Gaizo Moore et al., 2007; Yosef et al., 2016; Zhu et al., 2016; Thompson et al., 2019
Dasatinib and quercetin	Multiple pro-survival signaling pathways	Disruption of blood-tissue barriers with subsequent liver and perivascular tissue fibrosis and health deterioration	Schafer et al., 2017; Grosse et al., 2020
HSP90 inhibitors	PI3K/AKT pathway	Limited therapeutic response of single drug	Fuhrmann-Stroissnigg et al., 2017
FOXO4 peptide	FOXO4-p53	p53-dependent cytotoxicity	Baar et al., 2017; Serrano, 2017
Cardiac glycosides	NOXA	Frequent toxic reactions	Guerrero et al., 2019
Azithromycin and roxithromycin	Autophagy and glycolysis	Drug–drug interaction and adverse reaction	Ozsvari et al., 2018

BCL: B-cell lymphoma; BCL-X_L: BCL-extra large; HSP90: heat shock protein 90; PI3K: phosphatidylinositol-3-kinase; AKT: protein kinase B; FOXO4: forkhead box O4; NOXA: phorbol-12-myristate-13-acetate-induced protein 1.

Table 4 Targeting metabolic shifts for senolysis

Evidence	Potential target	Research model	Reference
TIS-competent lymphomas show increased glucose use and higher ATP production.	Glucose use or autophagy	Eμ-Myc transgenic mouse lymphoma	Dörr et al., 2013
Activation of PDH enhances the use of pyruvate, causing increased respiration and redox stress in OIS.	PDK1-PDP2-PDH	$BRAFV^{600E}$-driven melanoma	Kaplon et al., 2013
Senescent myeloid cells enhance glycogen synthesis and thus suffer from glucose insufficiency and are unable to support mitochondrial respiration.	PGE2-EP2	Neurodegenerative disease	Minhas et al., 2021
Increased ammonia is produced by glutaminolysis to neutralize redundant H^+ in SnCs, thereby protecting them from acidosis.	Glutaminolysis (such as by glutaminase)	Aged mice	Johmura et al., 2021
Glucose and glutamine undergo dramatic blockage in nucleotide synthesis pathways in senescent HMECs.	Nucleotide synthesis	Human mammary epithelial cells	Delfarah et al., 2019

TIS: therapy-induced senescence; ATP: adenosine triphosphate; PDK1: pyruvate dehydrogenase kinase 1; PDP2: pyruvate dehydrogenase phosphatase isoenzyme 2; PDH: pyruvate dehydrogenase; PGE2: prostaglandin E2; EP2: E-type prostaglandin receptor 2; SnCs: senescent cells; OIS: oncogene-induced senescence; HMECs: human mammary epithelial cells.

Immune cells are another main force in senescence-associated immunotherapy. First, there is a crosstalk between classic cellular senescence and immunity. p53 and p21 are two well-known growth regulators. Their role in senescence-associated immune promotion is to increase infiltration and recognition of immune cells (Xue et al., 2007; Li et al., 2011), and stimulate macrophage M1 polarization (Lujambio et al., 2013; Sturmlechner et al., 2021), eventually inducing clearance of SnCs. In addition, based on the specific chemokine secretion, tissue-specific receptor repertoire, and tissue microenvironment, distinct subsets of immune cells, such as NK cells, neutrophils, dendritic cells, monocytes/macrophages, B cells, and T cells, can be recruited for SnC surveillance and clearance.

As research progresses, more advanced approaches become available. For example, in CAR-T, T cells are equipped with a cell-specific CAR to precisely attack target cells. This approach has already shown superiority in hematological malignancies (Feins et al., 2019; Hong et al., 2020). Given their unique characteristics, CAR-T is a promising candidate for SnC elimination once senescence-specific surface markers are available. One example is urokinase-type plasminogen activator receptor (uPAR)-specifc CAR-T, in which the uPAR was adopted as the prey for T cells due to its universality in OIS cells (Amor et al., 2020). Results showed these modified cells ablate SnCs both in vitro and in vivo, promoting escape from aging-related diseases. However, this approach is limited given the absence of uPAR in some kinds of SnCs. Coincidentally, NK cells are another promising effector cell type in the SnCs-targeted CAR approach (Carlsten and Childs, 2015). Compared to CAR-T, CAR-NK reserves the function of native receptors for cell targeting and recognition, greatly reducing the possibility of tumor escape by decreased expression of the CAR antigen (Rezvani et al., 2017). Besides, because of the restricted persistence of NK cells in vivo, organisms are more likely to avoid life-threatening cytotoxicity, such as cytokine release syndrome (CRS) (Kale et al., 2020). Also, allogenic NK cells can bypass the major risk caused by CAR-T cells, namely graft-versus-host disease (GVHD), allowing them to be an off-the-shelf product for immediate clinical use (Ruggeri et al., 2002). All these factors emphasize the unique advantages of CAR-NK in SnC surveillance.

A suitable target is required for CAR-dependent therapy but is not yet available in the heterogenous SnCs. Alternatively, an antigen-nonspecific approach mediated by invariant natural killer T cells (iNKTs) was recently established to eliminate SnCs. iNKTs are a subset of mature T cells, having both innate and adaptive immune features. Unlike T cells, they express a semi-invariant TCR to recognize lipid antigens presented by CD1d (Crosby and Kronenberg, 2018). Once activated by lipid antigens, such as α-galactosylceramide (α-GalCer), iNKTs preferentially induce cytotoxicity in SnCs over proliferative cells without cell or tissue selectivity. This approach has already shown efficacy in the high-fat diet (HFD) mouse model and lung injury-induced fibrosis (Arora et al., 2021). Notably, the unique invariant TCR equips iNKTs with binding specificity towards lipid antigens, greatly reducing the

possibility of potential side effects. The short-life of iNKTs also helps avoid prolonged cytolytic effects, rendering iNKTs a promising candidate for SnC elimination (Crosby and Kronenberg, 2018).

3.3 Senomorphics

Complete SnC eradication is the end point of both senolysis and immune-mediated clearance. However, a recent study showed that continuous and acute removal of liver sinusoid endothelial cell (LSEC) $p16^{High}$ SnCs could unexpectedly contribute to liver and perivascular tissue fibrosis, reminding us of the value of therapeutic alternatives (Grosse et al., 2020). Considering the positive role of SnCs, one feasible strategy is to convert them into a more desirable state for long-term coexistence rather than aim for complete elimination.

Senomorphic change refers to the neutralization of SASP through SASP inhibitors or other inhibitors. Given the broad microenvironment remodeling during senescence, most of which surrounds the SASP, senomorphic change would definitely contribute to a reduction in the negative effects of SnCs. The combination of therapeutics with SASP inhibitors is becoming increasingly attractive and has already found application in certain fields where it effectively diminishes the deleterious components of SASP, thereby making SnCs coexistent (Schmitt, 2018; Lee and Schmitt, 2019). As for a strategy for developing senomorphics, blunting the production SASP factors and inhibiting their function are two main options (Table 5). However, the intricate regulating network in SASP and the heterogeneity of senescence undoubtedly add therapeutic complexity, and how we choose tactics is condition-dependent, which needs a full scale understanding of individual aging biology.

3.4 Function restoration

Restoring the function of SnCs to reverse the senescence phenotype seems attractive in some age-related pathologies, especially neurodegenerative diseases (Gan and Südhof, 2019; bin Imtiaz et al., 2021). SC redifferentiation serves as one such practical strategy. Neural stem cells (NSCs) were found to deepen quiescence to escape from eradication under the niche inflammatory signals and Wnt activation (Kalamakis et al., 2019). However, they barely differed from their young counterparts in terms of neuronal regeneration when activated, assisting the maintenance of brain function. Another population of multipotent SCs is oligodendrocyte progenitor cells (OPCs), also essential for brain function and myelin regeneration. Senescent OPCs seeing upregulation of PIEZO1, one receptor of OPC could help to sense the ECM stiffness, lost their cell proliferation and differentiation capacity which could be restored by PIEZO1 inhibition via neonate central nervous system (CNS) niche (Segel et al., 2019). In addition, certain endogenous factors are sometimes responsible for the restoration of SnC function. The general idea is that the composition of serum and niche is not immutable: loss or gain of molecule function occurs spontaneously with age. An increasing number of endogenous factors have been already found to mediate senility, including synapse-boosting factor (Gan and Südhof, 2019), α-Klotho (Sahu et al., 2018), sirtuin 1 (Sirt1) (Jeng et al., 2018), and ten-eleven translocation 1 (TET1) (Moyon et al., 2021). Each factor could be overcome to attenuate senescence if appropriate measures are taken, offering novel regenerative therapies (Segel et al., 2019).

Extracellular vesicles (EVs) are a type of cell-derived membranous vesicle that can be classified into two groups, micro vesicles (plasma membrane) and

Table 5 Strategies for developing senomorphics

Therapeutic strategy (SASP regulation)	Function in SASP regulation (senomorphics)	References
cGAS-STING and NF-κB signaling pathway	Two key regulators in SASP induction	Loo et al., 2020; Meyers and Zhu, 2020
Transcription	Transcription regulation carried out with the help of NF-κB and C/EBPβ	Sebastian et al., 2005; Kuilman et al., 2008; Chien et al., 2011
Post-transcription	p38 is linked to SASP mRNA stability; regulation of mTOR	Alspach et al., 2014; Herranz et al., 2015
Epigenetic dynamics	SASP-associated gene expression	Pazolli et al., 2012; Contrepois et al., 2017; Cheng et al., 2018

SASP: senescence-associated secretory phenotype; cGAS-STING: cyclic GMP-AMP synthase-stimulator of interferon genes; NF-κB: nuclear factor-κB; C/EBPβ: CCAAT/enhancer-binding protein β; mTOR: mammalian target of rapamycin.

exosomes (endosomal system), according to their biogenesis (van Niel et al., 2018). They are considered to function in intercellular communication and permit exchange of cellular ingredients, such as nucleic acids, proteins, lipids, and metabolites (Pathan et al., 2019). EVs show senescence-specific functional alteration. For example, SnCs can excrete toxic cytoplasmic DNA via exosomes to maintain cellular homeostasis (Takahashi et al., 2017). It was also reported that neonatal umbilical cord EVs (UC-EVs)-derived mesenchymal stem cells (UC-MSCs) are abundant in gero-protective signals which could rejuvenate senescent adult bone marrow-derived MSCs (AB-MSCs) to promote their osteogenic differentiation and repair capacity. The mechanism involved transferring proliferating cell nuclear antigen (PCNA) into AB-MSCs (Lei et al., 2021). A similar conclusion was obtained in EVs released by gingiva-derived MSCs (GMSC-EVs), thus making EVs another fascinating target in gero-protective intervention (Shi et al., 2021).

4 Conclusions

In the past, a plethora of markers were proposed to describe cellular senescence, but none was applicable to all scenarios, underlining the necessity to characterize senescence and determine the significance of broader senescence-specific events. Though cellular senescence was primarily depicted as a cell-autonomous tumor suppressor program, it has now been confirmed to participate in a series of non-cell-autonomous activities and can produce opposing effects on growth and disease. In this review, we describe the negative roles of senescence from several angles and provide examples of the currently available therapeutic strategies associated with senescence. However, some unanticipated outcomes of these therapeutics present barriers to clinical practice. Any strategies targeting SnCs should make a trade-off between the possible pros and cons. Existing therapeutic strategies are focused more on the permanent removal of SnCs, which may not always be beneficial. New strategies could achieve long-term coexistence or even function restoration of SnCs in the future. In all cases, it is important to obtain a more thorough understanding of senescence in the varied biological processes.

Author contributions

Yunzi ZHAO analyzed the literature and prepared the first draft of the manuscript. Hui LI contributed to the writing and design of the manuscript. Hui HUI and Qinglong GUO revised, edited, and checked the final version. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Yunzi ZHAO, Hui LI, Qinglong GUO, and Hui HUI declare that they have no conflict of interests.

This review does not contain any studies with human or animal subjects performed by any of the authors.

References

Aird KM, Zhang G, Li H, et al., 2013. Suppression of nucleotide metabolism underlies the establishment and maintenance of oncogene-induced senescence. *Cell Rep*, 3(4): 1252-1265. https://doi.org/10.1016/j.celrep.2013.03.004

Alspach E, Flanagan KC, Luo XM, et al., 2014. p38MAPK plays a crucial role in stromal-mediated tumorigenesis. *Cancer Discov*, 4(6):716-729. https://doi.org/10.1158/2159-8290.CD-13-0743

Amor C, Feucht J, Leibold J, et al., 2020. Senolytic CAR T cells reverse senescence-associated pathologies. *Nature*, 583(7814):127-132. https://doi.org/10.1038/s41586-020-2403-9

Arora S, Thompson PJ, Wang Y, et al., 2021. Invariant natural killer T cells coordinate removal of senescent cells. *Med (N Y)*, 2(8):938-950. https://doi.org/10.1016/j.medj.2021.04.014

Baar MP, Brandt RMC, Putavet DA, et al., 2017. Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. *Cell*, 169(1):132-147.e16. https://doi.org/10.1016/j.cell.2017.02.031

Bai ZS, Peng YL, Ye XY, et al., 2022. Autophagy and cancer treatment: four functional forms of autophagy and their therapeutic applications. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 23(2):89-101. https://doi.org/10.1631/jzus.B2100804

Batlle E, Clevers H, 2017. Cancer stem cells revisited. *Nat Med*, 23(10):1124-1134. https://doi.org/10.1038/nm.4409

bin Imtiaz MK, Jaeger BN, Bottes S, et al., 2021. Declining lamin B1 expression mediates age-dependent decreases of hippocampal stem cell activity. *Cell Stem Cell*, 28(5): 967-977.e8. https://doi.org/10.1016/j.stem.2021.01.015

Blokland KEC, Pouwels SD, Schuliga M, et al., 2020. Regulation of cellular senescence by extracellular matrix during chronic fibrotic diseases. *Clin Sci (Lond)*, 134(20):2681-2706. https://doi.org/10.1042/CS20190893

Carlsten M, Childs RW, 2015. Genetic manipulation of NK cells for cancer immunotherapy: techniques and clinical

implications. *Front Immunol*, 6:266. https://doi.org/10.3389/fimmu.2015.00266

Chaleckis R, Murakami I, Takada J, et al., 2016. Individual variability in human blood metabolites identifies age-related differences. *Proc Natl Acad Sci USA*, 113(16):4252-4259. https://doi.org/10.1073/pnas.1603023113

Chambers CR, Ritchie S, Pereira BA, et al., 2021. Overcoming the senescence-associated secretory phenotype (SASP): a complex mechanism of resistance in the treatment of cancer. *Mol Oncol*, 15(12):3242-3255. https://doi.org/10.1002/1878-0261.13042

Chen F, Long QL, Fu D, et al., 2018. Targeting SPINK1 in the damaged tumour microenvironment alleviates therapeutic resistance. *Nat Commun*, 9:4315. https://doi.org/10.1038/s41467-018-06860-4

Cheng H, Xuan HW, Green CD, et al., 2018. Repression of human and mouse brain inflammaging transcriptome by broad gene-body histone hyperacetylation. *Proc Natl Acad Sci USA*, 115(29):7611-7616. https://doi.org/10.1073/pnas.1800656115

Cheng T, Rodrigues N, Shen H, et al., 2000. Hematopoietic stem cell quiescence maintained by $p21^{cip1/waf1}$. *Science*, 287(5459):1804-1808. https://doi.org/10.1126/science.287.5459.1804

Chien Y, Scuoppo C, Wang XW, et al., 2011. Control of the senescence-associated secretory phenotype by NF-κB promotes senescence and enhances chemosensitivity. *Genes Dev*, 25(20):2125-2136. https://doi.org/10.1101/gad.17276711

Contrepois K, Coudereau C, Benayoun BA, et al., 2017. Histone variant H2A.J accumulates in senescent cells and promotes inflammatory gene expression. *Nat Commun*, 8: 14995. https://doi.org/10.1038/ncomms14995

Crespo J, Sun HY, Welling TH, et al., 2013. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol*, 25(2):214-221. https://doi.org/10.1016/j.coi.2012.12.003

Crosby CM, Kronenberg M, 2018. Tissue-specific functions of invariant natural killer T cells. *Nat Rev Immunol*, 18(9): 559-574. https://doi.org/10.1038/s41577-018-0034-2

Cruickshanks HA, Mcbryan T, Nelson DM, et al., 2013. Senescent cells harbour features of the cancer epigenome. *Nat Cell Biol*, 15(12):1495-1506. https://doi.org/10.1038/ncb2879

del Gaizo Moore V, Brown JR, Certo M, et al., 2007. Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. *J Clin Invest*, 117(1):112-121. https://doi.org/10.1172/JCI28281

Delfarah A, Parrish S, Junge JA, et al., 2019. Inhibition of nucleotide synthesis promotes replicative senescence of human mammary epithelial cells. *J Biol Chem*, 294(27):10564-10578. https://doi.org/10.1074/jbc.RA118.005806

di Mitri D, Alimonti A, 2016. Non-cell-autonomous regulation of cellular senescence in cancer. *Trends Cell Biol*, 26(3): 215-226. https://doi.org/10.1016/j.tcb.2015.10.005

Dörr JR, Yu Y, Milanovic M, et al., 2013. Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. *Nature*, 501(7467):421-425. https://doi.org/10.1038/nature12437

Ecker BL, Kaur A, Douglass SM, et al., 2019. Age-related changes in HAPLN1 increase lymphatic permeability and affect routes of melanoma metastasis. *Cancer Discov*, 9(1): 82-95. https://doi.org/10.1158/2159-8290.CD-18-0168

Erbe R, Wang ZY, Wu S, et al., 2021. Evaluating the impact of age on immune checkpoint therapy biomarkers. *Cell Rep*, 36(8):109599. https://doi.org/10.1016/j.celrep.2021.109599

Faget DV, Ren QH, Stewart SA, 2019. Unmasking senescence: context-dependent effects of SASP in cancer. *Nat Rev Cancer*, 19(8):439-453. https://doi.org/10.1038/s41568-019-0156-2

Feins S, Kong WM, Williams EF, et al., 2019. An introduction to chimeric antigen receptor (CAR) T-cell immunotherapy for human cancer. *Am J Hematol*, 94(S1):S3-S9. https://doi.org/10.1002/ajh.25418

Fuhrmann-Stroissnigg H, Ling YY, Zhao J, et al., 2017. Identification of HSP90 inhibitors as a novel class of senolytics. *Nat Commun*, 8:422. https://doi.org/10.1038/s41467-017-00314-z

Gan KJ, Südhof TC, 2019. Specific factors in blood from young but not old mice directly promote synapse formation and NMDA-receptor recruitment. *Proc Natl Acad Sci USA*, 116(25):12524-12533. https://doi.org/10.1073/pnas.1902672116

García-Prat L, Martínez-Vicente M, Perdiguero E, et al., 2016. Autophagy maintains stemness by preventing senescence. *Nature*, 529(7584):37-42. https://doi.org/10.1038/nature16187

Gomes AP, Ilter D, Low V, et al., 2020. Age-induced accumulation of methylmalonic acid promotes tumour progression. *Nature*, 585(7824):283-287. https://doi.org/10.1038/s41586-020-2630-0

Grosse L, Wagner N, Emelyanov A, et al., 2020. Defined $p16^{High}$ senescent cell types are indispensable for mouse healthspan. *Cell Metab*, 32(1):87-99.e6. https://doi.org/10.1016/j.cmet.2020.05.002

Guerrero A, Herranz N, Sun B, et al., 2019. Cardiac glycosides are broad-spectrum senolytics. *Nat Metab*, 1(11): 1074-1088. https://doi.org/10.1038/s42255-019-0122-z

Han L, Long QL, Li SJ, et al., 2020. Senescent stromal cells promote cancer resistance through SIRT1 loss-potentiated overproduction of small extracellular vesicles. *Cancer Res*, 80(16):3383-3398. https://doi.org/10.1158/0008-5472.CAN-20-0506

Haynes L, Eaton SM, Burns EM, et al., 2003. CD4 T cell memory derived from young naive cells functions well into old age, but memory generated from aged naive cells functions poorly. *Proc Natl Acad Sci USA*, 100(25):15053-15058. https://doi.org/10.1073/pnas.2433717100

Henson SM, Lanna A, Riddell NE, et al., 2014. p38 signaling inhibits MTORC1-independent autophagy in senescent human CD8$^+$ T cells. *J Clin Invest*, 124(9):4004-4016. https://doi.org/10.1172/JCI75051

Hernandez-Segura A, Nehme J, Demaria M, 2018. Hallmarks of cellular senescence. *Trends Cell Biol*, 28(6):436-453. https://doi.org/10.1016/j.tcb.2018.02.001

Herranz N, Gallage S, Mellone M, et al., 2015. mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. *Nat Cell Biol*, 17(9):1205-1217. https://doi.org/10.1038/ncb3225

Ho TT, Warr MR, Adelman ER, et al., 2017. Autophagy maintains the metabolism and function of young and old stem cells. *Nature*, 543(7644):205-210. https://doi.org/10.1038/nature21388

Hong MH, Clubb JD, Chen YY, 2020. Engineering CAR-T cells for next-generation cancer therapy. *Cancer Cell*, 38(4):473-488. https://doi.org/10.1016/j.ccell.2020.07.005

Hwang HJ, Lee YR, Kang D, et al., 2020. Endothelial cells under therapy-induced senescence secrete CXCL11, which increases aggressiveness of breast cancer cells. *Cancer Lett*, 490:100-110. https://doi.org/10.1016/j.canlet.2020.06.019

Jeng MY, Hull PA, Fei MJ, et al., 2018. Metabolic reprogramming of human CD8$^+$ memory T cells through loss of SIRT1. *J Exp Med*, 215(1):51-62. https://doi.org/10.1084/jem.20161066

Jeon OH, Kim C, Laberge RM, et al., 2017. Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nat Med*, 23(6):775-781. https://doi.org/10.1038/nm.4324

Jin WN, Shi KB, He WY, et al., 2021. Neuroblast senescence in the aged brain augments natural killer cell cytotoxicity leading to impaired neurogenesis and cognition. *Nat Neurosci*, 24(1):61-73. https://doi.org/10.1038/s41593-020-00745-w

Johmura Y, Yamanaka T, Omori S, et al., 2021. Senolysis by glutaminolysis inhibition ameliorates various age-associated disorders. *Science*, 371(6526):265-270. https://doi.org/10.1126/science.abb5916

Kalamakis G, Brüne D, Ravichandran S, et al., 2019. Quiescence modulates stem cell maintenance and regenerative capacity in the aging brain. *Cell*, 176(6):1407-1419.e14. https://doi.org/10.1016/j.cell.2019.01.040

Kale A, Sharma A, Stolzing A, et al., 2020. Role of immune cells in the removal of deleterious senescent cells. *Immun Ageing*, 17:16. https://doi.org/10.1186/s12979-020-00187-9

Kaplon J, Zheng L, Meissl K, et al., 2013. A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. *Nature*, 498(7452):109-112. https://doi.org/10.1038/nature12154

Kaur A, Webster MR, Marchbank K, et al., 2016. sFRP2 in the aged microenvironment drives melanoma metastasis and therapy resistance. *Nature*, 532(7598):250-254. https://doi.org/10.1038/nature17392

Kaur A, Ecker BL, Douglass SM, et al., 2019. Remodeling of the collagen matrix in aging skin promotes melanoma metastasis and affects immune cell motility. *Cancer Discov*, 9(1):64-81. https://doi.org/10.1158/2159-8290.CD-18-0193

Kirkland JL, Tchkonia T, 2020. Senolytic drugs: from discovery to translation. *J Intern Med*, 288(5):518-536. https://doi.org/10.1111/joim.13141

Kowald A, Passos JF, Kirkwood TBL, 2020. On the evolution of cellular senescence. *Aging Cell*, 19(12):e13270. https://doi.org/10.1111/acel.13270

Krtolica A, Parrinello S, Lockett S, et al., 2001. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc Natl Acad Sci USA*, 98(21):12072-12077. https://doi.org/10.1073/pnas.211053698

Kuilman T, Michaloglou C, Vredeveld LCW, et al., 2008. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell*, 133(6):1019-1031. https://doi.org/10.1016/j.cell.2008.03.039

Kuilman T, Michaloglou C, Mooi WJ, et al., 2010. The essence of senescence. *Genes Dev*, 24(22):2463-2479. https://doi.org/10.1101/gad.1971610

Lawrenson K, Grun B, Benjamin E, et al., 2010. Senescent fibroblasts promote neoplastic transformation of partially transformed ovarian epithelial cells in a three-dimensional model of early stage ovarian cancer. *Neoplasia*, 12(4):317-325. https://doi.org/10.1593/neo.91948

Lee S, Schmitt CA, 2019. The dynamic nature of senescence in cancer. *Nate Cell Biol*, 21(1):94-101. https://doi.org/10.1038/s41556-018-0249-2

Lee S, Yu Y, Trimpert J, et al., 2021. Virus-induced senescence is a driver and therapeutic target in COVID-19. *Nature*, 599(7884):283-289. https://doi.org/10.1038/s41586-021-03995-1

Lei Q, Gao F, Liu T, et al., 2021. Extracellular vesicles deposit *PCNA* to rejuvenate aged bone marrow-derived mesenchymal stem cells and slow age-related degeneration. *Sci Transl Med*, 13(578):eaaz8697. https://doi.org/10.1126/scitranslmed.aaz8697

Levi N, Papismadov N, Solomonov I, et al., 2020. The ECM path of senescence in aging: components and modifiers. *FEBS J*, 287(13):2636-2646. https://doi.org/10.1111/febs.15282

L'Hôte V, Courbeyrette R, Pinna G, et al., 2021. Ouabain and chloroquine trigger senolysis of BRAF-V600E-induced senescent cells by targeting autophagy. *Aging Cell*, 20(9):e13447. https://doi.org/10.1111/acel.13447

Li FM, Huangyang P, Burrows M, et al., 2020. FBP1 loss disrupts liver metabolism and promotes tumorigenesis through a hepatic stellate cell senescence secretome. *Nat Cell Biol*, 22(6):728-739. https://doi.org/10.1038/s41556-020-0511-2

Li H, Lakshmikanth T, Garofalo C, et al., 2011. Pharmacological activation of p53 triggers anticancer innate immune

response through induction of ULBP2. *Cell Cycle*, 10(19): 3346-3358.
https://doi.org/10.4161/cc.10.19.17630

Lian JY, Yue Y, Yu WN, et al., 2020. Immunosenescence: a key player in cancer development. *J Hematol Oncol*, 13:151.
https://doi.org/10.1186/s13045-020-00986-z

Liu Y, Elf SE, Miyata Y, et al., 2009. p53 regulates hematopoietic stem cell quiescence. *Cell Stem Cell*, 4(1):37-48.
https://doi.org/10.1016/j.stem.2008.11.006

Liu ZY, Leung D, Thrush K, et al., 2020. Underlying features of epigenetic aging clocks in vivo and in vitro. *Aging Cell*, 19(10):e13229.
https://doi.org/10.1111/acel.13229

Lizardo DY, Lin YL, Gokcumen O, et al., 2017. Regulation of lipids is central to replicative senescence. *Mol BioSyst*, 13(3):498-509.
https://doi.org/10.1039/c6mb00842a

Loo TM, Miyata K, Tanaka Y, et al., 2020. Cellular senescence and senescence-associated secretory phenotype via the cGAS-STING signaling pathway in cancer. *Cancer Sci*, 111(2):304-311.
https://doi.org/10.1111/cas.14266

Lujambio A, Akkari L, Simon J, et al., 2013. Non-cell-autonomous tumor suppression by p53. *Cell*, 153(2):449-460.
https://doi.org/10.1016/j.cell.2013.03.020

Manfredi R, 2004. HIV infection and advanced age: emerging epidemiological, clinical, and management issues. *Ageing Res Rev*, 3(1):31-54.
https://doi.org/10.1016/j.arr.2003.07.001

Mavrogonatou E, Pratsinis H, Papadopoulou A, et al., 2019. Extracellular matrix alterations in senescent cells and their significance in tissue homeostasis. *Matrix Biol*, 75-76: 27-42.
https://doi.org/10.1016/j.matbio.2017.10.004

Meyers AK, Zhu XW, 2020. The NLRP3 inflammasome: metabolic regulation and contribution to inflammaging. *Cells*, 9(8):1808.
https://doi.org/10.3390/cells9081808

Milanovic M, Fan DNY, Belenki D, et al., 2018a. Senescence-associated reprogramming promotes cancer stemness. *Nature*, 553(7686):96-100.
https://doi.org/10.1038/nature25167

Milanovic M, Yu Y, Schmitt CA, 2018b. The senescence-stemness alliance–a cancer-hijacked regeneration principle. *Trends Cell Biol*, 28(12):1049-1061.
https://doi.org/10.1016/j.tcb.2018.09.001

Minhas PS, Latif-Hernandez A, Mcreynolds MR, et al., 2021. Restoring metabolism of myeloid cells reverses cognitive decline in ageing. *Nature*, 590(7844):122-128.
https://doi.org/10.1038/s41586-020-03160-0

Montes CL, Chapoval AI, Nelson J, et al., 2008. Tumor-induced senescent T cells with suppressor function: a potential form of tumor immune evasion. *Cancer Res*, 68(3):870-879.
https://doi.org/10.1158/0008-5472.CAN-07-2282

Moyon S, Frawley R, Marechal D, et al., 2021. TET1-mediated DNA hydroxymethylation regulates adult remyelination in mice. *Nat Commun*, 12:3359.
https://doi.org/10.1038/s41467-021-23735-3

Muñoz-Galván S, Lucena-Cacace A, Perez M, et al., 2019. Tumor cell-secreted PLD increases tumor stemness by senescence-mediated communication with microenvironment. *Oncogene*, 38(8):1309-1323.
https://doi.org/10.1038/s41388-018-0527-2

Nacarelli T, Fukumoto T, Zundell JA, et al., 2020. NAMPT inhibition suppresses cancer stem-like cells associated with therapy-induced senescence in ovarian cancer. *Cancer Res*, 80(4):890-900.
https://doi.org/10.1158/0008-5472.CAN-19-2830

Oltra SS, Peña-Chilet M, Flower K, et al., 2019. Acceleration in the DNA methylation age in breast cancer tumours from very young women. *Sci Rep*, 9(1):14991.
https://doi.org/10.1038/s41598-019-51457-6

Otero-Albiol D, Carnero A, 2021. Cellular senescence or stemness: hypoxia flips the coin. *J Exp Clin Cancer Res*, 40:243.
https://doi.org/10.1186/s13046-021-02035-0

Ozsvari B, Nuttall JR, Sotgia F, et al., 2018. Azithromycin and roxithromycin define a new family of "senolytic" drugs that target senescent human fibroblasts. *Aging*, 10(11): 3294-3307.
https://doi.org/10.18632/aging.101633

Pathan M, Fonseka P, Chitti SV, et al., 2019. Vesiclepedia 2019: a compendium of RNA, proteins, lipids and metabolites in extracellular vesicles. *Nucleic Acids Res*, 47(D1): D516-D519.
https://doi.org/10.1093/nar/gky1029

Pawelec G, 2019. Does patient age influence anti-cancer immunity? *Semin Immunopathol*, 41(1):125-131.
https://doi.org/10.1007/s00281-018-0697-6

Pazolli E, Alspach E, Milczarek A, et al., 2012. Chromatin remodeling underlies the senescence-associated secretory phenotype of tumor stromal fibroblasts that supports cancer progression. *Cancer Res*, 72(9):2251-2261.
https://doi.org/10.1158/0008-5472.CAN-11-3386

Pereira BI, Devine OP, Vukmanovic-Stejic M, et al., 2019. Senescent cells evade immune clearance via HLA-E-mediated NK and $CD8^+$ T cell inhibition. *Nat Commun*, 10:2387.
https://doi.org/10.1038/s41467-019-10335-5

Perrigue PM, Rakoczy M, Pawlicka KP, et al., 2020. Cancer stem cell-inducing media activates senescence reprogramming in fibroblasts. *Cancers*, 12(7):1745.
https://doi.org/10.3390/cancers12071745

Poblocka M, Bassey AL, Smith VM, et al., 2021. Targeted clearance of senescent cells using an antibody-drug conjugate against a specific membrane marker. *Sci Rep*, 11:20358.
https://doi.org/10.1038/s41598-021-99852-2

Qing YJ, Li H, Zhao YZ, et al., 2021. One-two punch therapy for the treatment of T-cell malignancies involving p53-dependent cellular senescence. *Oxid Med Cell Longev*, 2021:5529518.
https://doi.org/10.1155/2021/5529518

Rajendran P, Alzahrani AM, Hanieh HN, et al., 2019. Autophagy and senescence: a new insight in selected human diseases. *J Cell Physiol*, 234(12):21485-21492.
https://doi.org/10.1002/jcp.28895

Rezvani K, Rouce R, Liu EL, et al., 2017. Engineering natural killer cells for cancer immunotherapy. *Mol Ther*, 25(8):

1769-1781.
https://doi.org/10.1016/j.ymthe.2017.06.012

Ritschka B, Storer M, Mas A, et al., 2017. The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. *Genes Dev*, 31(2):172-183.
https://doi.org/10.1101/gad.290635.116

Ruggeri L, Capanni M, Urbani E, et al., 2002. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science*, 295(5562):2097-2100.
https://doi.org/10.1126/science.1068440

Sahu A, Mamiya H, Shinde SN, et al., 2018. Age-related declines in α-Klotho drive progenitor cell mitochondrial dysfunction and impaired muscle regeneration. *Nat Commun*, 9:4859.
https://doi.org/10.1038/s41467-018-07253-3

Salazar G, Cullen A, Huang JW, et al., 2020. SQSTM1/p62 and PPARGC1A/PGC-1alpha at the interface of autophagy and vascular senescence. *Autophagy*, 16(6):1092-1110.
https://doi.org/10.1080/15548627.2019.1659612

Schafer MJ, White TA, Iijima K, et al., 2017. Cellular senescence mediates fibrotic pulmonary disease. *Nat Commun*, 8:14532.
https://doi.org/10.1038/ncomms14532

Schmitt CA, 2018. UnSASPing senescence: unmasking tumor suppression? *Cancer Cell*, 34(1):6-8.
https://doi.org/10.1016/j.ccell.2018.06.009

Sebastian T, Malik R, Thomas S, et al., 2005. C/EBPβ cooperates with RB:E2F to implement Ras^{V12}-induced cellular senescence. *EMBO J*, 24(18):3301-3312.
https://doi.org/10.1038/sj.emboj.7600789

Segel M, Neumann B, Hill MFE, et al., 2019. Niche stiffness underlies the ageing of central nervous system progenitor cells. *Nature*, 573(7772):130-134.
https://doi.org/10.1038/s41586-019-1484-9

Serrano M, 2017. Ageing: tools to eliminate senescent cells. *Nature*, 545(7654):294-295.
https://doi.org/10.1038/nature22493

Shah Y, Verma A, Marderstein AR, et al., 2021. Pan-cancer analysis reveals molecular patterns associated with age. *Cell Rep*, 37(10):110100.
https://doi.org/10.1016/j.celrep.2021.110100

Sharma A, Almasan A, 2021. Autophagy and PTEN in DNA damage-induced senescence. *Adv Cancer Res*, 150:249-284.
https://doi.org/10.1016/bs.acr.2021.01.006

Shi HZ, Zeng JC, Shi SH, et al., 2021. Extracellular vesicles of GMSCs alleviate aging-related cell senescence. *J Dent Res*, 100(3):283-292.
https://doi.org/10.1177/0022034520962463

Storer M, Mas A, Robert-Moreno A, et al., 2013. Senescence is a developmental mechanism that contributes to embryonic growth and patterning. *Cell*, 155(5):1119-1130.
https://doi.org/10.1016/j.cell.2013.10.041

Sturmlechner I, Zhang C, Sine CC, et al., 2021. p21 produces a bioactive secretome that places stressed cells under immunosurveillance. *Science*, 374(6567):eabb3420.
https://doi.org/10.1126/science.abb3420

Suda M, Shimizu I, Katsuumi G, et al., 2021. Senolytic vaccination improves normal and pathological age-related phenotypes and increases lifespan in progeroid mice. *Nat Aging*, 1(12):1117-1126.
https://doi.org/10.1038/s43587-021-00151-2

Sugrue VJ, Zoller JA, Narayan P, et al., 2021. Castration delays epigenetic aging and feminizes DNA methylation at androgen-regulated loci. *eLife*, 10:e64932.
https://doi.org/10.7554/eLife.64932

Takahashi A, Okada R, Nagao K, et al., 2017. Exosomes maintain cellular homeostasis by excreting harmful DNA from cells. *Nat Commun*, 8:15287.
https://doi.org/10.1038/ncomms15287

Thompson PJ, Shah A, Ntranos V, et al., 2019. Targeted elimination of senescent beta cells prevents type 1 diabetes. *Cell Metab*, 29(5):1045-1060.e10.
https://doi.org/10.1016/j.cmet.2019.01.021

Toso A, Revandkar A, di Mitri D, et al., 2014. Enhancing chemotherapy efficacy in *Pten*-deficient prostate tumors by activating the senescence-associated antitumor immunity. *Cell Rep*, 9(1):75-89.
https://doi.org/10.1016/j.celrep.2014.08.044

van Niel G, D'Angelo G, Raposo G, 2018. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol*, 19(4):213-228.
https://doi.org/10.1038/nrm.2017.125

Venkei ZG, Yamashita YM, 2018. Emerging mechanisms of asymmetric stem cell division. *J Cell Biol*, 217(11):3785-3795.
https://doi.org/10.1083/jcb.201807037

Wang C, Vegna S, Jin HJ, et al., 2019. Inducing and exploiting vulnerabilities for the treatment of liver cancer. *Nature*, 574(7777):268-272.
https://doi.org/10.1038/s41586-019-1607-3

Wang RW, Viganò S, Ben-David U, et al., 2021. Aneuploid senescent cells activate NF-κB to promote their immune clearance by NK cells. *EMBO Rep*, 22(8):e52032.
https://doi.org/10.15252/embr.202052032

Weber R, Groth C, Lasser S, et al., 2021. IL-6 as a major regulator of MDSC activity and possible target for cancer immunotherapy. *Cell Immunol*, 359:104254.
https://doi.org/10.1016/j.cellimm.2020.104254

Wiley CD, Campisi J, 2021. The metabolic roots of senescence: mechanisms and opportunities for intervention. *Nat Metab*, 3(10):1290-1301.
https://doi.org/10.1038/s42255-021-00483-8

Xu QX, Long QL, Zhu DX, et al., 2019. Targeting amphiregulin (AREG) derived from senescent stromal cells diminishes cancer resistance and averts programmed cell death 1 ligand (PD-L1)-mediated immunosuppression. *Aging Cell*, 18(6):e13027.
https://doi.org/10.1111/acel.13027

Xue W, Zender L, Miething C, et al., 2007. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature*, 445(7128):656-660.
https://doi.org/10.1038/nature05529

Yager EJ, Ahmed M, Lanzer K, et al., 2008. Age-associated decline in T cell repertoire diversity leads to holes in the repertoire and impaired immunity to influenza virus. *J Exp Med*, 205(3):711-723.

https://doi.org/10.1084/jem.20071140

Yosef R, Pilpel N, Tokarsky-Amiel R, et al., 2016. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. *Nat Commun*, 7:11190. https://doi.org/10.1038/ncomms11190

Yoshida S, Nakagami H, Hayashi H, et al., 2020. The CD153 vaccine is a senotherapeutic option for preventing the accumulation of senescent T cells in mice. *Nat Commun*, 11:2482. https://doi.org/10.1038/s41467-020-16347-w

Zhang BY, Fu D, Xu QX, et al., 2018. The senescence-associated secretory phenotype is potentiated by feedforward regulatory mechanisms involving Zscan4 and TAK1. *Nat Commun*, 9:1723. https://doi.org/10.1038/s41467-018-04010-4

Zhu HY, Li QQ, Liao TP, et al., 2021. Metabolomic profiling of single enlarged lysosomes. *Nat Methods*, 18(7):788-798. https://doi.org/10.1038/s41592-021-01182-8

Zhu Y, Tchkonia T, Pirtskhalava T, et al., 2015. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell*, 14(4):644-658. https://doi.org/10.1111/acel.12344

Zhu Y, Tchkonia T, Fuhrmann-Stroissnigg H, et al., 2016. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell*, 15(3):428-435. https://doi.org/10.1111/acel.12445