



Review

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Multiple characteristic alterations and available therapeutic strategies of cellular senescence

Yunzi ZHAO, Hui LI, Qinglong GUO, Hui HUI[✉]

State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Carcinogenesis and Intervention, China Pharmaceutical University, Nanjing 210009, China

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- κ B (NF- κ B), 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 drug-gable 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

✉ Hui HUI, moyehh@163.com

Yunzi ZHAO, <https://orcid.org/0000-0001-7466-3833>
Hui HUI, <https://orcid.org/0000-0002-3257-0708>

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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 “metaclocks,” 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 therapeutic 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

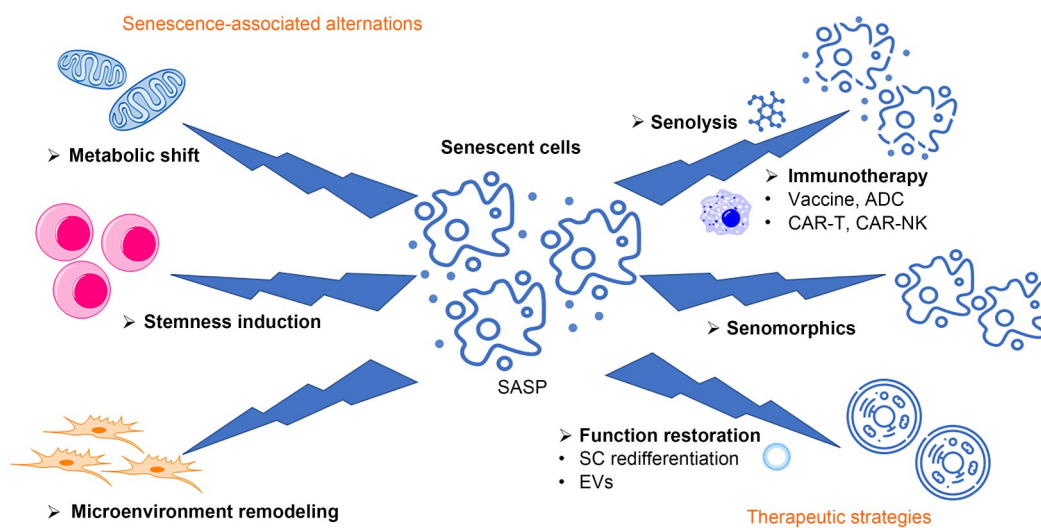


Fig. 1 Senescence-associated alterations and therapeutic strategies. Senescent cells (in the middle) with SASP undergo a series of specific property and function changes (on the left). Currently available therapeutic strategies are shown on the right. SASP: senescence-associated secretory phenotype; 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 (Nacarello 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 (Battle 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 up-regulation 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 Tchkoia, 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 | E μ -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)-specific 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- κ B 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- κ B and C/EBP β | 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- κ B: nuclear factor- κ B; C/EBP β : 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.

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