SCIENCE CHINA
Life Sciences

FROM CAS & CAE MEMBERS
•REVIEW•

March 2020 Vol.63 No.3: 332–342
https://doi.org/10.1007/s11427-019-1629-6

Cellular senescence: from anti-cancer weapon to anti-aging target

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Received November 12, 2019; accepted January 3, 2020; published online February 13, 2020

Cellular senescence (CS) is a state of stable cell cycle arrest characterized by the production and secretion of inflammatory molecules. Early studies described oncogene-induced senescence (OIS) as a barrier to tumorigenesis, such that the therapeutic induction of CS might represent a rational anti-cancer strategy. Indeed, the validity of this approach has been borne out by the development and approval of the cyclin-dependent kinase (CDK) inhibitor palbociclib for the treatment of breast cancer. Apart from tumors, senescent cells have also been shown to accumulate during natural mammalian aging, where they produce detrimental effects on the physiology of surrounding tissues. Thus, pharmacological senescent cell depletion has been proposed as an approach to delay age-related functional decline; this has been formally demonstrated in animal models. In this review article, we describe the current mechanistic understanding of cellular senescence at the molecular level and how it informs the development of new therapeutic strategies to combat cancer and aging.

cellular senescence, cancer, healthy aging, pro-senescence cancer therapy, senolytic therapies

Citation: Yuan, L., Alexander, P.B., and Wang, X.F. (2020). Cellular senescence: from anti-cancer weapon to anti-aging target. Sci China Life Sci 63, 332–342. https://doi.org/10.1007/s11427-019-1629-6

Cellular senescence (CS) is a fundamental cellular fate underlying eukaryotic life. Under normal growth conditions, cells can undergo proliferation, quiescence, or differentiation. However, in response to environmental stress, severely damaged cells permanently exit the cell cycle and enter a state of either senescence or cell death. Initially, the phenomenon of CS was identified when cultured primary cells were shown to undergo a limited number of cell divisions *in vitro* (Hayflick, 1965). Today, built upon decades of studies, a clearer picture has emerged of this aged cell fate, which can be triggered by multiple types of cellular stress including irreparable DNA damage, telomere attrition, oncogenic mutations, deregulated metabolism, and oxidative stress (Campisi, 2013; Collado et al., 2007). Unlike the apoptotic fate, senescent cells remain metabolically active and enter a long-lasting non-dividing state, with profound physiological impacts on surrounding cells and tissues. Indeed, current studies indicate that CS plays major roles in many fundamental biological processes such as development, wound healing, tumorigenesis, and aging (Herbig et al., 2006; Jun and Lau, 2010; Muñoz-Espín et al., 2013; Storer et al., 2013; Xue et al., 2007).

In this review article, we summarize recent mechanistic insights into the senescent fate, highlight the physiological impacts of CS on cancer and aging, and discuss the future directions of senescence research with a focus on the development of anti-cancer and anti-aging therapeutics.

Molecular mechanisms of cellular senescence

Characteristics of cellular senescence

Cells undergoing senescence display distinct senescence-

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associated features. Although no known single characteristic is specific to senescence, the simultaneous display of multiple markers is often sufficient to define senescence (Sharpless and Sherr, 2015). In general, the fundamental characteristic of senescence is permanent cell cycle arrest, under which cells cannot reenter the cell cycle through any known physiological stimulation. In addition, senescent cells exhibit a flattened morphology and increased cell size. Notably, senescence-associated beta-galactosidase (SA-β-Gal) staining is widely used to detect senescent cells both in culture and in tissues. This method is based on activity of the GLB1-encoded lysosomal beta-galactosidase enzyme, whose expression is commonly increased upon senescence induction (Debacq-Chainiaux et al., 2009). Persistent DNA damage foci can also be observed when senescence is induced by genomic damage. Moreover, some senescent cells contain SAHF, which help to establish and maintain cell cycle arrest by suppressing proliferation-related gene expression (Narita et al., 2003). Finally, unlike the dormant cell fate, senescent cells remain highly active metabolically and can modulate the surrounding microenvironment. These cells develop a senescence-associated secretory phenotype (SASP), which mediates the robust secretion of numerous pro-inflammatory cytokines, proteases, growth factors, and other extracellular proteins (Acosta et al., 2008; Kuilman et al., 2008). Importantly, the potent autocrine and paracrine activities of the SASP have been causatively associated with some of the physiological impacts of CS on cancer and aging, as described later in this review.

Because replicative and oncogene-induced senescence were first characterized in mammalian cells grown in culture, some scientists initially questioned the physiological relevance of the senescent cell fate. However, many subsequent studies observed the central features of senescent cells in various *in vivo* contexts (Hornsby, 2002) ranging from normal aging (Herbig et al., 2006; Wang et al., 2009) to disease states such as tumor formation (Prieur and Peeper, 2008), cardiovascular disease (Fyhrquist et al., 2013), and fibrosis (Krizhanovsky et al., 2008; Schafer et al., 2017). To develop CS-dependent therapeutic strategies to combat these diseases, it is critical to reliably and accurately identify senescent cells in mammalian tissues (Sharpless and Sherr, 2015). Historically, the two major markers utilized to detect CS *in vivo* have been SA-β-Gal activity and $p16^{INK4a}$ expression. Although the underlying reason for SA-β-Gal's upregulation in senescent cells remains somewhat mysterious, because of its convenience and relative specificity, SA-β-Gal cytochemical staining remains a standard assay for identifying senescent cells *in vivo* (Debacq-Chainiaux et al., 2009; Itahana et al., 2007). In addition, expression of $p16^{INK4a}$ (further discussed in Causes of cellular senescence below) has been shown to increase in tissues from aged rodents (Krishnamurthy et al., 2004), primates (Herbig et al., 2006), and humans (Ressler et al., 2006), such that it is considered to represent a biomarker of aging; $p16^{INK4a}$ expression can be analyzed using standard assays such as antibody-based immunohistochemistry and RNA *in situ* hybridization. For CS detection in live animals, Sharpless and colleagues generated a $p16^{INK4a}$-luciferase knock-in reporter mouse model, which they used to monitor levels of CS during aging, tumorigenesis, and inflammation (Burd et al., 2013; Liu et al., 2019). Finally, a SA-β-Gal-activatable near-infrared molecular probe was recently developed and demonstrated to be capable of quantifying levels of senescent cells in tumor xenografts (Wang et al., 2019b). While these new tools represent exciting advances for the field, to most confidently detect and quantify senescent cell populations *in vivo*, it remains important to utilize multiple established markers of CS.

Causes of cellular senescence

CS is a cellular response to severe environmental stress. For the aforementioned replicative exhaustion of primary cell cultures, CS is now understood to be triggered by telomere erosion, which can elicit a DNA damage response (DDR) when telomeres become critically short and dysfunctional after multiple rounds of cell division (d'Adda di Fagagna et al., 2003; Harley et al., 1990). In addition to telomere shortening, irradiation, oxidative stress, and various other DNA damaging agents can also stimulate potent DDR signaling to induce CS (Nakamura et al., 2008). Furthermore, epigenetic-associated interference can induce CS in a DDR-independent manner. For example, polycomb repressive complex 2 (PRC2) creates an inactive chromatin state at the cyclin-dependent kinase Inhibitor 2A (*CDKN2A*) locus encoding $p16^{INK4a}$; this is partly mediated by PRC2's histone lysine methyltransferase EZH2. During CS induction, epigenetic disruptions in PRC2 functionality lead to decreased levels of lysine 27 trimethylation on histone H3 (H3K27me3). As a result, expression of $p16^{INK4a}$, an important tumor suppressor and aging biomarker, is activated to mediate CS initiation (Bracken et al., 2007).

In addition to transcriptional mechanisms, oncogenic signaling activation triggers a type of senescence termed oncogene-induced senescence (OIS). The first report of OIS showed that, rather than transforming cells into a tumorigenic state, oncogenic HRAS induction in primary cells causes permanent G1 arrest (Serrano et al., 1997). Subsequently, several other oncogenic signals including activation of oncogenic KRAS, NRAS, BRAF, and AKT, or loss of tumor suppressive PTEN, have been shown to be capable of triggering CS (Collado and Serrano, 2010). Collectively, these findings highlight CS as an important tumor suppressor pathway.

Reprogramming of cellular metabolism represents a final prominent initiator of CS. For example, mitochondrial dys-

function can lead to overproduction of reactive oxygen species (ROS), causing the onset of CS (Jiang et al., 2013). Mitochondrial damage also decreases the cytosolic NAD+/NADH ratio (Wiley et al., 2016), leading to 5′ AMP-activated protein kinase (AMPK) activation and the phosphorylation and stabilization of p53 to induce cell cycle arrest. In sum, it is now known that a variety of environmental stresses acting on DNA, protein signaling networks, and mitochondria can all lead to induction of the senescent cell fate.

To counter these diverse stress signals collectively referred to as senescent stimuli, natural anti-senescence factors are known to maintain healthy rates of proliferation by stimulating pro-growth signal transduction cascades. The first such protein discovered with this function was trefoil factor 1 (TFF1), which was identified as a soluble factor capable of promoting prostate and pancreatic cancer tumorigenesis by suppressing OIS (Radiloff et al., 2011). TFF1's anti-senescence activity was further shown to be dependent upon signal transduction through the epidermal growth factor receptor (EGFR), such that targeted EGFR inhibition fully impaired TFF1's capacity to suppress OIS. This provided an important clue that other molecules signaling through EGFR might possess similar anti-senescence capacities. Indeed, subsequent studies revealed that EGF itself has potent anti-senescence activity in a wide range of normal mammalian cell types including bronchial and mammary epithelial cells (Alexander et al., 2015). Although this finding went against the prevailing dogma which considered EGF as a simple mitogen stimulating cell growth rates, it has important implications for major physiological processes ranging from embryonic development to cancer, since targeted EGFR inhibitors such as erlotinib and gefitinib are currently utilized as anti-cancer therapies. Moreover, because these pharmacological agents can rapidly induce CS in untransformed cell types (Alexander et al., 2015), they can be employed as tools to trigger CS and dissect molecular mechanisms underlying the initiation and establishment of the senescent state. Several such studies successfully employing this approach are described below (Chong et al., 2018; Xiang et al., 2017; Yuan et al., 2018). In addition to TFF1 and EGF, it will be interesting to see whether other naturally occurring senescence suppressors remain to be discovered and how the activities of these anti-senescence factors are coordinated to counteract senescent stimuli and maintain healthy levels of cell proliferation.

Effector pathways of cellular senescence

Senescence stimuli produce activation of either or both of the p53-p21 and $p16^{INK4a}$-pRB signaling pathways, which function to initiate and maintain the senescence state (Campisi, 2013). In general, genomic damage activates DDR signaling towards the p53-p21 axis, which prevents RB inactivation and thereby blocks cell proliferation. Meanwhile, activated RB signaling can also be mediated by $p16^{ink4a}$ via targeted interference with the formation of cyclin D–CDK4 and cyclin D–CDK6 complexes, which are the upstream suppressive regulators of RB. Notably, while the p53 and $p16^{INK4a}$ signaling pathways represent the primary regulators of CS, many other factors work in parallel or in a complementary fashion to promote senescence. For example, activated RB can bind to E2F-responsive promoters in conjunction with the Suv39h1 histone methyltransferase to induce heterochromatin formation associated with histone H3 lysine 9 methylation (H3K9me), termed senescence-associated heterochromatic foci (SAHF). Consequently, SAHF formation to suppress pro-proliferative gene expression can lead to the permanent cell cycle arrest underlying senescence. This formation of SAHF at pro-proliferative gene loci appears to be a unique feature of senescence distinguishing it from reversibly arrested states such as quiescence (Braig et al., 2005; Narita et al., 2003).

To support an irreversible cell cycle arrest phenotype, senescent cells are known to reprogram several major metabolic pathways. For example, in OIS, decreased phosphorylation of the pyruvate dehydrogenase complex (PDH) can increase pyruvate usage by the TCA cycle, leading to accelerated catabolic metabolism as well as redox stress generation. These pathways collectively promote cell cycle arrest as a barrier against malignant transformation (Kaplon et al., 2013). While this decline of PDH phosphorylation is critical for OIS, the molecular events regulating mitochondrial oxidative phosphorylation have remained unclear. Our recently published work indicates that the mitochondrial PKC-δ-PDK axis acts upstream of PDH phosphorylation for cell fate determination (Yuan et al., 2018).

Besides irreversible cell cycle arrest, another prominent characteristic of CS is the production and secretion of numerous pro-inflammatory cytokines, proteases, growth factors, and other proteins, termed the SASP (Coppé et al., 2010). While it is well known that the NF-κB transcription factor plays a role in generating these inflammatory molecules, the signaling pathways leading to NF-κB activation in the context of CS have been more difficult to pinpoint. Recently, we discovered a role for the multi-ligand scavenger receptor CD36 in initiating the SASP (Chong et al., 2018). Although normally expressed exclusively in certain immune cell types, upon exposure to senescent stimuli non-immune cells upregulate cell surface expression of CD36, which then signals through the canonical Src–p38 pathway to activate NF-κB and the SASP. Very interestingly, this process is dependent on the CD36 ligand amyloid-beta (Aβ); Aβ-mediated CD36 activation thus represents the first description of an extrinsic signaling event leading to SASP molecule production. Moreover, the SASP was further shown to develop in a temporal manner,

such that a small subset of secreted factors are upregulated initially whereas the complete spectrum of components is produced only after CS establishment, suggesting a feed-forward mechanism in which early SASP signaling events drive the full senescent phenotype (Chong et al., 2018). The precise nature of these early signaling events and how they function to establish and reinforce the senescent phenotype is a topic worthy of further investigation.

In addition to our discovery of CD36's role in extrinsic SASP initiation (Chong et al., 2018), other recent studies suggest that the intrinsic cGAS (cyclic GMP-AMP synthase)-STING (stimulator of interferon genes) pathway is also critically involved in this process (Dou et al., 2017; Glück et al., 2017; Yang et al., 2017). Briefly, upon senescence induction, due in part to compromised integrity of the nuclear envelope, chromatin fragments are released from the nucleus into the cytoplasm. These are recognized by the cytosolic DNA sensor cGAS, which activates the STING pathway, leading to NF-κB-mediated production of inflammatory cytokines.

Recent research in this field has further investigated the source of cytosolic DNA acting upstream of the cGAS-STING pathway for SASP production (De Cecco et al., 2019) and revealed a role for the retrotransposable element LINE-1. Especially during late-stage CS, transcriptional activation and subsequent reverse transcription of LINE-1 promotes the production of cytoplasmic LINE-1 DNA, leading to cGAS-STING pathway activation for producing SASP factors such as type I interferons. Notably, considering that derepression of LINE-1 occurs mainly during the late phase of CS, it will be of interest to explore if the signaling pathways activated during early-stage senescence can subsequently compromise the host surveillance program and promote the re-activation of LINE-1 for senescence reinforcement. For example, findings from us and others suggest that loss of the epigenetic modulator ubiquitin-like with PHD and ring finger domain containing protein 1 (UHRF1), a multi-functional regulator of DNA and histone modifications, is critical for initiating CS (Jung et al., 2017; Xiang et al., 2017). Interestingly, in mouse germ cells, UHRF1 is known to be actively involved in silencing *Line1* and other retrotransposons via CpG promoter methylation (Dong et al., 2019). Therefore, loss of UHRF1 during CS initiation could represent an upstream event contributing to retrotransposon activation for senescence progression.

Besides its capacity to silence retrotransposons and thereby limit SASP factor production, UHRF1 possesses additional anti-senescence functions by virtue of its epigenetic regulatory activities: UHRF1 reads encoded histone modifications (Arita et al., 2008; Arita et al., 2012), links this code to DNA methylation by binding to hemimethylated CpG dinucleotides (Arita et al., 2008; Avvakumov et al., 2008), and recruits DNA methyltransferase 1 (DNMT1) to maintain DNA methylation (Bostick et al., 2007). Furthermore, UHRF1 is overexpressed in many cancer types and has been considered an oncogene due to its epigenetic silencing function, which is thought to predominantly target cell cycle inhibitors including major tumor suppressors (Alhosin et al., 2016). Finally, loss of UHRF1 has been shown to induce G1 cell cycle arrest by abrogating replication factory formation, leading to the conclusion that UHRF1 is required for DNA replication initiation (Xiang et al., 2017). Therefore, UHRF1 functions to suppress CS by at least four distinct mechanisms: (i) by suppressing retrotransposon activation and subsequent STING-dependent SASP initiation; (ii) by directly repressing the transcription of important cell cycle regulators; (iii) by maintaining proper levels of DNA methylation after replication; and (iv) by directly promoting DNA replication in response to cell growth signals. Together, these mechanisms appear adequate to explain why UHRF1 down-regulation is sufficient to trigger CS in normal cells (Jung et al., 2017; Xiang et al., 2017). Therapeutically, these multi-functional activities render UHRF1 a prime target for senescence-related anti-cancer therapy (discussed in Cellular senescence and anti-cancer treatments below); indeed, a prototype inhibitor targeting UHRF1 has recently been developed and validated (Myrianthopoulos et al., 2016).

In addition to the epigenetic and signaling mechanisms described above, alteration of other fundamental biological processes can also support the senescent cell fate. For example, autophagy plays context-dependent roles during senescence, being both pro-senescence and anti-senescence. During the onset of CS, autolysosomes and the mammalian target of rapamycin (mTOR) work together to form the TOR-autophagy spatial coupling compartment (TASCC). Recycled amino acids derived from autophagy are then used for mTOR-mediated biosynthesis of SASP factors (Narita et al., 2011). In contrast with this pro-senescence role of general autophagy, selective autophagy needs to be switched off to stabilize GATA4, a key transcription factor responsible for the activation of NF-κB and the SASP (Kang et al., 2015). Under normal conditions, GATA4 is degraded by p62-mediated selective autophagy. Suppression of this degradative machinery during CS leads to stabilization of GATA4, SASP initiation, and senescence. Like general autophagy, activation of an unfolded protein response (UPR) has also been associated with senescence, partly due to its contribution to the SASP (Catanzaro et al., 2014). Specifically, during OIS, upregulation of Clade B serine protease inhibitors 3 (SERPINB3) and 4 (SERPINB4) compomise protein turnover in both lysosomal and proteasomal compartments. As a result, enhanced ER stress and an UPR trigger the stabilization of SASP factors.

While the p53-p21 and $p16^{INK4a}$-pRB pathways are currently thought to represent the major senescence pathways, many additional signaling pathways and biological processes

shape the complex features of senescent cells. However, it should be noted that most pathways involved in senescence also play important roles in other cell fates including quiescence, terminal differentiation, and cell death. Accordingly, there is a need to further characterize the specific molecular determinants driving CS rather than other non-dividing cell fates. Recently, we discovered the inner mitochondrial membrane peptidase subunit 2 (IMMP2L) as an important switch controlling the proliferative, senescent, and apoptotic cell fates via its regulation of metabolic and apoptotic signaling processes (Yuan et al., 2018). Briefly, in dividing cells, IMMP2L processes the metabolic enzyme glycerol-3-phosphate dehydrogenase (GPD2) to promote proper mitochondrial function, whereas after CS induction senescent cells lose this peptidase activity, resulting in metabolic alterations and elevated levels of ROS. A second important substrate for IMMP2L is the cell death regulator apoptosis-inducing factor (AIF): upon exposure to severe oxidative stress, IMMP2L cleaves AIF into its pro-apoptotic form, leading to clearance of irreparably damaged cells. In this way, IMMP2L-dependent mitochondrial reprogramming determines whether cells will undergo senescence or apoptosis in response to stress stimuli (Yuan et al., 2018). These findings provide insight into a new molecular pathway specifying the senescent cell fate, with potential relevance for human longevity and age-related disease.

Cellular senescence and cancer

Cellular senescence functions as a barrier against malignant transformation

CS plays complex and context-specific roles throughout the process of tumor development. During tumor initiation, OIS acts as a prominent barrier against cancer progression. OIS was first reported more than two decades ago, when it was shown that introducing a single causative mutation for tumorigenesis, HrasG12V, into primary cells provoked cell cycle arrest and CS instead of malignancy (Serrano et al., 1997). Today, various alterations in oncogenic or tumor suppressive signaling are known to trigger CS *in vitro* and *in vivo* (Gorgoulis and Halazonetis, 2010). In these settings, CS inhibits early-stage tumor progression through both intrinsic and extrinsic mechanisms, suggesting that CS is a prominent tumor suppression pathway. These oncogenic stimuli activate the p53 and $p16^{INK4a}$ signaling pathways to induce cell cycle arrest and block tumor initiation. Moreover, OIS can also reprogram cellular metabolism to favor proliferative arrest. For example, OIS induction through either BRAFV600E or HrasG12V enhances the activity of mitochondrial pyruvate dehydrogenase to promote pyruvate flux into the tricarboxylic acid (TCA) cycle. Consequently, an increased catabolism-to-anabolism ratio along with high levels of mitochondrial ROS together establish a metabolic state less supportive for proliferation (Kaplon et al., 2013).

Besides these intracellular pathways, SASP induction can also be tumor suppressive. Release of the SASP factors IL-1a, IL-6, and IL-8 can generate a positive feedback loop to reinforce the senescence program that includes upregulation of the cell cycle inhibitor $p15^{INK4b}$, thereby sustaining pro-inflammatory signaling (Acosta et al., 2008; Kuilman et al., 2008). Moreover, the secreted SASP component plasminogen activator inhibitor-1 (PAI-1) has been shown to play an important role in senescence-associated cell cycle arrest through its interference with growth factor-mediated mitogenic signaling (Elzi et al., 2012; Kortlever et al., 2006). SASP factors also modulate the microenvironment to activate immunosurveillance leading to senescent cell clearance. For example, the release of certain SASP factors can elicit an innate immune response that involves the recruitment of macrophages, neutrophils, and natural killer cells for senescent cell elimination (Xue et al., 2007). In addition to this immune cell recruitment for an innate response, SASP can also activate an adaptive immune response involving the infiltration of $CD4^+$ T cells and professional antigen presenting cells (APCs), such as monocytes/macrophages, to clear pre-malignant senescent cells expressing specific antigens (Kang et al., 2011). In sum, the current evidence suggests that CS functions to impede cancer formation at early stages of tumorigenesis through both tumor cell intrinsic (cell cycle arrest) and extrinsic (SASP-dependent) mechanisms.

Cellular senescence promotes late-stage tumor progression

Rather than functioning as a barrier against tumor progression, in some contexts CS has been shown to instead promote tumorigenesis. While SASP components can sometimes activate immunosurveillance programs to eliminate cells as described above, in other settings a diminished capacity of the immune system may instead allow SASP factors to promote tumor growth. For example, the aforementioned core SASP components IL-6 and IL-8 can drive an epithelial-mesenchymal transition (EMT) associated with a cancer stem cell phenotype (Ortiz-Montero et al., 2017). The SASP can also modulate the microenvironment to benefit cancer cells through secretion of vascular endothelial growth factor (VEGF) for angiogenesis (Coppé et al., 2006) or matrix metalloproteinases (MMPs) for cell invasion (Hassona et al., 2014; Kim et al., 2017). Furthermore, CS can also directly modulate the immunosurveillance system to prevent cancer cell clearance. As mentioned earlier, the SASP enables pre-cancerous senescent cells to be recognized and eliminated by immune cells. However, during advanced stages of cancer development in which senescent and malignant cancer cells co-exist within the same microenvironment, the senescent

cell secretome can recruit and guide immunosuppressive myeloid cells to protect established cancer cells from immune clearance (Eggert et al., 2016). In this regard, the distinct impacts of CS on an anti-cancer immune response versus tumor progression appear to be largely dependent on the stage of tumorigenesis.

Cellular senescence and anti-cancer treatments

Because CS represents a state of long-lasting cell cycle arrest with SASP-mediated immune cell activation, senescence induction has been considered as a promising therapeutic strategy for cancer treatment (Acosta and Gil, 2012). Multiple strategies have now been tested to safely and effectively induce cancer cell senescence based on known senescence inducers and effector pathways (Acosta and Gil, 2012). Namely, these strategies have included telomerase inhibition, CDK blockage, PTEN suppression, mutant p53 reactivation, and p53 stabilization. Among these, selective pharmacological targeting of CDK4/6 with palbociclib has shown promising outcomes for blocking tumor growth via senescence induction. These studies have resulted in the recent approval by the U.S. Food and Drug Administration (FDA) for palbociclib as a treatment for advanced hormone receptor positive (HR+) breast cancer, with additional clinical trials in other cancer types underway (Anders et al., 2011; Sherr et al., 2016; Vijayaraghavan et al., 2017).

Pro-senescence induction represents an emerging strategy for cancer treatment considering its prominent effects on inducing cell cycle arrest and immune surveillance. However, if therapy-induced senescent cancer cells persist in surviving in the body without being effectively eliminated, this could potentially lead to detrimental consequences. For example, continuous production and secretion of SASP factors could result in a pro-inflammatory microenvironment. Moreover, secondary mutations in senescence-associated effector pathways may place senescent cancer cells at risk for re-entry into the cell cycle, thereby leading to relapse and disease progression.

Thus, senescence-focused cancer treatments may need to ultimately ensure the elimination or death of senescent cancer cells in order to maximize therapeutic efficiency. Our recently published work (Yuan et al., 2018) demonstrates that senescent cells can acquire unique pro-survival mechanisms under stressful conditions, pathways which are not required for keeping healthy cells alive. This supports using a sequential intervention strategy to treat cancer in a two-step fashion as illustrated in Figure 1: first, inducing cancer cell senescence with palbociclib or another senescence inducer; and second, after cancer cells enter CS, applying secondary treatments to interfere with specific anti-apoptotic pathways required for senescent cancer cell survival. In this approach, only senescent cancer cells undergo cell death, such that

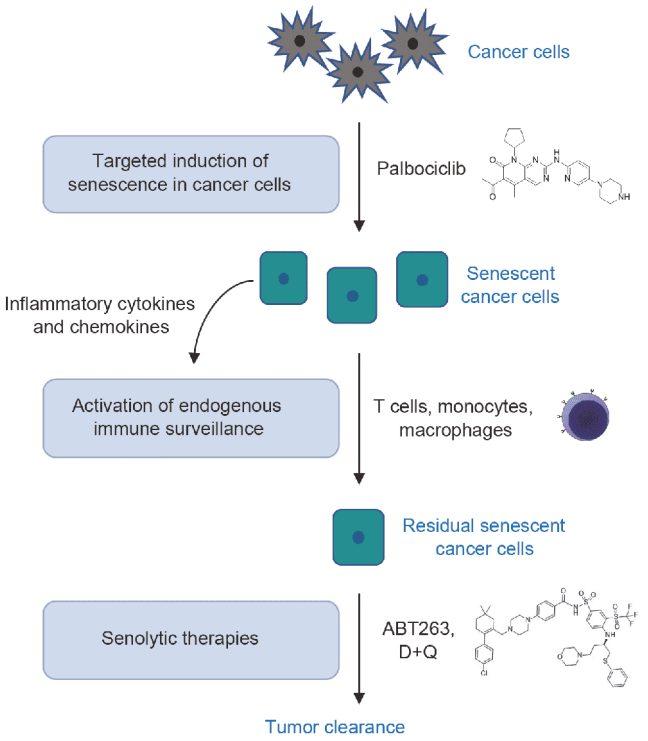

Figure 1 Harnessing cellular senescence to develop novel anti-cancer therapeutic strategies. CS-inducing agents such as the CDK4/6 inhibitor palbociclib have shown great promise for the treatment of various solid tumors. However, these therapies leave behind long-lived senescent tumor cells, which can deleteriously impact the surrounding microenvironment via the SASP. Recent strategies to clear these residual tumor cells have employed senolytic agents such as the Bcl-2 inhibitor ABT263 or the dasatinib and quercetin combination, with the goal of achieving improved long-term patient responses.

normal non-senescent cells remain viable and healthy. Meanwhile, this strategy could bring additional benefits if applied *in vivo*, because non-cancerous senescent cells, which are associated with age-related disorders, may be killed simultaneously. Indeed, a recent study explored the potential of a similar sequential senescence-to-apoptosis strategy for liver cancer treatment (Wang et al., 2019a). Here, a pharmacological inhibitor of the DNA-replication kinase CDC7 was employed to selectively induce senescence in cancer cells with mutated TP53. The investigators further demonstrated that, by suppressing mTOR signaling, the antidepressant compound sertraline can specifically induce apoptosis in senescent liver cancer cells but not in proliferating cells. Although further studies are needed, sequential pro-senescence therapy aimed at eliminating senescent cancer cells represents a promising new anti-cancer therapeutic strategy.

Cellular senescence and aging

The world's population is rapidly aging

The aged population is currently growing at a fast rate

worldwide. According to a recent report released from the U.S. Census Bureau, while 8.5% of the global population were aged 65 and older in 2015, this population is projected to more than double by 2050 to represent 16.7% of the total world population. Natural aging is a complex process of functional decline that progressively occurs at many levels ranging from gene expression to bodily operation throughout the lifespan of living organisms. In developed countries, aging is considered the primary risk factor associated with the development of many chronic diseases underlying severe illness and mortality in later life, including type 2 diabetes, cancer, neurodegeneration, and cardiovascular disease (Niccoli and Partridge, 2012). Accordingly, the projected steady increase in the aged population will bring many challenges related to well-being and societal prosperity.

In the last few decades, benefiting from advances in medical science and healthcare, human life expectancy has significantly increased around the world. Particularly, the average life expectancy for males born in the U.S. has progressively increased since the 1960s (Dong et al., 2016). However, this increased lifespan does not directly translate into additional years of active and healthy living: rather, many years at the later stage of lifespan are often accompanied by ill health (DALYs and Collaborators, 2017). For instance, in 1990 a U.S.-born male was projected to live until 72 with nine years of poor health, whereas in 2016, this life expectancy has increased to 76 but is nevertheless accompanied by 10 years of living with functional health loss.

Across the world, this large gap between lifespan and health span is a common issue. To meet the needs of a graying world, basic age-related research and clinical practice should aim to effectively expand the healthy and productive years of living for aging populations. Furthermore, the most prevalent chronic diseases including cancer, dementia, diabetes, heart diseases, and osteoporosis are all regarded as age-associated diseases that are now being investigated and treated separately. Therefore, an improved understanding of aging at the molecular level may hold great potential in the development of geriatric medicine to intervene in the aging process, with the goal of simultaneously preventing the onset and progression of age-related disease (Kaeberlein et al., 2015).

Cellular senescence as a hallmark of aging

Among all known cellular and molecular characteristics underlying the complex mammalian aging process, CS is regarded as one of the major hallmarks contributing to age-related phenotypes and disorders (López-Otín et al., 2013). Since the discovery of CS in cultured primary cells more than five decades ago, significant efforts have been made to characterize CS at the molecular level. Accordingly, several senescence-associated markers have been established enabling routine CS detection *in vivo*. In this regard, recent studies indicate that, during the course of natural aging, senescent cells accumulate in various tissues of rodents, primates, and human beings (Burd et al., 2013; Herbig et al., 2006; Kang et al., 2015; Wang et al., 2009).

Current research aims to explore whether senescent cell accumulation is merely a consequence of normal aging or whether those senescent cells are causatively involved in age-related pathologies. While the impact of senescent cell accumulation on many age-related diseases needs to be further investigated, emerging studies suggest that CS can contribute to these disorders. For example, during the decline of body mass and skeletal muscle strength (sarcopenia), the muscle stem cells responsible for muscle regeneration enter a senescent cell fate (Doherty, 2003). In a cell autonomous-manner, senescence renders those stem cells incapable of dividing, thereby leading to a diminished capacity for muscle regeneration (Sousa-Victor et al., 2014).

Causative roles of CS for age-related disorders have also been attributed to SASP factors acting in a paracrine manner. In developed counties, atherosclerosis is now the most common cause of mortality and disability; mechanisms underlying this disease involve the development of atheromatous plaques, which are degenerative materials residing in and pathogenically remodeling the arteries. A recent study revealed that these atherosclerotic lesions consist of numerous senescent cells, the presence of which is detrimental for advancing atherosclerosis. Regarding the precise paracrine mechanism, these senescent cells have been shown to release SASP factors such as IL-1 into the surrounding microenvironment to amplify inflammation in the plaques. Meanwhile, secreted proteolytic MMP factors may also destabilize plaques leading to potential rupture and subsequent thrombosis (Childs et al., 2016).

Eliminating senescent cells to extend healthy lifespan

Although transient CS activation can be beneficial to suppress the onset of tumorigenesis, the accumulation of viable senescent cells *in vivo* due to declined immunesurveillance is deleterious and contributes to age-related disorders as described above. Accordingly, efficient elimination of senescent cells in tissues and organs represents a promising therapeutic strategy to prevent the onset of chronic diseases and extend healthy lifespan. The first proof-of-concept study conducted in prematurely aged mice suggested that selective clearance of $p16^{INK4a}$-positive senescent cells can delay and ameliorate many age-related pathologies (Baker et al., 2011). A few years later, consistent results were obtained in naturally aged mice such that genetic elimination of senescent cells expressing $p16^{INK4a}$ preserved the functionality of multiple organs, thereby prolonging both lifespan and healthy lifespan (Baker et al., 2016).

Built upon these promising studies linking senescent cell clearance to healthy aging, current research is aimed toward developing therapeutic interventions targeting senescent cells *in vivo* with the goal of preserving body fitness throughout the aging process (Figure 2). Several approaches have been conceived that focus on employing pharmacological agents termed senolytic drugs to induce senescent cell death (Baar et al., 2017; Fuhrmann-Stroissnigg et al., 2017; Zhu et al., 2015). One prominent first-in-class compound identified for this purpose is ABT263, which is a potent inhibitor of the anti-apoptotic proteins BCL-2, BCL-XL, and BCL-W. ABT263 has been shown to kill senescent cells residing in sublethally irradiated or naturally aged mice, leading to the rejuvenation of certain stem cell populations (Chang et al., 2016). Moving forward, some of the first small-scale clinical trials aimed at evaluating the impact of targeted senescent cell killing have delivered promising results. In particular, combinatory intermittent administration of the senolytic compounds dasatinib and quercetin (D+Q) can alleviate physical dysfunction associated with idiopathic pulmonary fibrosis, which has been identified as a CS-associated disease (Justice et al., 2019). Further clinical trials revealed that D+Q decreases senescent cell abundance in multiple tissues including adipose and skin (Hickson et al., 2019). Taken together, stemming from the characterization of the detrimental impacts of CS on age-related pathologies, ongoing innovation in senolytic therapy to effectively and safely eliminating senescent cells holds great promise to provide fundamental support for healthy aging.

In addition to pharmacological elimination of detrimental senescent cells, suppressing the onset of CS could represent an additional avenue leading to health preservation. One recent study suggests that the natural polyamine agent named spermidine, which is prevalent in daily nutrition, could be beneficial to suppress senescence (García-Prat et al., 2016). As mentioned above, senescence induction in muscle stem cells compromises muscle's regenerative capacity and leads to the onset of sarcopenia. In a geriatric mouse model, spermidine administration was shown to trigger autophagy and decrease protein aggregation in muscle stem cells, thereby restoring their regenerative capacity. It remains to be further explored whether increased uptake of this daily nutrient can suppress senescence to benefit human health. Although this and many other questions remain, pharmacologically reducing the level of CS, via either senescent cell ablation or suppression of pro-senescence pathways, represents an exciting approach for extending the healthy lifespan of the world's aging population.

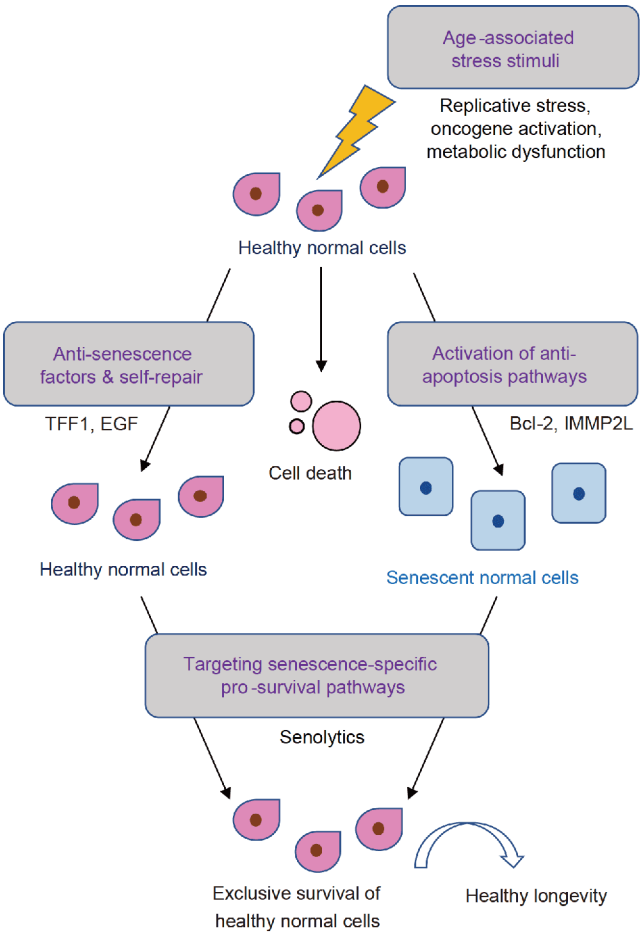

Figure 2 Cellular senescence as an anti-aging target to promote healthy longevity. A variety of age-associated cell-intrinsic stresses and environmental insults are known to induce CS in normal cells, including certain stem cell populations, and this has been correlated with age-related functional decline. These stress stimuli trigger CS rather than cell death via induction of senescence-specific pro-survival signaling cascades. Pharmacological targeting of these survival pathways to clear senescent cells may protect against the negative impacts of CS to promote healthy longevity.

Conclusion and perspective

During the past decade, firm links between CS and the aging process have been established such that delaying the onset of CS or ablating senescent cells can now be considered a rational approach to extend human healthspan. At the same time, progress has also been made in developing new targeted anticancer treatments that act by inducing stable cell cycle arrest in cancer cells. During the next 10 years, we anticipate the rapid development of therapeutic strategies combining CDK inhibitors or other CS inducers with senolytic agents to achieve selective tumor cell apoptosis. While targeted ablation of senescent cells to combat aging remains a more distant goal, the aging world population dictates that this will also remain an active area of research. Importantly, the roles of CS in mammalian aging and tumor progression were first elucidated from fundamental biological discoveries; thus, CS illuminates how research in basic science can have major unanticipated therapeutic implications. As such, an improved understanding of the precise molecular

mechanisms governing CS will be critical for fully leveraging the senescent cell fate for the advancement of human health.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

Acknowledgements *The authors apologize to colleagues whose work was not cited due to space limitations or our oversight. We thank the current and past members of the Wang laboratory whose studies contributed to the development of concepts presented in this review. This work was supported by the National Institute of Health (R01-CA233205 to X.F.W.).*

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