To clear, or not to clear (senescent cells)?
That is the question

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Cellular senescence is an anti-proliferative program that restricts the propagation of cells subjected to different kinds of stress. Cellular senescence was initially described as a cell-autonomous tumor suppressor mechanism that triggers an irreversible cell cycle arrest that prevents the proliferation of damaged cells at risk of neoplastic transformation. However, discoveries during the last decade have established that senescent cells can also impact the surrounding tissue microenvironment and the neighboring cells in a non-cell-autonomous manner. These non-cell-autonomous activities are, in part, mediated by the selective secretion of extracellular matrix degrading enzymes, cytokines, chemokines and immune modulators, which collectively constitute the senescence-associated secretory phenotype. One of the key functions of the senescence-associated secretory phenotype is to attract immune cells, which in turn can orchestrate the elimination of senescent cells. Interestingly, the clearance of senescent cells seems to be critical to dictate the net effects of cellular senescence. As a general rule, the successful elimination of senescent cells takes place in processes that are considered beneficial, such as tumor suppression, tissue remodeling and embryonic development, while the chronic accumulation of senescent cells leads to more detrimental consequences, namely, cancer and aging. Nevertheless, exceptions to this rule may exist. Now that cellular senescence is in the spotlight for both anti-cancer and anti-aging therapies, understanding the precise underpinnings of senescent cell removal will be essential to exploit cellular senescence to its full potential.

Keywords:
- cancer; immune surveillance; SASP; senescence.

Introduction

Cellular senescence is a complex stress response in which a proliferation-competent cell undergoes irreversible growth arrest, even in the presence of mitogenic stimulation [1]. Senescent cells are therefore distinct from post-mitotic and terminally differentiated cells, which have lost the capability to divide as part of their developmental programs, and from quiescent cells, which can re-enter the cell cycle when exposed to mitogenic cues [2]. The concept of cellular senescence was first introduced by Hayflick and Moorhead more than five decades ago to describe the finite replicative potential of human diploid cells after extensive serial passaging in culture [1]. This particular phenomenon was later linked to telomere attrition, which leads to chromosomal instability and can potentially cause cancer, supporting the original role of cellular senescence in tumor suppression [3].

The senescence response is elicited by multiple distinct stimuli connected to cellular damage [4, 5] and is accompanied by broad morphological and phenotypic changes [6, 7]. In addition to the permanent cell cycle arrest, senescent cells present increased cell size and a flat morphology in vitro and accumulate a lysosomal enzyme, which is termed senescence-associated β-galactosidase and can be easily detected by conventional staining [8]. Senescent cells also display extensive transcriptional changes, including up-regulation of tumor suppressor genes and down-regulation of cell-cycle promoting genes [6, 7, 9]. Furthermore, most senescent cells secrete inflammatory cytokines, growth factors and matrix metalloproteinases as part of the senescence-associated secretory phenotype (SASP) [10–12] also known as senescence-messaging-secretome [13]. Finally, senescent cells are also characterized by substantial chromatin and nuclear changes [14, 15]. Considering that none of these traits is exclusive to senescent cells, a combination of several markers is required to accurately define senescence, which can be a limitation when assessing senescence in vivo [2].
Beyond tumor suppression, it is now recognized that cellular senescence is functionally linked to many other biological processes, such as wound healing, tissue remodeling, embryonic development, aging and paradoxically, tumorigenesis [2, 9, 16]. The involvement of cellular senescence in these processes requires both cell-autonomous and non-cell-autonomous activities [16]. Most of the non-cell-autonomous effects are mediated by the SASP, which can impact tissue microenvironment, affect neighboring cells and recruit immune cells that can subsequently eliminate senescent cells [9, 16]. It is generally accepted that transient induction of senescence, followed by tissue remodeling and removal of senescent cells by the immune system is beneficial, because it contributes to the disposal of damaged cells [9]. On the other hand, chronic senescence or the inability to eliminate senescent cells is adverse, because the accumulation of senescent cells can lead to cancer and/or aging [9]. Therefore, the clearance of senescent cells seems to play a pivotal role in determining the net organismal effects of cellular senescence.

This review dissects the roles that different components of the senescence program (cell cycle arrest, SASP and immune clearance) play in cancer and other pathological states. The review initially focuses on the more established anti-proliferative roles of senescence, followed by recent advances shedding light on SASP regulation and its pleiotropic effects. Special emphasis is given to the clearance of senescent cells by the immune system, which depends on the senescence trigger, cellular context and cell type, and seems to dictate the ultimate outcome of the senescence program. How senescence can be induced to restrict or treat cancer and improve tissue remodeling, and how senescence and SASP can be targeted to avoid their deleterious effects are also discussed.

**Permanent cell cycle arrest**

Cell-cycle arrest is the most prominent feature of senescent cells, which permanently withhold replication by expressing cell-cycle inhibitors and repressing cell-cycle promoting genes [17]. Cellular senescence and associated proliferative arrest can be triggered by different cellular stressors, such as telomere shortening, oxidative damage, DNA damaging agents or oncogene activation [2, 6, 9]. Depending on the cell type and the trigger, different pathways are activated, and fundamentally distinct senescence states are achieved [2, 6, 9].

The initial cell culture phenomenon observed by Hayflick and Moorhead [1] is now defined as ‘replicative senescence’ and is the result of consecutive cell divisions that fail to maintain telomere length [18]. Telomeres are repetitive sequences (TTAGGG) that cover several kilobases of DNA located at the ends of chromosomes where they exert a protective function preserving genomic instability [19]. Once they reach a critically short length, telomere erosion is sensed by the cells as a type of DNA damage, therefore triggering a DNA-damage response (DDR) [20]. The main mediators of the DDR are the DNA damage kinases ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related and checkpoint kinases 1 and 2 [21, 22], which block cell-cycle progression through phosphorylation and stabilization of p53. The activation of p53 leads to the up-regulation of the cyclin-dependent kinase (CDK) inhibitor p21CIP1 (encoded by CDKN1A) (Fig. 1) [23, 24]. In parallel, ‘replicative senescence’ also results in expression of the CDKN2A locus [25], which comprises two important tumor suppressor genes: p14\(^{ARF}\) and p16\(^{INK4A}\) [26, 27]. While p14\(^{ARF}\) (p19\(^{ARF}\) in mice) further reinforces the activation of the p53/p21CIP1 pathway by degrading MDM2 proto-oncogene [28–30], p16\(^{INK4A}\) represses both CDK4 and CDK6 [27]. The combined inhibition of CDKs by p21CIP1 and p16\(^{INK4A}\) induces hypo-phosphorylation and activation of the tumor suppressor RB1 (retinoblastoma) [31], which binds and inhibits E2F transcription factors, permanently arresting cells in the G1 phase (Fig. 1) [32].

In addition to telomere shortening, cellular senescence can also be initiated following prolonged stress, such as DNA damage, hypoxia or accumulation of reactive oxygen species (ROS), which gives rise to ‘stress-induced senescence’ [33]. All these triggers activate the tumor suppressor p53 (Fig. 1), which can then drive either apoptosis or transient growth arrest through its target p21CIP1. In the case of increased ROS production, p53/p21CIP1 axis activation is mediated by p38 MAPK (mitogen-activated protein kinase 14) (Fig. 1) [34]. If the stress is not resolved by cellular repair mechanisms, this temporary growth arrest can progress to senescence, via activation of p16\(^{INK4A}\). As explained earlier, up-regulation of p16\(^{INK4A}\) and p21CIP1 leads to hypo-phosphorylated RB1, which slams the cell cycle to a stop in G1 phase (Fig. 1) [9].

**Figure 1. Senescence triggers and effector pathways.** A variety of stresses can activate the cellular senescence program. These stressors can engage the DNA-damage response (DDR) pathway, which activates the p53 and p16\(^{INK4A}\) pathways. This results in the inhibition of cyclin-dependent kinases (CDKs) and activation of the tumor suppressor retinoblastoma (RB1), which induces a permanent cell cycle arrest. There are also DDR-independent mechanisms, and all of them converge in the p53 and p16\(^{INK4A}\) pathways. ROS, reactive oxygen species; mTOR, mammalian target of rapamycin.
Activated oncogenes are also well-known inducers of senescence. ‘Oncogene-induced senescence’ was first observed when an oncogenic form of RAS (H-ras-V12) was expressed in human fibroblasts and led to permanent cell cycle arrest [35]. Mechanistically, activation of the MAP kinase signaling pathway can result in elevated ROS levels, halting the cell cycle (Fig. 1) [34, 36]. In addition, oncogenic RAS causes aberrant DNA replication, formation of double-stranded DNA breaks and activation of the DDR pathway, which can also drive senescence (Fig. 1) [4, 5]. ‘Oncogene-induced senescence’ also induces derepression of the CDKN2A locus (Fig. 1) [37, 38]. Interestingly, different oncogenes can trigger senescence through diverse mechanisms. For example, activation of BRAF (V600E) leads to p16INK4A induction and activation of pyruvate dehydrogenase (PDH) [39]. PDH activation is followed by an increased mitochondrial metabolism, which, in turn, results in high levels of ROS and induction of senescence (Fig. 1) [39]. Contrary to RAS-induced senescence, oncogenic BRAF-mediated cellular senescence seems to be DDR-independent [40].

Similar to oncogene activation, loss of tumor suppressor genes can also trigger senescence. Homozygous deletion of the tumor suppressor RB1 induces senescence via stimulation of the DDR and requires the activation of other RB family members that compensate for inactivation of RB1 [41]. The total absence of the tumor suppressor PTEN (phosphatase and tensin homologue) can lead to senescence without DDR activation or hyper-replication. Thus, its loss induces senescence in prostate lesions via mammalian target of rapamycin complex 1 and p53 activation (Fig. 1) [42, 43]. Finally, inactivation of neurofibromin 1 (NF1) and von Hippel-Lindau tumor suppressor activate the CDKN2A locus in a DDR-independent fashion [44, 45]. Other less-studied inducers of senescence include epigenetic, nucleolar and mitotic spindle stresses, which are also mediated by p53 and p16INK4A pathways [46]. The majority of the discoveries on cellular senescence have been made through experiments performed in vitro, using a single senescence-inducing stimulus [46]. However, individual cells in vivo are subjected to multiple cellular stressors simultaneously, suggesting that the activation of the downstream effector pathways may be more complex [46]. Moreover, the stimulated effector pathways also seem to depend on cell type and cellular context, which has led to define distinct senescence types [9]. Unexpectedly, some studies have suggested that senescent cells could re-enter the cell division cycle. For example, inactivation of PTEN or PDH in melanocytic nevi can reverse ‘oncogene-induced senescence’ [39, 47], while inactivation of p53 and p16INK4A or RB1 can reverse ‘replicative senescence’ [48–50]. A comprehensive catalog of senescence triggers, and activated pathways in different cell types will be useful to fully understand the nuances of the regulation of cellular senescence and its net organismal consequences.

**Interaction with the microenvironment**

Only a fraction of the effects of cellular senescence can be attributed to the lack of proliferative capacity. Senescent cells actively communicate with neighboring cells and the tissue microenvironment through the secretion of an array of molecules that constitute the SASP. The SASP includes pro-inflammatory cytokines, chemokines, growth factors and extracellular matrix proteases. This secretory program was initially noticed by studying the transcriptional profile of senescent cells [51, 52]. The concept of ‘senescence-associated secretory phenotype’ was later introduced by the Campisi group when they realized that secreted factors from senescent fibroblasts could promote the transformation of pre-malignant but not of normal cells [53]. This was an unanticipated discovery, because senescence was believed to just be an anti-tumoral program [35].

The SASP is regulated at both the transcriptional and post-transcriptional levels. The expression of SASP factors is controlled by the transcription factors NF-κB (nuclear factor of kappa light) and C/EBPβ (CCAAT/enhancer-binding protein-β), which have increased activity in senescence [10–12]. The regulation of the SASP program involves complex regulatory circuits aiming at both amplifying and restricting the secretory phenotype. On the one hand, interleukin 1α (IL1A) can stimulate the expression of NF-κB, which at the same time induces the expression of IL1A (Fig. 2). Similarly, NF-κB orchestrates the transcription of SASP factors, which in turn can amplify the activity and expression of NF-κB as part of a positive regulatory loop (Fig. 2) [10, 12, 54]. One the other hand, senescent cells with a strong SASP up-regulate microRNAs miR-146a and miR-146b through NF-κB, and these microRNAs repress NF-κB-regulator IRAK1 restricting the SASP response (Fig. 2) [55]. The purpose of this negative regulatory loop may be to restrain the

![Figure 2. Senescence-associated secretory phenotype (SASP) regulation. The SASP is mediated through DNA-damage response (DDR)-dependent and DDR-independent mechanisms. The transcription factor NF-κB is the main regulator of the transcription of SASP genes, and different pathways can control NF-κB expression and activation. Moreover, there are intricate positive and negative regulatory loops, which allow enhancing and restricting the SASP response, respectively. SASP is involved in different biological processes. mTOR, mammalian target of rapamycin.](image-url)
excessive secretion of molecules that could induce inflammation [55] or to prevent the accidental activation of the pathway as a result of stochastic transcription factor fluctuations [56].

Senescence-associated secretory phenotype regulation is mediated through DDR-dependent and DDR-independent mechanisms. On one hand, the DDR proteins ATM, NBN (nibrin, also known as NBS1) and checkpoint kinases 2 can be involved in the initiation and maintenance of the SASP [57]. The mechanistic link between DDR and SASP was recently elucidated [56]. By using a miR-146a expression reporter, which can inform of SASP induction [55], GATA4 (GATA binding protein 4) was identified as a regulator of cellular senescence and SASP response (Fig. 2) [56]. In normal cells, GATA4 binds the p62 autophagy adaptor and is degraded by selective autophagy. However, upon senescence induction, this selective autophagy is suppressed through decreased interaction between GATA4 and p62. Increased levels of GATA4 induce TRAF3IP2 (tumor necrosis factor receptor-associated factor protein 2) and IL1A, which activate NF-κB to initiate and maintain the SASP. GATA4 also activates the expression of miR-146a, which was previously shown to dampen the activation of NF-κB [55]. GATA4 pathway activation depends on the key DDR kinases ATM and ataxia telangiectasia and Rad3-related. However, how the DDR inhibits the basal autophagy-mediated degradation of GATA4 remains an open question.

On the other hand, it is now clear that several senescence-inducing stimuli produce SASPs independent of DNA damage [39, 58–62]. For example, SASP can be regulated by p38 MAPK, which can act independently of the canonical DDR [60]. Mechanistically, p38 MAPK induces the SASP by strongly increasing NF-κB transcriptional activity (Fig. 2). In addition, mutant BRAF and PTEN-loss-induced senescence programs are accompanied by the secretion of multiple components of the SASP [39, 61]. Finally, developmental senescence seems to also produce a SASP independent of DDR [58, 59]. These studies raise the question of whether DDR-dependent and DDR-independent SASPs could be inherently different. Moreover, not all senescent cells produce a SASP [63], further emphasizing the complexity of this process.

The number of proteins involved in SASP regulation is rapidly increasing and includes Nemo (IKBKG; inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma) [64], RIG1 (retinoic acid-inducible gene I) and Klotho [65], MacroH2A1 [66] or HMGB1 (high mobility group box 1) [67], further suggesting that different types of senescence may depend on different regulators. Recently, the role of mTOR (mechanistic target of rapamycin) on SASP regulation was unveiled by two independent studies, which propose two distinct but complementary mechanisms (Fig. 2) [68, 69]. The first study shows that mTOR can control the translation of IL1A, and therefore, regulate the SASP [69]. The authors observed that IL1A mRNA levels were almost unaffected by rapamycin, an mTOR inhibitor, whereas protein levels on the surface of senescent cells were significantly lower. Treatment of senescent cells with rapamycin significantly altered SASP production and suppressed the ability of senescent fibroblasts to stimulate tumor growth, suggesting that similar inhibitors could be used to block the deleterious effects of the SASP. In parallel, the other study demonstrates that mTOR can also orchestrate the SASP by regulating the translation of MK2 (MAPKAPK2; mitogen-activated protein kinase-activated protein kinase 2) [68]. MK2 phosphorylated the RNA-binding protein ZFP36L1 (ZFP36 ring finger protein-like 1), restraining its ability to degrade the transcripts of numerous SASP components. Interestingly, pharmacological mTOR inhibition impaired the ability of senescent cells to either suppress or promote tumor growth. These studies suggest that pharmacological inhibition of SASP can have both advantageous and disadvantageous effects, and further experimentation will be required to determine the specific scenarios that could benefit from SASP inhibition.

Senescence-associated secretory phenotype factors are highly pleiotropic and can impact various biological processes (Fig. 2) [2]. For example, SASP components are able to influence the senescence-cell cycle arrest, in a cell-autonomous manner, possibly to help establish a more stable growth arrest [10, 12]. Recent studies have shown that SASP can also transmit senescence to the normal neighboring cells in a paracrine manner [64, 70]. Paracrine senescence is mediated by a complex secretory program that is orchestrated by the inflammasome and IL-1 signaling, and involves TGFβ (transforming growth factor beta), which generates ROS and DNA damage and subsequent permanent cell cycle arrest [64, 70]. The effects of the SASP are not restricted to tumor suppression, but can also involve tumor promotion [53] or wound healing [71, 72]. The role of SASP on aging has not been fully elucidated [73], and strategies that ablate SASP in vivo in aged organisms will be required to address this question. Finally, SASP can also induce inflammatory cell recruitment, which can in turn affect tissue microenvironment, promote or repress tumorigenesis and remove senescent cells [74–77]. Taken together, SASP composition seems variable and depends on the cell type, the senescence trigger, the cellular context and the intensity and the duration of the program [11, 62], and could therefore have different organismal effects.

**Immunoclearance of senescent cells**

Senescent cells secrete various immune-cell attracting chemokines, activating cytokines, adhesion molecules and immune modulators, implying that attracting immune cells and inducing local inflammation are common properties of senescent cells [78]. Depending on the secreted molecules and the tissue microenvironment, senescent cells can potentially interact with different immune cell subtypes. Interestingly, senescent cells can directly transfer proteins to immune cells, and this novel mode of senescent cell communication could potentially regulate immune surveillance [79]. Nevertheless, different studies support the notion that senescent cell elimination is required for the full beneficial effects of cellular senescence in different biological processes such as tumor regression, tumor suppression, tissue remodeling, development and aging (Fig. 3) [9, 78].

The first evidence for the clearance of senescent cells by the immune system was provided by studies performed in a mouse model of hepatocellular carcinoma in which endogenous p53 could be restored in established tumors by turning off an shRNA (short hairpin RNA) against p53 [75]. Re-expression of endogenous p53-induced senescence and subsequent elimination of senescent tumor cells by innate immune cells, including macrophages, neutrophils and natural killer cells (NK cells).
Pharmacological inhibition of NK cells or depletion of macrophages delayed tumor regression, suggesting that senescent cell removal is necessary to fully benefit from the induction of cellular senescence in liver cancer cells. Using a similar system and focusing on NK cells, it was later shown that the recognition of senescent tumor cells expressing the ligand RAE1ε (retinoic acid early inducible ε) by NK cells expressing the receptor NKG2D (also known as KLRK1; killer cell lectin-like receptor subfamily K, member 1) was crucial for the successful disposal of senescent tumors [80]. In a different model involving orthotopic implantation of metastatic melanoma tumors taken from patients, pharmacological inhibition of aurora kinases impaired mitosis, induced senescence and markedly blocked proliferation of patient-derived xenografts [81]. Macrophage recruitment was dependent on senescence induction and was a requisite for tumor regression and to avoid tumor re-growth. Altogether, these data demonstrate that triggering senescence in tumor cells coupled with immune surveillance can lead to effective tumor regression (Fig. 3).

The disposal of senescent cells in vivo is also crucial to prevent tumor initiation (Fig. 3). In a mouse liver model of oncogenic NrasG12V-induced senescence, senescent premalignant cells were found surrounded by various immune cells and were cleared by infiltrating monocytes and macrophages [77]. Interestingly, the elimination of senescent cells by innate immune cells required the CD4+TH1 cell response. Indeed, CD4+ T cells were able to recognize the new epitope formed by the G12V mutation in Nras. Most importantly, the targeting of senescent cells was compromised in immunodeficient mice lacking NK cells or NK cells and macrophages, and this reduced clearance led to the development of full-blown liver tumors, indicating that senescent cell removal is key for the tumor suppressor function of senescent cells in vivo. In a different setting, radiation-induced senescence in the bone, which depends on RB1, was associated with the secretion of multiple bioactive factors, including interleukin-6 (IL6), as well as with the infiltration of natural killer T (NKT) cells [82]. Importantly, the absence of senescent cell clearance predisposed mice to radiation-induced osteosarcomas, further emphasizing the importance of senescent immunosurveillance for the positive effects of cellular senescence in tumor suppression.

Cellular senescence and subsequent clearance are not just required for cell-autonomous tumor suppression but are also critical for the additional beneficial aspects of cellular senescence (Fig. 3). For example, during liver injury, hepatic stellate cells (HSCs) are activated to become proliferative and fibrogenic myofibroblasts, which contribute to liver fibrosis [83]. HSCs eventually become senescent and display a SASP that includes extracellular matrix proteases and pro-inflammatory cytokines [74]. On one hand, the secretion of extracellular matrix-degrading enzymes helps resolve fibrosis. On the other hand, the SASP attracts innate immune cells that eliminate the senescent HSCs and facilitate the resolution of fibrosis. Pharmacological inhibition of NK cells does not affect the initial number of senescent HSCs but delays fibrosis resolution, supporting the importance of senescent cell clearance for fibrosis reversion. In a subsequent study, granule exocytosis was shown to be essential for NK-cell-mediated killing of senescent cells [84]. Accordingly, mice with defects in granule exocytosis accumulated senescent HSCs and displayed more liver fibrosis in response to a fibrogenic agent, further emphasizing the benefits of senescent cell removal in maintaining tissue homeostasis. Senescent HSCs can also be killed by macrophages in a process that is dependent on p53 [76]. Thus, p53-expressing senescent HSCs produce a SASP that induces M1 polarization of the macrophages, which is the tumor-suppressive class of macrophages, therefore creating an anti-tumor microenvironment. In the absence of p53, HSCs continue to proliferate and stimulate tumor-promoting M2 macrophages, creating a pro-tumorigenic microenvironment. Taken together, the removal of senescent cells can have non-cell-autonomous effects in maintaining tissue homeostasis and restricting tumorigenesis (Fig. 3).

Senescent cell elimination is also beneficial for other biological processes, such as tissue regeneration and embryonic...
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Experiments will shed light on all these unanswered questions. Thus, the genetic elimination of p16-expressing senescent cells could also be important to dictate the net effects. As highlighted before, it could depend on the senescence trigger, tissue context and secreted molecules. Furthermore, the timing of senescent cell removal could also be important to dictate the net effects. Thus, the genetic elimination of p16-expressing senescent cells was recently shown to impair wound healing [72]. Future experiments will need to further confirm the relevance of the senescent cell clearance for the successful completion of these physiological processes [9].

Finally, evidence of the positive effects of the removal of senescent cells comes from settings in which senescent cells are not eliminated and therefore lead to detrimental consequences. The most notorious example is aging, which is characterized by the progressive accumulation of senescent cells [86–88]. This accumulation is not just a marker of organismal aging, but has important functional consequences. Thus, genetic elimination of senescent cells in a model of accelerated aging led to delay and reversal of aging, demonstrating that the accumulation of senescent cells actively contributes to aging [89]. One plausible explanation is that the efficient turnover of senescent stem or progenitor cells facilitates tissue regenerative capacity [62]. It is also possible that ablation of senescent cells attenuates the local and perhaps systemic effects of the SASP, which might contribute to various aspects of aging [46, 73]. These results support that senolytic agents that are selectively toxic to senescent cells could trigger their clearance and reverse some aspects of aging [90]. In the case of melanocytic nevi, accumulation of senescent cells is in general not accompanied by immune cell infiltration and inflammation [46]. Even if nevi are stable for many years, they pose a risk for the subsequent development of melanoma [91], and in fact, PTEN inactivation in melanocytic nevi leads to melanoma formation in vivo [47], further highlighting the advantages of eliminating senescent cells. Moreover, senescent cells have been shown to aggravate pulmonary fibrosis, which is also associated with their accumulation [92]. Why some senescent cells accumulate while others are eliminated is unknown. As highlighted before, it could depend on the senescence trigger, tissue context and secreted molecules. Furthermore, the timing of senescent cell removal could also be important to dictate the net effects. Thus, the genetic elimination of p16-expressing senescent cells was recently shown to impair wound healing [72]. Future experiments will shed light on all these unanswered questions.

Therapeutic opportunities

Considering that cellular senescence can exert both beneficial and detrimental effects, both pro-senescence and anti-senescence approaches are being developed. Pro-senescence therapies could be useful for the treatment of cancer and for ongoing tissue repair processes, whereas anti-senescence therapies could be beneficial to remove senescence and fibrosis in ‘resolved’ injuries or to rejuvenate the aged muscle [9]. The challenge lies on suppressing the negative aspects of cellular senescence while enhancing the positive ones (Fig. 3).

The potential of pro-senescence therapies was unveiled by using the Eμ-Myc mouse model of B-cell lymphoma, in which Myc is expressed under an immunoglobulin heavy-chain enhancer–promoter and restricted to the B-cell compartment [93]. Upon cyclophosphamide (CTX) treatment, Eμ-Myc; Trp53−/− tumors showed a delayed regression followed by recurrence, while Eμ-Myc; Bcl2 tumors, which are resistant to apoptosis, presented a significantly better prognosis through the induction of cellular senescence [94]. The senescent response was not accompanied by immune clearance, and as a result, Eμ-Myc; Bcl2 lymphomas eventually progressed. By using similar systems, cellular senescence could be induced in Eμ-NrasG12D; Bcl2 lymphomas that were treated with chemotherapy [95] and in Myc-driven lymphomas in response to Myc inactivation [96]. Furthermore, endogenous p53 reactivation triggers senescence in various tumor models, such as HrasG12V-driven liver cancer, sarcomas and angiosarcomas [75, 97, 98].

Cellular senescence can also be induced pharmacologically (Fig. 3) [99]. For example, CDK4 inhibitors result in senescence in many cancer cells and are showing promising activity in clinical trials of breast cancer [100–104]. Furthermore, pro-senescence therapies could be exploited for non-tumoral pathologies. For example, palbociclib could be used for the treatment of renal fibrosis [105], while CCN1 administration could have potential for liver and cutaneous fibrosis [71, 106]. Several novel compounds have recently been identified as senescence inducers. By performing a cell-based pro-senescence screen, AURKB inhibitors were identified as promising pro-senescence candidates [107, 108]. In addition, sunitinib, a known multi-targeted receptor tyrosine kinase (RTK) inhibitor, can also trigger senescence in cancer cells [109].

Given that senescent cell accumulation is in general detrimental, strategies to clear senescent cells in those cases in which they are not naturally eliminated will be required (Fig. 3) [9]. For example, chemotherapeutic treatments can promote disease progression through the induction of senescence and associated SASP in the tumor stroma [110] or in the tumor cells [111]. One option would be to manipulate the SASP or the immune cells to facilitate the clearance of senescent cells. Work from Alimonti laboratory recently demonstrated that senescent PTEN null tumors in mice are massively infiltrated by CD11b+Gr-1+ myeloid cells that protect proliferating tumor cells from senescence, therefore sustaining tumor growth [61]. Mechanistically, Gr-1+ cells antagonized senescence in a paracrine manner by interfering with the SASP through the secretion of interleukin-1 receptor antagonist (IL-1RA). Therapeutically, the authors reduced the percentage of tumor-infiltrating CD11b+Gr-1+ myeloid cells by using an antagonist of CXC chemokine receptor 2 (CXCRL2), enhancing the senescent response. In a subsequent study, they demonstrated that the immunosuppressive microenvironment is established by the Jak2/Stat3 pathway in prostate senescent tumor cells [112]. Treatment with docetaxel in combination with a JAK2 inhibitor reprogrammed the SASP and improved the efficacy of docetaxel-induced senescence by triggering a strong antitumor immune response. Another possibility is to combine pro-senesecence therapies with senolytic strategies. In lymphoma, therapy-induced senescence (TIS) improves long-term outcomes, but tumors eventually regrow [113]. TIS-competent lymphomas show increased glucose utilization and much higher ATP production in a process that also involves...
increased autophagy [113]. By selectively blocking glucose utilization or autophagy, senescent tumor cells underwent an apoptotic response. Altogether, these data demonstrate that the SASP, immune cells or senescent cells can be manipulated to improve therapeutic response to pro-senescent therapies (Fig. 3).

Finally, anti-senescent therapies could be used in settings in which senescent cells accumulate (Fig. 3). On one hand, several pharmacological agents may function through inhibition of senescence [9]. For example, lung fibrosis can be improved by decreasing senescence through the chemical inhibition of NOX4 [114] or with the anti-inflammatory agent rutipatidine [115]. On the other hand, targeting senescent cells would also be promising to improve lifespan because the genetic elimination of senescent cells can delay and reverse aging in a mouse model of accelerated aging [103]. In a recent study, dasatinib (a Bcr-Abl tyrosine kinase inhibitor) and quercetin (a flavonol) were able to selectively kill senescent cells [90]. In vivo, this combination reduced senescent cell burden in chronologically aged mice, radiation-exposed mice and progeroid mice. And periodic drug administration extended healthspan in progeroid mice. These results demonstrate that senolytic therapies are feasible and could be used to extend healthspan and possibly lifespan.

Conclusions

Cellular senescence was initially defined as a cell-autonomous tumor suppressor program. It is now clear that cellular senescence presents non-cell-autonomous activities and is involved in many distinct biological processes, where it plays both positive and negative roles. Cellular senescence is an anti-proliferative program that restricts the propagation of damaged cells. However, although arrested, those senescent damaged cells are metabolically active and secrete a variety of molecules to communicate with the tissue microenvironment and the neighboring cells, with either favorable or unfavorable consequences. These secreted factors can also attract immune cells, which can target and eliminate the senescent cells. In general, senescent cell clearance gives rise to beneficial outcomes, such as tumor suppression, tumor regression, tissue remodeling and embryonic development. In contrast, senescent cell accumulation tends to be associated with detrimental effects, such as cancer or aging. What determines the accumulation or disposal of senescent cells remains elusive. One plausible hypothesis is that depending on the trigger, the cell type and the cellular context, distinct senescence responses are unleashed and that could affect the recruitment and activity of immune cells. Another possibility is related to the ability of the immune cells to eliminate senescent cells, which can be compromised by the senescent cells, their location or the age of the individual. Dissecting the mechanisms involved in senescent cell clearance in each particular scenario will be critical to better exploit cellular senescence with therapeutic purposes.

Pro-senescent and anti-senescent therapies hold promise, but also entail significant risks. For example, therapy-induced senescence is being exploited for cancer treatment and could potentially be used to prevent cancer and improve tissue homeostasis. However, to be fully beneficial, therapy-induced senescence should be coupled to senolytic strategies. This could be achieved by manipulating the immune system or the SASP, or by directly targeting senescent cells. Finding the best strategy to combine pro-senescent and senolytic therapies will be critical to avoid undesirable side effects. Similarly, senolytic therapies could be harnessed to delay or even revert aging. To clear, or not to clear (senescent cells), that is the question that will need to be addressed in the years to come.

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