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Review Article

Impact of senescence-associated secretory phenotype and its potential as a therapeutic target for senescence-associated diseases

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"Cellular senescence" is a state in which cells undergo irreversible cell cycle arrest in response to a variety of cellular stresses. Once cells senesce, they are strongly resistant to any mitogens, including oncogenic stimuli. Therefore, cellular senescence has been assumed to be a potent anticancer mechanism. Although irreversible cell-cycle arrest is traditionally considered the major characteristic of senescent cells, recent studies have revealed some additional functions. Most noteworthy is the increased secretion of various secretory proteins, such as inflammatory cytokines, chemokines, growth factors, and MMPs, into the surrounding extracellular fluid. These newly recognized senescent phenotypes, termed senescence-associated secretory phenotypes (SASPs), reportedly contribute to tumor suppression, wound healing, embryonic development, and even tumorigenesis promotion. Thus, SASPs appear to be beneficial or deleterious, depending on the biological context. As senescent cells are known to accumulate during the aging process *in vivo*, it is quite possible that their accumulation in aged tissues promotes age-associated functional decline and various diseases, including cancers, at least to some extent. Here, we focus on and discuss the functional and regulatory network of SASPs toward opening up new possibilities for controlling aging and aging-associated diseases.

Conceptual vicissitudes of cellular senescence

Unlike germ cells and certain tissue stem cells, most, if not all, normal human somatic cells stop dividing permanently after a finite number of cell divisions in culture and enter a state of stable cell cycle arrest termed "cellular (or replicative) senescence."[1,2] Senescent cells are essentially irreversibly arrested in either the G_1 or G_2/M phase of the cell cycle and are no longer able to divide, despite remaining viable and metabolically active for long periods (Fig. 1).[3–7] Growth arrest plays an important role in preventing the extensive cell divisions required for malignant transformation; accordingly, cellular senescence has long been considered a barrier to cancer.[3,8–10] Although a number of hypotheses have been proposed to explain the mechanisms imposing cellular senescence, much evidence suggests that replicative senescence in human cells is provoked by telomere erosion after extensive cell division.[11] Moreover, subsequent studies have revealed that a similar phenotype can be induced more rapidly when normal cells are exposed to a variety of potentially oncogenic stimuli, such as excessive levels of reactive oxygen species, treatment with DNA-damaging agents, or activation of certain oncogenes.[12,13] These cells undergo stable cell cycle arrest, accompanied by morphological changes typically associated with cellular senescence, including a flat and enlarged cellular morphology,[12,14] upregulation of senescence-associated β-galactosidase activity,[15] and senescence-associated heterochromatic foci,[16] although these changes are not required for cell-cycle arrest.[14,17–19]

Note that excessive or irreparable DNA damage in non-telomeric lesions, in addition to eroded telomeres, causes persistent activity of the DNA damage response (DDR), which triggers the onset of senescence processes.[20] Indeed, persistent DDR contributes to the establishment of irreversible cell-cycle arrest through the induction of $p21^{Waf1/Cip1}$ and $p16^{Ink4a}$ cyclin-dependent kinase inhibitors[21–23] and consequent activation of the retinoblastoma tumor suppressor protein pathway (Fig. 1).[24] Thus, the concept of cellular senescence has been expanded to include phenotypically similar cell-cycle arrest provoked by a variety of stresses, eliciting persistent activation of DDR.

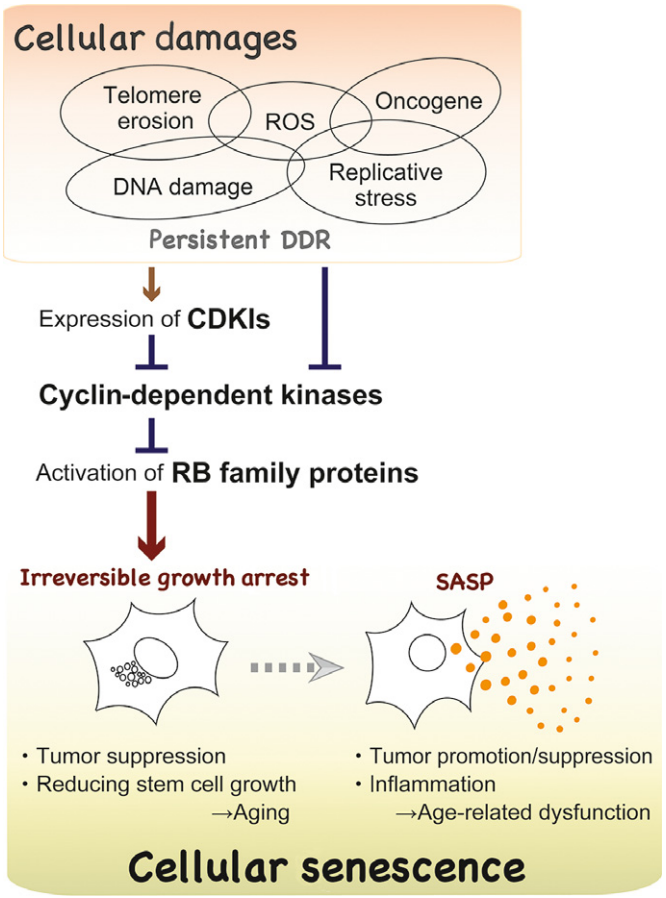

Fig. 1. Overview of cellular senescence characterized by permanent cell-cycle arrest and the senescence-associated secretory phenotype (SASP). A variety of stimuli can lead to cellular senescence by the activation of retinoblastoma protein (pRB) through the upregulation of cyclin-dependent kinase inhibitors (CDKIs), followed by the inactivation of cyclin-dependent kinases. Senescent cells are characterized by irreversible cell-cycle arrest and paracrine activity of the SASP. DDR, DNA damage response; ROS, reactive oxygen species.

Senescence-associated secretory phenotype and its physiological roles

Despite their stable non-dividing state, senescent cells are metabolically active and exhibit the upregulation of a wide range of genes. The most noteworthy upregulated genes encode a series of secreted proteins, such as inflammatory cytokines, chemokines, extracellular matrix remodeling factors, and growth factors. These secreted proteins function physiologically in the tissue microenvironment, in which they could propagate the stress response and communicate with neighboring cells. This phenotype, termed the senescence-associated secretory phenotype (SASP),[25,26] uncovers the paracrine function of senescent cells (Fig. 1) and is an important characteristic that distinguishes senescent cells from non-senescent, cell cycle-arrested cells, such as quiescent cells and terminally differentiated cells.

Some SASP factors reportedly play important roles in the onset of stable cell-cycle arrest in senescent cells, which presumably contributes to the tumor-suppressive function of cellular senescence.[27,28] However, many SASP factors have the potential to cause chronic inflammation and/or tumorigenesis, depending on the biological context.[25,29] It is therefore possible that SASP has deleterious effects in organismal homeostasis[26] (Fig. 2), although these detrimental outcomes of SASP cannot explain why the cell non-autonomous phenotype of senescent cells has been maintained during evolution. Notably, however, recent reports have revealed additional beneficial roles for the SASP during embryonic development[30,31] and in limiting fibrosis after tissue injury or suppressing tumorigenesis by promoting the elimination of senescent cells.[32–34] Furthermore, SASP has an important role in accelerating wound healing. In the skin, wound closure occurs during the proliferation phase as a consequence of wound contraction,[35] which is due primarily to the formation of newly synthesized granulation tissue and induction of specialized contractile fibroblasts termed myofibroblasts.[36] In response to a cutaneous wound, senescent fibroblasts and endothelial cells appear and accelerate wound closure by inducing myofibroblast differentiation through the secretion of platelet-derived growth factor AA,[37] an SASP factor, although the upstream signals that induce senescence after wounding are not fully understood. Thus, it is tempting to speculate that SASP was selected during evolution owing to these beneficial effects.

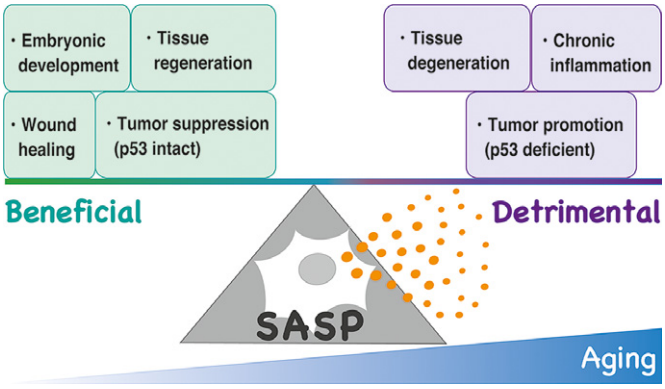

Fig. 2. Dual roles of senescence-associated secretory phenotype (SASP) in cellular senescence. SASP could have beneficial or detrimental outcomes in physiological and pathological processes during aging.

Pathological impact of SASP

Owing to aging-associated alterations of innate and adaptive immunity,[38] clearance of senescent cells by the immune system could fail to function properly, resulting in the accumulation of senescent cells in aged tissues. Thus, it is possible that increased levels of pro-inflammatory cytokines secreted by senescent cells promote chronic inflammation, thereby accelerating age-associated functional declines and diseases, such as osteoarthritis, pulmonary fibrosis, Alzheimer's disease, and cancer (Fig. 2). In line with this idea, the elimination of $p16^{Ink4a}$-positive senescent cells from aged mice reportedly delays the onset of age-related dysfunction, including sarcopenia, cataracts, atherosclerosis, loss of adipose tissue, and tumorigenesis, and extends their healthy lifespan,[39–41] suggesting that the accumulation of senescent cells *in vivo* is likely to contribute to the onset of aging and aging-associated diseases. While these studies have utilized transgenic mice lines expressing a suicide gene driven by a 1.6-kb fragment of the $p16^{Ink4a}$ gene promoter, the regulation of $p16^{Ink4a}$ gene expression is known to be controlled not only by the promoter region but also by intronic regions and upstream regions of the $p16^{Ink4a}$ promoter.[42] Thus, this suggests a potent additional effect of actually targeting $p16^{Ink4a}$ in aged tissue.

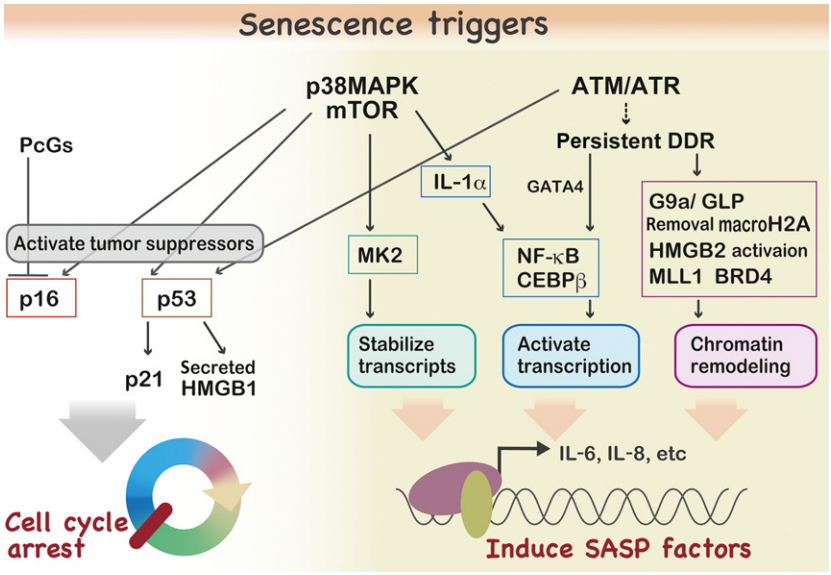

Fig. 3. Multilevel control of senescence-associated secretory phenotype (SASP) induction in cellular senescence. The expression of SASP factors is upregulated by multilevel control mechanisms, including transcriptional activation, stabilization of transcripts, and chromatin remodeling. Persistent DNA damage response (DDR) signaling could induce SASP without p53-dependent signaling related to senescent growth arrest. ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; BRD4, bromodomain-containing protein 4; CEBPβ, CCAAT/enhancer binding protein-β; GATA4, GATA binding protein 4; HMGB2, high mobility group box 2; IL, interleukin; MK2, MAPK-activated protein kinase 2; MLL1, mixed lineage leukemia 1; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; PcG, polycomb group protein.[42]

Furthermore, certain cell types might express $p16^{Ink4a}$ independently of cellular senescence in mice.[43] These findings therefore suggest that alternative approaches are needed to firmly confirm this idea.

With pathological relevance to tumor-promoting effects of senescent cells *in vivo,* dermal fibroblasts in aged mice increase expression of secreted Frizzled-related protein 2, a Wnt antagonist, which augments angiogenesis, metastasis, and chemotherapy resistance of melanoma cells.[44] Moreover, we found that dietary or genetic obesity provoke the SASP in hepatic stellate cells (HSCs) through increased levels of enterohepatically recirculated deoxycholic acid (a DNA-damaging gut bacterial metabolite), and SASP factors secreted by HSCs facilitate hepatocellular carcinoma (HCC) development in mice.[45] Of note, a recent report from Lowe's group has reported that senescent HSCs suppress, rather than promote, HCC development through SASP in mice treated with diethyl nitrosamine plus carbon tetrachloride (CCl_4).[46] These seemingly disparate results may reflect, at least in part, the status of the *p53* gene in hepatocytes. It should be noted that our HCC mouse model possessed a loss-of-function mutation in the *p53* gene (our unpublished data, 2013), in contrast to the HCC arising in mice treated with diethyl nitrosamine plus CCl_4.[46] Moreover, several lines of evidence have shown that SASP suppresses or promotes tumorigenesis depending on p53 status.[25,47,48] Thus, this promotional effect of SASP on tumor growth could be limited by functional p53, which is often deficient in tumor cells.

Regulation of SASP induction

The factors secreted by SASP vary depending on cell type and triggers of cellular senescence.[49] Among many SASP factors, major pro-inflammatory cytokines, such as interleukin-1α (IL-1α), IL-1β, IL-6, and IL-8, appear to be more common compared with other SASP factors.[25,28,50–52] These factors are reportedly induced by multiple mechanisms, including nuclear factor-κB (NF-κB)[50] and CCAAT/enhancer binding protein-β[28] transcription factors, p38MAPK,[53] and mammalian target of rapamycin (mTOR) signaling,[51,52] in senescent cells (Fig. 3). Furthermore, autophagic activity correlated with negative feedback in the mTOR pathway has been shown to contribute to the production of secretory factors.[51,54,55] Autophagy-mediated protein degradation might provide raw materials for facilitating protein translation and consequent protein turnover to establish the SASP. However, the precise mechanisms regulating SASP induction are far from complete. In contrast to senescence cell-cycle arrest, SASP is not induced by the ectopic expression of $p16^{Ink4a}$ or $p21^{Waf1/Cip1}$, suggesting an involvement of non-core senescence signaling pathway (s) in SASP induction.[25,26,56] Indeed, a recent report revealed that the transcription factor GATA binding protein 4 (GATA4), which is a substrate of selective autophagy, is stabilized in senescent cells, depending on the DDR kinases ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related), but not p53 or $p16^{Ink4a}$, and that GATA4 acts as an upstream activator of NF-κB to initiate the SASP.[57] We have also reported that the persistent activation of ATM triggers the degradation of G9a and GLP histone methyltransferases, thereby causing the epigenetic de-repression of a subset of SASP genes.[58] Along similar lines, the activation of ATM has been shown to trigger the removal of macroH2A.1, which is a tumor-suppressive histone variant, from the chromatin of SASP genes, leading to SASP induction.[59] Collectively, persistent DDR appears to provoke SASP through ATM signaling, which transduces DNA damage signals into transcriptional machinery.

Senescent cells also undergo chromatin reorganization, which is crucial for modulating gene expression, including SASP genes. For instance, Aird and colleagues have shown that high mobility group box 2 (HMGB2), which is a non-histone chromatin-bound protein that activates transcription by altering chromatin architecture, preferentially localizes to and

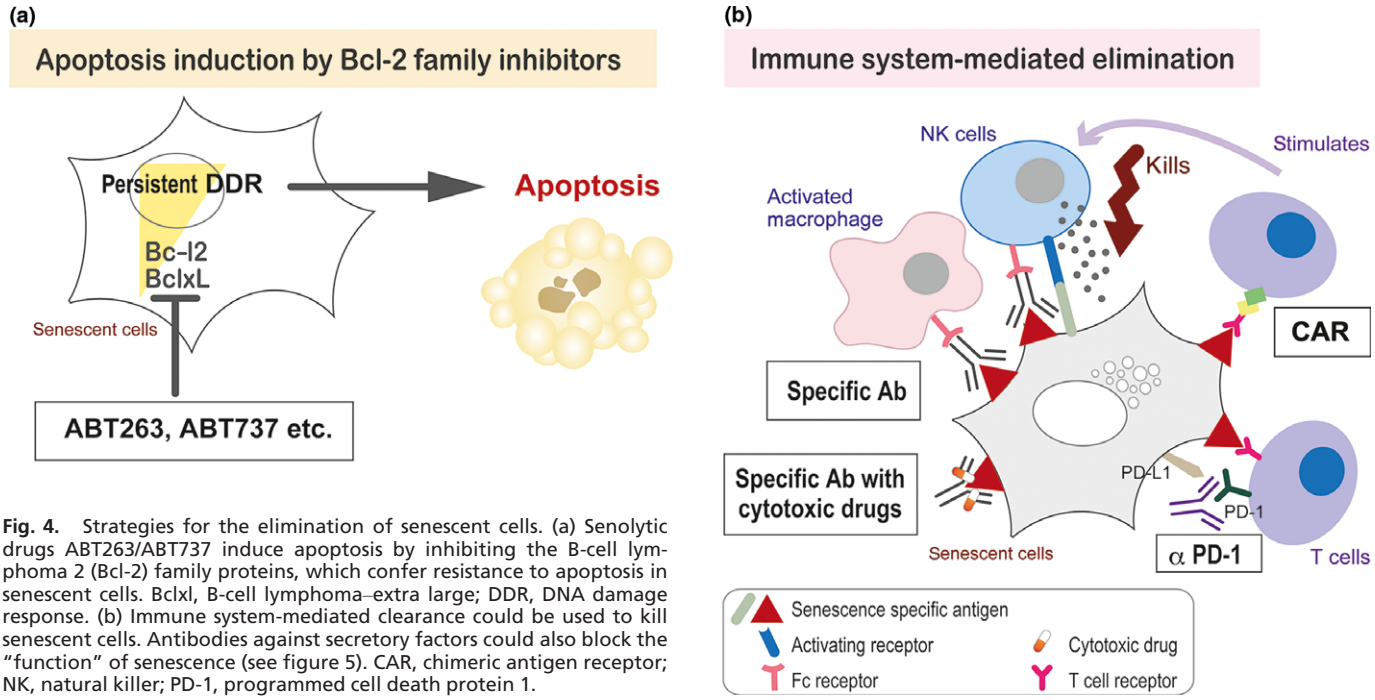

Fig. 4. Strategies for the elimination of senescent cells. (a) Senolytic drugs ABT263/ABT737 induce apoptosis by inhibiting the B-cell lymphoma 2 (Bcl-2) family proteins, which confer resistance to apoptosis in senescent cells. Bclxl, B-cell lymphoma–extra large; DDR, DNA damage response. (b) Immune system-mediated clearance could be used to kill senescent cells. Antibodies against secretory factors could also block the "function" of senescence (see figure 5). CAR, chimeric antigen receptor; NK, natural killer; PD-1, programmed cell death protein 1.

upregulates SASP loci, including IL-6 and IL-8.[60] It should also be noted that the loss of HMGB2 during senescence represses SASP gene expression, but does not affect cell cycle arrest, consistent with the idea that senescence cell-cycle arrest and SASP are regulated by different machineries.[25,26,56] However, HMGB1, another HMGB family protein, is a secreted factor that induces senescent growth arrest in the senescence-initiating phase.[61] In contrast to SASP induction, HMGB1 secretion itself relies on p53, but not ATM. These reports imply that extracellular HMGB1 secreted through p53-dependent signaling might have a role in cell-cycle arrest at the senescence-initiating step, and that HMGB2 remained to chromatin participates in SASP-inducible chromatin remodeling at the maturation step of senescence. Other epigenetic regulators, mixed-lineage leukemia 1 (MLL1) and bromodomain-containing protein 4 (BRD4), also have important roles in the regulation of SASP induction.[62,63] It has been reported that MLL1 is critical for proliferation-promoting gene expression required to trigger the DDR, thus inducing SASP.[62] In contrast, BRD4 "directly" binds to super-enhancer regions enriched in close proximity to pivotal SASP genes and accelerates SASP induction, resulting in immune cell-mediated targeting and the elimination of premalignant senescent cells *in vitro* and *in vivo*.[63] Of note, neither MLL1 nor BRD4 suppression significantly alters tumor suppressor gene activation leading to cell cycle arrest during oncogene-induced senescence. Together, these findings suggest that SASP induction could be blunt, without affecting senescence cell-cycle arrest, and consequently could be a potential therapeutic target for various aging-related diseases, including cancer.

Clinical applications targeting cellular senescence and SASP

Given evidence that the healthy lifespan of mice is extended by eliminating senescent cells in a transgenic mouse model,[39,40,64] targeting senescent cells is an attractive strategy to prevent and treat age-associated dysfunctions and thereby achieve healthy aging. Moreover, as a recent report has shown that clearing therapy-induced senescence cells reduces several side-effects of the drugs, including bone marrow suppression and cardiac dysfunction, and even cancer recurrence,[65] it is anticipated that the removal of senescent cells could prevent the toxicity of anticancer treatment and enhance therapeutic benefit. In this regard, there are several possible approaches, such as selectively ablating senescent cells or blocking SASP induction. In particular, senolytic compounds, which specifically induce cell death in senescent cells, are

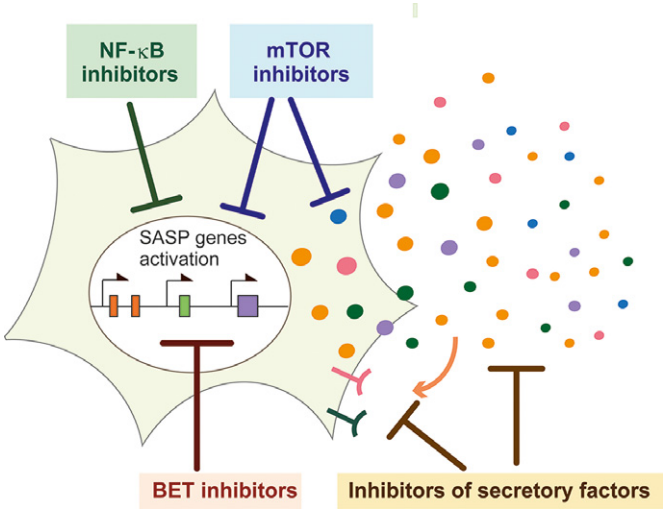

Fig. 5. Strategies for senescence-associated secretory phenotype (SASP) attenuation. SASP factors could be blocked by targeting its induction (e.g., nuclear factor-κB [NF-κB] inhibitor, mammalian target of rapamycin [mTOR] inhibitor, or bromo and extra terminal domain [BET] inhibitor) or its activity (e.g., interleukin [IL]-1 receptor antagonist, anti-IL-6 receptor antibody, IL-6 inhibitor, or tumor necrosis factor-α inhibitor).

likely to represent a new therapeutic avenue (Fig. 4). These drugs are dependent on senescence-specific vulnerabilities compared with normal dividing or differentiated cells. For example, ABT263 (navitoclax) and ABT737 specifically inhibit the anti-apoptotic proteins B-cell lymphoma 2 and B-cell lymphoma–extra large, which are upregulated in some, but not all, senescent cells, and can potently and rapidly induce apoptosis in senescent cells, both in culture and *in vivo*.[66–68] These findings are a significant step towards the clinical application that targets cellular senescence. Although the approach is promising, the senolytic drugs identified to date might have limitations for clinical applications. For example, in a phase II study of ABT263 applied to advanced and recurrent small-cell lung carcinoma patients, transient thrombocytopenia and neutropenia have been reported as side-effects.[69] Thus, the identification of additional senolytic drugs is needed.

Another potential option for eliminating senescent cells is utilizing the immune surveillance system.[70] Antibodies against senescence-specific surface antigens, such as CD44 in endothelial cells,[71] could induce a direct immune response or deliver cytotoxic drugs[72] to the site of a senescent lesion. Furthermore, chimeric antigen receptor-modified T cells, which were originally developed for efficient cancer therapy,[73] have potential applications for senescent cell clearance, after their specificity and safety are confirmed. Furthermore, immune checkpoint blockers, such as anticytotoxic T lymphocyte-associated protein 4 or anti-programmed cell death protein 1, might invoke a tumor-suppressive immune surveillance program for cancer cells, especially in combination with therapy-induced senescence.[49]

Controlling the induction of SASP is also a promising approach to prevent the negative effects of senescence (Fig. 5). This could be achieved by targeting its transcriptional regulatory systems, such as NF-κB,[50] mTOR signaling,[51,52] or BRD4,[63] or by blocking the function of several key inflammatory mediators such as IL-6 or tumor necrosis factor-α.[74] It is worth mentioning that rapamycin, an mTOR inhibitor, reduces the secretion of inflammatory cytokines in senescent cells by suppressing the translation of downstream drivers, such as IL1-α[52] and MAPK-activated protein kinase 2.[51] It is also notable that rapamycin and other mTOR inhibitors have been shown to extend the lifespan of mice.[75] These findings, together with the fact that mTOR inhibitors are already used in clinical settings,[76,77] suggest that mTOR inhibitors are highly promising drugs for the prevention of deleterious effects of cellular senescence.

To block the enhancer effect of BRD4 on the expression of SASP genes, small molecule inhibitors of the bromo and extra terminal domain family of proteins, so-called BET inhibitors, are feasible. While BET inhibitors reportedly disrupt a tumor-suppressive immune surveillance program,[63] current studies on inflammation-driven cancers, however, give rise to other more positive therapeutic implications regarding the inhibitory effect on SASP-promoted tumorigenesis.[45] Speculatively, BET inhibitors might suppress senescence-associated secretion in senescent stromal cells,[44,45,78,79] which present around cancer cells to promote their growth, especially in p53-deficient conditions. Indeed, BRD4 has been reported as an oncoprotein, and its effects can be blocked by BET inhibitors.[80] To enable clinical applications of BET inhibitors for age-related diseases, further studies are necessary to clarify the mechanisms underlying the dual oncogenic and tumor-suppressive roles of BRD4 and to improve the specificity and selectivity of BET inhibitors.

Notably, some agents that target SASP components are already used for clinical applications and could be candidates for drug repositioning. An IL-1 receptor antagonist, anakinra, an anti-IL-6-receptor antibody, tocilizumab, and tumor necrosis factor-α inhibitors, such as etanercept and infliximab, are currently used to treat rheumatoid arthritis.[81,82] Additionally, an IL-6 inhibitor, sirukumab, is awaiting approval by the US FDA, with positive phase III data for rheumatoid arthritis.[83] As the IL-1 receptor is a key inducer of many other SASP factors and IL-6 and IL-8 subsequently appear to propagate senescence as a common part of SASP,[25,28,50–52] these drugs may be useful for selectively blocking deleterious SASP. In fact, the specific inhibition of IL-6 or IL-8 signaling weakens the inflammatory response associated with senescence in mice.[27,28]

However, because SASP also has some beneficial effects, such as wound healing, tissue regeneration, and immune surveillance, and most SASP factors have roles independent of SASP, it is possible that drugs targeting SASP will have unforeseen side-effects. Thus, special caution should be exercised, particularly in establishing clinical applications of anti-SASP drugs.

Conclusions

In this review, we described recent findings related to the physiological and pathological impact of SASP, focusing on therapeutic opportunities for controlling senescence-associated diseases. Although research on the biological significance of the upregulation of secretory proteins in senescent cells has lagged since their discovery,[84–87] many investigators are now revisiting senescence-associated secretory proteins in the context of SASP, and these studies have broadened our view of cellular senescence. Functioning only as a tumor-protection mechanism, apoptosis appears to be safer than cellular senescence. Nevertheless, cellular senescence was also selected during evolution, suggesting that cellular senescence might have important advantages that are not associated with apoptosis, that is, through the SASP. Thus, understanding the SASP has the potential to resolve the paradoxical mechanisms of cellular senescence both in aging and cancer, for example, regeneration *versus* degeneration and immune clearance *versus* growth promotion, respectively.

In the face of an aging society, increasing accumulation of senescent cells in aged tissues presumably promotes chronic inflammation and age-related diseases, including cancer. Thus, emerging therapeutic strategies, including selectively ablating senescent cells or inhibiting the SASP, are promising for future clinical translation promoting healthy aging.

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Disclosure Statement

The authors have no conflict of interest.

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