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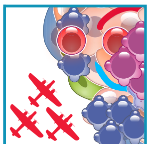
CELLULAR SENESCENCE: AGING, CANCER, AND INJURY

Arianna Calcinotto, Jaskaren Kohli, Elena Zagato, Laura Pellegrini, Marco Demaria, and Andrea Alimonti

Institute of Oncology Research (IOR), Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; University of Groningen, European Research Institute for the Biology of Ageing, University Medical Center Groningen, Groningen, The Netherlands; IOR, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; Università della Svizzera Italiana, Faculty of Biomedical Sciences, Lugano, Italy; Faculty of Biology and Medicine, University of Lausanne UNIL, Lausanne, Switzerland; and Department of Medicine, Venetian Institute of Molecular Medicine, University of Padova, Padova, Italy



Calcinotto A, Kohli J, Zagato E, Pellegrini L, Demaria M, Alimonti A. Cellular Senescence: Aging, Cancer, and Injury. *Physiol Rev* 99: 1047–1078, 2019. Published January 16, 2019; doi:10.1152/physrev.00020.2018.—Cellular senescence is a permanent state of cell cycle arrest that occurs in proliferating cells subjected to different stresses. Senescence is, therefore, a cellular defense mechanism that prevents the cells to acquire an unnecessary damage. The senescent state is accompanied by a failure to re-enter the cell cycle in response to mitogenic stimuli, an enhanced secretory phenotype and resistance to cell death. Senescence takes place in several tissues during different physiological and pathological processes such as tissue remodeling, injury, cancer, and aging. Although senescence is one of the causative processes of aging and it is responsible of aging-related disorders, senescent cells can also play a positive role. In embryogenesis and tissue remodeling, senescent cells are required for the proper development of the embryo and tissue repair. In cancer, senescence works as a potent barrier to prevent tumorigenesis. Therefore, the identification and characterization of key features of senescence, the induction of senescence in cancer cells, or the elimination of senescent cells by pharmacological interventions in aging tissues is gaining consideration in several fields of research. Here, we describe the known key features of senescence, the cell-autonomous, and noncell-autonomous regulators of senescence, and we attempt to discuss the functional role of this fundamental process in different contexts in light of the development of novel therapeutic targets.

I. INTRODUCTION

Cellular senescence is a stable cell cycle arrest that occurs in diploid cells and limits their proliferative life span. The first description of this phenomenon dates back to 1960s, when Hayflick and Moorhead observed that human diploid fibroblasts in culture could reach a maximum number of cell divisions before arresting their growth (150). This biological clock, known as the “Hayflick limit,” is caused by a progressive shortening of telomeres upon each cell division and represents a physiological response to prevent genomic instability and therefore accumulation of DNA damage (79, 150). This phenomenon is currently defined as replicative senescence. Senescent cells can accumulate with age and at sites of age-related pathologies, such as in osteoarthritis (261) and atherosclerosis (47), and can have an impact on the normal physiology of the tissues, causing a progressive functional deterioration. However, diploid cells can also experience an accelerated senescence response, independent from the telomere shortening, known as premature senescence (79, 82, 283). This senescence response occurs immediately after certain insults, such as genotoxic stress or metabolic shock, triggered in cells by culture conditions. Oncogenic stress triggered by the overexpression of certain oncogenes or loss of tumor suppressor genes (TSGs) in primary and tumor cells also induces senescence (32, 77). It has been demonstrated that senescence occurs in vivo in different tumors, where it arrests tumor development and progression. Thus, because of its antiproliferative effects, senescence also appears to be a potent antitumor mechanism. This tumor-suppressive function of senescence has paved the way for treatments that enhance senescence for cancer therapy, a process termed prosenescence therapy for cancer. Despite their involvement in various pathological conditions, senescent cells play key roles in physiological processes such as embryogenesis, tissue remodeling, and tissue repair (85). Here, we provide an overview of the causes that induce cellular senescence in different organisms with a particular focus on the various roles that senescent cells play in the human body.

II. CELLULAR SENESCENCE

A. Hallmarks of Cellular Senescence

Senescent cells are not characterized by universal or specific biomarkers, but rather by a number of nonexclusive markers. Cell cycle arrest is a crucial characteristic for the identification of all types of senescence, but it cannot be considered a unique marker because of the fact that multiple cellular mechanisms can drive a stable replicative arrest. However, the inability to express genes required for proliferation, even in a promitogenic environment (96, 98), allows distinguishing senescence from quiescence, a nonproliferative state of the cells that is readily reversed in response to mitogens. Senescent cells are characterized by a higher activity of senescence-associated β-galactosidase (SA-β-gal) at pH 6 and can be identified by flow cytometry using fluorescein di-D-galactopyranoside, a substrate that can be cleaved by galactosidase (44). In senescent cells, cell cycle arrest correlates with an augmented level of cell cycle inhibitors, including p16INK4a, p21CIP1, and p27. Moreover, elevated expression of p19ARF, p53, and PAI-1 are observed in senescent cells and used as miscellaneous senescence biomarkers (44) **(FIGURE 1)**. In addition, senescent cells are commonly characterized by an altered cell size with a more smoothed shape compared with proliferating cells and exhibit senescence-associated heterochromatin foci formation (242), accumulation of lipofuscin (136), DNA damage foci (159), loss of lamin B1 (296), senescence-associated distension of satellites (308), expression of embryonic chondrocyte–expressed 1 (DEC1) and decoy death receptor 2 (DCR2) (71), upregulation of some microRNAs (miRNAs) and secretion of a large number of factors, including growth factors, cytokines, chemokines, and proteases, known as the senescence-associated secretory phenotype (SASP) or senescence-messaging secretome **(FIGURE 1)**. All the above-mentioned features define the gold-standard markers to identify senescent cells and represent the actual hallmarks of senescence (70, 157, 192). Nonetheless, there is growing interest in finding novel markers of senescence that could have also a prognostic potential in aging and cancer (107, 108, 113). One of the characteristics of senescent cells is that they remain metabolically active and able to produce and secrete a plethora of factors that can affect the tissue microenvironment in different modalities (3, 291). A key feature of the senescence phenotype is the acquisition of this altered cell metabolism indispensable for the accomplishment of the senescence program (249). Depletion of the catabolic enzyme glycogen phosphorylase in cells results in glycogen accumulation, which is associated with reduced proliferation and a corresponding induction of senescence (254). Growing literature on the metabolism of cellular senescence reports that both glucose consumption and lactate production are elevated during senescence (57, 101, 179). Characteristic changes in the metabolism of senescent cells in the context of cancer are discussed in sect. V.

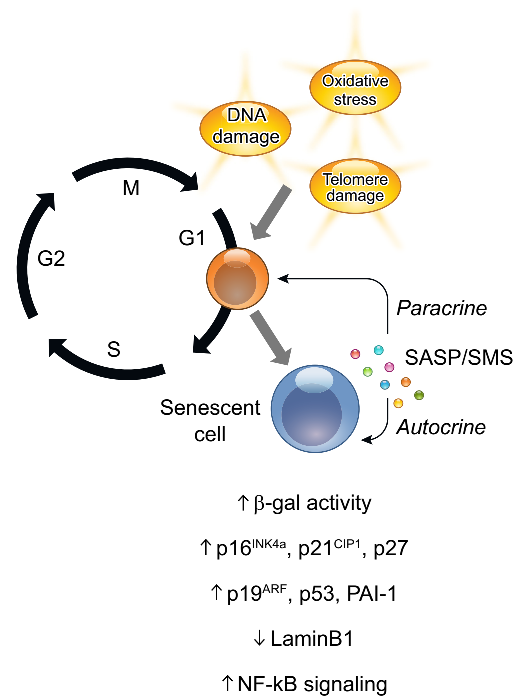


FIGURE 1. Characteristics of cellular senescence. Cellular senescence can be triggered by multiple genetic alterations induced by oxidative stress, DNA, or telomere damage. Senescent cells exhibit permanent growth arrest, increased expression of cell cycle inhibitors, and changes in cellular structures and protein expression. Senescence can be reinforced in an autocrine manner or spread through paracrine mechanisms to neighboring tumor cells by the release of senescence-associated secretory phenotype (SASP) or senescence-messaging secretome (SMS).

B. Role of Senescence in Evolution and Different Organisms

Senescence is one of the major causes of aging and aging-related disorders (214). For many years, scientists were puzzled about the reason why natural selection, which designs an organism for optimal survival and reproductive success, would allow cellular senescence to be transmitted to offspring. The recent discoveries that cellular senescence is required, although not essential, for the regulation of embryogenesis and acts as a checkpoint that limits the proliferation of tumor cells may explain why evolution does not prevent cellular senescence to disappear in a population from generation to generation. This explanation is coherent with the "antagonist pleiotropy" hypothesis, which theorized that genes that have beneficial effects early in life can be detrimental at later ages and therefore can be favored by evolution and passed to the offspring. Intriguingly, there are several animals, including mammals, that seem to have evolved without cellular senescence. In 1990, Finch and colleagues analyzed the possibility of the existence of organisms that exhibit negligible senescence. They found that organisms such as rockfish, sturgeon, turtles, bivalve mollusks, and certain perennial trees, and possibly lobsters, survived until considerable ages in the wild without detectable reduction in the fitness, such as in their reproductive capability or functional activities and without showing marker of senescence, such as telomerase shortening or increased oxidative damage (35, 119). Interestingly, some organisms are considered biologically immortal. A small fresh-water Cnidarian, the Hydra, seems to show negative senescence at younger age and negligible senescence at older age (220), whereas the *Turritopsis nutricula*, a small species of hydrozoan, once reaching adulthood, is able to transfer its cells back to childhood (259). This ability to reverse the mitotic cycle is unique in the animal kingdom and allows the jellyfish to bypass death. Of note, these animals do not develop cancers, and embryogenesis and tissue remodeling are regulated in different modalities compared with mammals. A better understanding of the different senescence trajectories in different animals could lead to a deeper comprehension of the evolutionary forces that shape the life of an organism, and it is currently under investigation by many laboratories.

C. Causes and Effector Pathways of Senescence

Cellular senescence is induced in physiological and pathological contexts by a number of different causes. Among them, telomere shortening represents one of the most important (289, 290, 292). Telomeres are repetitive nucleotide-sequence motifs that protect the ends of chromosomes from deterioration or fusion with adjacent chromosomes. Each cell division leads to the loss of 50–200 bp of unreplicated DNA at the 3′ end. The enzyme telomerase (also called terminal transferase) is responsible for adding bases to the end of telomeres to compensate telomere erosion. However, telomerase activity is not sufficient to balance the rapid rate of cell proliferation that results in telomere shortening and cell aging (249, 340). Moreover, telomere erosion triggers the DNA damage response (DDR; **FIGURE 1**), a signaling pathway in which ataxia-telangiectasia mutated (ATM) or ATM- and Rad3-related (ATR) kinases (83) block cell-cycle progression through stabilization of the p53 protein (130) and the transcriptional activation of the cyclin-dependent kinase (Cdk) inhibitor p21 (51). As a demonstration of that, senescent cells depict positive to γ-H2AX (a phosphorylated form of the histone variant H2AX) and to the DDR proteins 53BP1, NBS1, and MDC1. Indeed, together, these molecular events can induce a transient proliferation arrest that can evolve in senescence if cells are not able to repair the damage. DNA damage mediated by hit of oxidative stress participates in telomere erosion (269) (**FIGURE 1**). In addition to telomere shortening, physiological stresses imposed to healthy and cancer cells are also reported to induce cellular senescence. Abnormal O_2 levels induce shortening of telomeres, leading the cells to senescence (331, 345). Also, the culturing condition of both human and mouse cells can cause cellular senescence, a phenomenon called "culture shock" (269). Oxidative stress, endoplasmic reticulum stress or interferon (IFN)-related responses also induce cellular senescence (48, 58, 251) (**FIGURE 1**). Treatment with DNA damage agents such as UV, γ-irradiation (82), tert-butyl hydroperoxide (82) or anticancer chemotherapy agents (27, 90, 276) are known to induce senescence in both normal and cancer cells, a phenomenon named "therapy-induced senescence" (TIS) (79, 114, 273, 302). Although TIS arrests cancer proliferation, it also accelerates the aging process in the normal cells of the patient (see sect. V for more details). The discrimination between TIS, replicative senescence, and stress-induced senescence is very arduous, because the nomenclature merely mirrors the spectrum of different stimuli that can induce the cells to a senescent phenotype. Senescence can also be triggered by the activation of oncogenes [oncogene-induced senescence (OIS)] and loss of TSGs, as will be discussed in detail in sect. V. In addition, the immune response can also drive senescence. A systemic proinflammatory state that occurs with aging (termed inflammaging) (125) has been implicated in the induction of senescence in the chondrocytes, a condition that is responsible of osteoarthrosis, and in additional aging-related disorders linked to inflammation (155, 265, 313) (see sect. IV of this review for more details).

D. Autocrine and Paracrine Senescence and Its Impact on the Tissue Microenvironment

Senescent tumor cells secrete a plethora of immune modulators, inflammatory cytokines, growth factors, chemokines, and proteases commonly referred to as the SASP (72) or senescence-messaging secretome (194) (**FIGURE 1**). Key elements of the SASP are the proinflammatory cytokines interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-1α (IL-1α). Additional chemokines binding to the IL-8 receptor C-X-C motif chemokine receptor 2 (CXCR2), such as CXCL-2, CXCL-3, and CXCL-5, are also important components of the SASP in OIS. CCL-2 (MCP-1), CCL-20 (MIP-3α), CCL-7 (MCP-3), CXCL-4 (PF-4), CXCL1 (Gro-α), and CXCL-12 (SDF-1) have been described in the SASP of cells undergoing to OIS and replicative senescence (72). Importantly, IL-1α is considered one of the master regulators of the SASP. The release of IL-1α by senescent cells transmits senescence to normal and tumor cells. IFN can also induce senescence by triggering DNA damage in the target cells (232, 312). Senescent cells also secrete growth factors, such as many insulin-like growth factor–binding proteins (IGFBPs) that can modulate the insulin-like growth factor (IGF) pathway. As demonstrated, IGF can act as a potent inducer of senescence (74). Important elements of the SASP are also matrix metalloproteinases (MMPs), such as MMP-1 and -3, that can also act as regulatory elements of senescence, as they can cleave IL-8, IL-1, VEGF, and other CXCL/CCL family chemokines (72). In addition, senescent cells secrete serine proteases like urokinase- or tissue-type plasminogen activators, the respective uPA receptor, and inhibitors of these serine proteases (PAI-1 and -2). Finally, the SASP is composed of nonmacromolecular elements such as nitric oxide (NO) and reactive oxygen species (ROS) that can affect the phenotype of neighboring cells (72). Most of the SASP components are regulated by the nuclear factor kappa light-chain–enhancer of activated B cells (NF-κB), CCAAT/enhancer-binding protein beta (CEBP/β) and by mTOR (5, 62, 132, 193, 196, 281). The transcription factor GATA4, acting upstream of NF-κB, is also required for senescence establishment and SASP induction (177). Another regulator of SASP is the bromodomain and extraterminal domain (BET) family member bromodomain-containing protein 4 (BRD4) that positively regulates the senescence secretome and promotes senescence immune clearance (315). The SASP is also regulated by signal transducer and activator of transcription 3 (STAT3) in certain tissues. Indeed, inhibition of the JAK pathway results in a reprogramming of the SASP that abolishes the negative components of these factors (319). In addition, the mixed-lineage leukemia 1 (MLL1) has also been reported to enable the SASP, mainly by inducing genes required for the DNA replication and for the DDR activation (49). Other SASP regulators include NOTCH1 (160) and the high mobility group B proteins (HMGB1 and HMGB2) (7, 86). Finally, recent data demonstrate that the SASP can be controlled by the cGAS/STING pathway. cGAS is a DNA sensor that, through the adaptor protein STING, triggers cellular senescence and the transcription of genes that control the SASPs (104, 139, 353). By means of the SASP, senescent cells can influence the tissue microenvironment via paracrine mechanisms (92). They can influence neighboring proliferating cells and the recruitment and activation of immune cells in aging tissues and tumors (92, 339), as is detailed in sect. V of the present review. Being that the SASP is an important player in tuning the balance of the complex tissue microenvironment, several investigators are currently trying to identify compounds that can reprogram the SASP (SASP reprogramming) in cancer to boost the anticancer immune response (see sects. V and VII of this review for more details). Similarly, inhibition of the SASP by either elimination of senescent cells or compounds that block the senescence secretome has been proposed for the cure of aging-related disorders (64).

III. SENESCENT CELLS IN TISSUE REMODELING

The senescence program is engaged in a number of physiological and pathological processes that require tissue remodeling. The persistence of senescent cells during these processes determines their positive or negative role: transient accumulation of senescent cells in tissues are mainly covering beneficial functions, whereas persistent senescence seems to negatively impact the restoration of tissue homeostasis **(FIGURE 2)**.

A. Embryogenesis

Programmed senescence has been shown to play a beneficial role during mammalian embryogenesis (85, 235, 305). SA-β-gal$^+$ and K_i-67$^-$ senescent cells are characterized by activation of WNT and Hedgehog pathways and are present throughout various regions of the embryo during development, including the apical ectodermal ridge, neural roof plate, mesonephros, and endolymphatic sac. In the mouse embryo, these cells appear in a coordinated fashion at embryonic days 10.5–11.5 and undergo apoptosis or are cleared by macrophages at embryonic day 17.5 (235, 305). During this developmental phase, senescent cells coordinate limb patterning and tissue remodeling mainly via paracrine activation of phospho-extracellular signal–regulated kinase (pERK) pathways in adjacent mesenchymal cells. A feedback loop is initiated where pERK signaling in turn maintains senescence, and interference with this loop leads to mild developmental abnormalities (305). Interestingly, developmental senescent cells are p21$^+$, but p53$^-$ or p16$^-$, and SA-β-gal$^+$ cells are lost in *p21* null, but not *p53*- or *p16*-null, embryos. In accordance, p21 upregulation is p53 independent and is mediated by TGF-β/SMAD and PI3K/FOXO signaling. The *p21* null embryos display mild morphological defects, suggesting compensatory mechanisms exist for limb patterning if senescent cells are absent.

Therefore, embryonic development is a robust process in which senescence plays an important, albeit nonessential, role (235, 305). Senescent cells are also observed during amphibian development in which they are induced by transforming growth factor-β (TGF-β) signaling. It is therefore possible that senescence may have originally arisen as a developmental mechanism during evolution (85).

B. Tissue Repair

In response to tissue injury or wounding, sophisticated mechanisms exist in mammals to prevent infections by foreign pathogens and to repair the damaged tissue. Tissue repair is a phenomenon consisting of four primary phases [hemostasis, inflammation, proliferation, and remodeling (121)], and senescence has been described to influence these processes via the SASP.

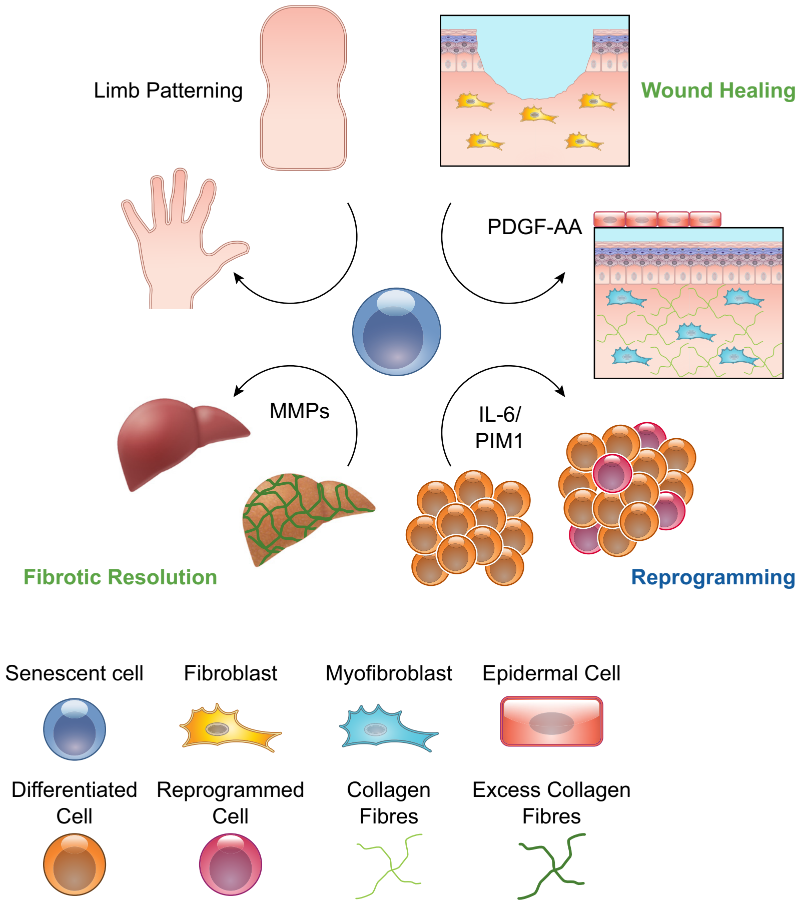


FIGURE 2. Senescent cells in tissue remodeling. Senescent cells secrete PDGF-AA in cutaneous wounds to induce myofibroblast differentiation and wound closure. They facilitate fibrotic resolution through matrix metalloproteinase (MMP) secretion and induce reprogramming in neighboring cells via an IL-6/PIM1 axis. Senescent cells also ensure limb patterning is correct during embryogenesis.

A role for senescent cells in promoting wound healing was first discovered using an engineered mouse model (p16–3MR) in which senescent $p16^+$ cells can be visualized, sorted, and selectively eliminated. Inducing cutaneous wounds in untreated mice results in a transient appearance of senescent fibroblasts and endothelial cells at the site of injury, and elimination of senescent cells delays wound repair kinetics. During their presence in wounds, senescent cells secrete platelet-derived growth factor AA, which induces differentiation of nearby fibroblasts into myofibroblasts, driving wound contraction during the proliferative phase and optimizing tissue repair (89) **(FIGURE 2)**.

Cellular senescence also plays a proregenerative function as the SASP can induce cellular plasticity and stemness. $HRAS^{V12}$-expressing keratinocytes upregulate many genes associated with stemness, which are regulated by the SASP regulator NF-κB. $HRAS^{V12}$-expressing hepatocytes induce stemness gene expression in neighboring cells in vivo. Transient exposure of newborn keratinocytes to the SASP produced by $HRAS^{V12}$-expressing keratinocytes also induces upregulation of stemness genes in vitro. When SASP-exposed newborn keratinocytes are grafted into wounds in nude mice, they promote hair growth and increased number of hair follicles, confirming a proregenerative function. Interestingly, prolonged exposure to the SASP results in cell-cycle arrest and paracrine-induced senescence. This intrinsic mechanism could be a cellular response to prevent tumorigenesis in response to excessive regenerative stimuli (270).

Mammals are incapable of regenerating complex structures such as entire limbs. However, salamanders do display this ability, and senescent cells are reported to influence regeneration. Senescent cells transiently appear during intermediate stages of salamander limb regeneration before being cleared via macrophages upon limb maturation (362). It is unclear whether senescent cells cover a positive role and how they contribute to limb regeneration. It is likely that macrophage-mediated clearance is required for tissue remodeling, similar to observations in developing embryos (235, 305), as depleting macrophages in salamander leads to regenerative defects (140).

C. Fibrosis

A fibrotic response is activated during reparative processes and entails the formation of excessive connective tissue. Accumulation of extracellular matrix (ECM) proteins results in permanent scarring and affects tissue structure and functionality, which can lead to organ failure and death in extreme cases (172). In different tissues, either a promoting or interfering role for cellular senescence in the formation of scar tissue have been demonstrated.

During the remodeling phase of cutaneous wound healing, the matricellular protein cysteine-rich angiogenic inducer 61 (CYR61), otherwise known as CCN1, induces p16 and p53-mediated senescence of dermal fibroblasts. CCN1 activates NADPH oxidase 1 (NOX1) through Ras-related C3 botulinum toxin substrate 1 (RAC1). NOX1 induces ROS levels, which activates p53 via the DDR and p16 via ERK and p38 MAPK (173). These CCN1-induced senescent fibroblasts secrete antifibrotic MMPs to degrade ECM components and curb fibrosis. Accordingly, mice carrying a mutant form of *CCN1*, thus unable to induce senescence, or p16–3MR mice deprived of senescent cells display increased collagen deposition and enhanced fibrosis (89, 173).

Chronic tissue damage to the liver can result in cirrhosis, in which excessive fibrosis compromises the organ's function, leading to liver failure. The most common insults to the liver are from hepatitis viral infections, excess alcohol consumption, and nonalcoholic steatohepatitis, in which excess fat leads to liver inflammation. These damaging stimuli can activate hepatic stellate cells (HSCs) to differentiate ECM-producing myofibroblasts (25).

Administration of CCl_4 to mice induces liver damage and fibrotic scarring, but also senescent HSCs along the periphery of the scar. These senescent HSCs facilitate fibrotic resolution through decreased production of ECM components as well as increased expression of antifibrotic SASP factors such as proteases and MMPs. Importantly, $p53^{-/-}$*;INK4A/ARF*$^{-/-}$ mice treated with CCl_4 displayed fewer numbers of senescent HSCs and extensive liver cirrhosis (190).

During liver damage, cellular senescence is reported to be induced by the matricellular protein CCN1, which activates the RAC1/NOX1 mechanism to promote p16 and p53 activation, in a similar manner to cutaneous wound healing (182). Additional mechanisms for the induction of senescence in liver damage are IL-22, which promotes HSC senescence in a p53-dependent manner through STAT3 and SOCS3 (186), and IGF-1, which induces HSC senescence in a p53-dependent manner (245). Mice treated with recombinant CCN1, IL-22, or IGF-1 displayed accelerated fibrotic resolution (182, 186, 245), suggesting that prosenescence therapies could be promising agents to resolve liver fibrosis.

Senescence also plays an important role in limiting fibrosis in infarcted hearts. In a mouse model in which infarction is induced by ligation of the left coronary artery or by transverse aortic constriction, cardiac myofibroblasts enter senescence (227, 366). This process limits further fibrosis as $p53^{-/-}$*;INK4A*$^{-/-}$ mice, which are unable to induce senescent myofibroblasts, display enhanced collagen deposition and overall decreased cardiac function compared with wild-type mice during transverse aortic constriction (227). Interestingly, in the left coronary artery model, only *p53* loss is required (366). It is possible that senescence pathways in cardiac myofibroblasts depend upon the type of damage. Nonetheless, induction of senescence via CCN1 in infarcted hearts resolved fibrosis and improved heart function (227). Therefore, as in the case of liver fibrosis, therapies that induce senescence may also be attractive for myocardial infarctions.

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterized by decreased lung function due to persistent scarring. Common risk factors for IPF include smoking and exposure to environmental toxins (221).

Senescent biomarkers have been observed in human IPF samples, suggesting a pathological role for senescence in this disease (152, 277). In a mouse model of IPF, bleomycin administration induces senescence in epithelial cells and fibroblasts (15, 152, 277). Senescent lung fibroblasts can induce myofibroblast differentiation in a paracrine manner, suggesting that they express a profibrotic SASP (277). This may explain why senescent cell accumulation and persistence exacerbate pulmonary fibrosis rather than resolve it, in contrast to other fibrotic lesions. Pulmonary senescence is mediated by an increase in NOX4 and decrease in antioxidant response NFE2-related factor 2 (Nrf2) expression. As a result, ROS levels increase, leading to DNA damage and senescence (152).

Interestingly, genetic variants are reported to contribute to up to one third of IPF cases, and the genes associated with telomere maintenance, the telomerase reverse transcriptase (*TERT*) and the telomerase RNA component (*TERC*), are mutated in ~25% of these patients. These mutations are associated with short telomeres, which is likely to induce senescence in lung cells and aggravate IPF (10, 17, 221). ROS are known to accelerate telomere shortening, and therefore, it is also possible that telomere damage is a factor in sporadic IPF cases (75). Eliminating senescent cells or inhibiting ROS alleviates IPF in bleomycin-treated mice (152, 277). This approach may also be attractive to therapeutically alleviate IPF in human patients.

D. Tissue Reprogramming

The seminal findings that differentiated somatic fibroblasts can be reprogrammed into a pluripotent state in vitro by expression of the four Yamanaka factors (OCT3/4, SOX2, c-MYC, and KLF4) have opened up exciting new potentials in regenerative medicine. However, the extremely low efficiency of this process (~0.02%) suggests the existence of intrinsic barriers for reprogramming, potentially including cellular senescence (310, 311).

Indeed, expression of the four Yamanaka factors in mouse and human fibroblasts activates markers of cellular senescence such as SA-β-gal and senescence-associated heterochromatin foci formation. Interestingly, the individual expression of the four factors is also sufficient to induce reprogramming-induced senescence via p16 and p21 activation. The histone demethylase JMJD3 is recruited to the *INK4A* promoter upon reprogramming-induced senescence and decreases levels of the repressive H3K27me3 modification, thus leading to p16 induction. c-MYC and KLF4 trigger p21 expression via p53, whereas SOX2 expression does it via p53-independent mechanisms (23).

Mouse and human fibroblasts silenced for *p21* or *p53* generate a greater number of induced pluripotent stem (iPS) cell colonies with an accelerated rate, indicating that reprogramming is more efficient when senescence is ablated (23, 162, 180, 219, 322). Silencing *INK4A* only improved reprogramming efficiency in human fibroblasts, whereas silencing *ARF* affects only mouse fibroblasts (204). These results are likely due to human and murine fibroblasts differences in the pathways engaged for senescence induction (287).

As expression of *INK4A/ARF* increases with organismal aging (189), it is likely that cells from old individuals would be less prone to reprogramming in vitro. Indeed, skin fibroblasts from old mice (>2 yr) cannot be reprogrammed as efficiently as cells from young mice (2 mo) unless the *INK4A/ARF* locus is silenced (1, 204). Another approach to reprogram old cells is by using a six-factor cocktail (OCT4, SOX2, KLF4, c-MYC, NANOG, and LIN28). This method has been described to successfully generate iPS cells from centenarian adult fibroblasts as well as from fibroblasts serially passaged to replicative senescence (199). How this six-factor cocktail can reprogram senescent and very old cells is currently unknown, although NANOG and/or LIN28 may counteract the senescence program. LIN28 expression improves reprogramming efficiency in mouse fibroblasts, whereas NANOG expression only increases reprogramming kinetics (148). Therefore, LIN28 expression likely results in senescence bypass, and a possible mechanism could be that LIN28 inhibits production of the *let-7* miRNA, thereby preventing downstream translation of *HMGA2* (146), a transcriptional repressor of *INK4A/ARF* (244). It is also possible that LIN28 functions independently of the *INK4A/ARF* locus by enhancing *CDK4* translation (9, 348), which may negate p16-mediated senescence.

Owing to INK4A/ARF forming a barrier to reprogramming in vitro, it has been suggested that transient silencing of the locus during cellular reprogramming could be an effective approach for regenerative medicine in old individuals without increasing risk of malignancy (204). However, the role of INK4A/ARF differs vastly during in vivo reprogramming. Mice engineered to transiently express the four Yamanaka factors upon doxycycline administration (i4F) display NANOG positive cells in multiple tissues, indicating successful iPS cell generation. Despite this, in vivo reprogramming efficiencies are still very low (2), and therefore, intrinsic reprogramming barriers must also exist in vivo. However, in contrast to in vitro conditions, senescence, and more specifically INK4A/ARF, promote in vivo iPS cell generation. Upon doxycycline administration, i4F mice containing a heterozygous *INK4A/ARF* locus are resistant to teratoma formation and do not display NANOG-positive cells in tissues normally permissive to reprogramming in i4F wild-type mice. Interestingly, reprogrammable tissues in i4F wild-type mice display coexisting SA-β-gal and NANOG-positive cells, which are absent in heterozygous *INK4A/ARF* mice. These results indicate that senescent cells, which arise from DNA damage induced by expression of the four Yamanaka factors, generate a favorable environment for reprogramming in neighboring cells. This is mediated by the secretion of the SASP factor IL-6 (36). IL-6 activates the JAK/STAT target PIM1 downstream to induce reprogramming and cellular plasticity (36, 234). Aged mice were also more permissible to in vivo reprogramming than young mice because of the increased presence of senescent cells (234) **(FIGURE 2)**. This may suggest therapies for tissue regeneration would actually be more successful in elderly patients.

Other stimuli of senescence such as tissue injury create a permissible niche for in vivo cellular reprogramming. The i4F mice treated with bleomycin and subsequent doxycycline displayed a greater number of NANOG-positive cells in lungs than in uninjured mice (234). Inducing injury before reprogramming has therefore been discovered to be an effective method of iPS cell generation in tissues not typically susceptible to reprogramming such as skeletal muscle. These findings could help direct future strategies in regenerative medicine for repair of skeletal muscle or other difficult to reprogram tissues (61).

IV. SENESCENT CELLS IN AGING

A. Senescence in Age-Related Disease

Almost all multicellular organisms display features of aging, currently defined as a progressive loss in tissue and organ functions over time. Eventually, loss of tissue functions can lead to the generation of numerous chronic and age-related pathologies. As the frequency of all these disorders exponentially increase later in life, common basic molecular and cellular mechanisms could underlie how they arise (46).

Various markers of senescence, including SA-β-gal, p16, and DDR, accumulate in tissues of aged mammals including rodents (42, 189, 335), baboons (156, 168), and humans (67, 97, 211, 225, 267), suggesting that senescent cells could play a detrimental role in age-associated pathologies. Moreover, it has been suggested that the development and progression of these diseases could be ascribed to the decline of the regenerative functions of stem cells with advancing age (288). In vivo, the first causal link between senescence and aging has been proven in the progeroid mice BubR1 (22). These mice express extremely low levels of *BubR1*, a spindle checkpoint gene responsible for proper chromosome segregation during mitosis, and display an early onset of several age-associated disorders including sarcopenia, cataracts, cachexia, lordo kyphosis, cerebral gliosis, and decreased arterial wall thickness and elasticity (21, 149, 222). When *BubR1* mice were engineered so that *p16* expressing cells can be induced to undergo selective apoptosis (a model known as INK/ATTAC), mice displayed a significantly delayed onset in some of these disorders, but overall lifespan was not increased (22). In a subsequent study using naturally aging INK/ATTAC mice, the same authors showed that elimination of $p16^+$ cells delays onset of age-associated diseases in later life, but also increases median and maximum lifespans, suggesting senescent cells limit longevity (20).

B. Atherosclerosis

Atherosclerosis is initiated when lipoproteins amass in the intima of arteries and induces activation of endothelial and vascular smooth muscle cells (VSMCs). Activated cells trigger an inflammatory response in which recruited monocytes are converted into lipid-containing, foamy macrophages, which then accumulate and form plaques. VSMCs initially form a fibrous cap over the plaque to provide a barrier to circulating platelets, but over time this cap can erode, causing plaque to be released into the bloodstream. This results in downstream thrombosis and damage to organs fed by the circulatory system like the heart, brain, and kidney. Many vascular diseases arise because of this sequence of events including myocardial infarction, stroke, unstable angina, and sudden cardiac death (63, 309).

VSMCs and endothelial cells from human atherosclerotic plaques upregulate a number of senescence markers including SA-β-gal, p16, and p21 (133, 223, 229, 336). Interestingly, genetic associations in humans suggest a protective role for cellular senescence during atherosclerosis. Indeed, individuals carrying polymorphisms in *CDKN2A*, which result in decreased *p16* expression and potentially inability to enter senescence, are at increased risk for developing the disease (212). Similarly, low-density lipoprotein receptor–deficient ($Ldlr^{-/-}$) mice with exclusive *p16* deficiencies in bone marrow (BM) cells display increased monocyte and macrophage proliferation and accelerated atherogenesis (195).

Conversely, cellular senescence is reported to play deleterious roles in early and late stages of the disease. Fatty streaks are observed in $Ldlr^{-/-}$ mice after only 9 days on an atherogenic diet. Surprisingly, these streaks contained SA-β-gal–positive foam cell macrophages, which also upregulate expression of *CCL2* and *VCAM1*. These factors recruit monocytes, thereby stimulating greater conversion into foamy macrophages. $Ldlr^{-/-}$ mice fed an atherogenic diet for longer periods (88 days) display plaque-rich aortas consisting of senescent endothelial cells, VSMCs, and foam cell macrophages. These cells upregulate proteolytic SASP factors *MMP12* and *MMP13*, which promote plaque instability (63) **(FIGURE 3)**. It is, therefore, possible that senescence plays a dual role during atherosclerosis: on one side, proliferative arrest of monocytes and macrophages limits plaque growth, but on the other side, SASP factors secreted from these cells can also induce disease progression (151). Nevertheless, therapies that act to eliminate senescent cells could be used to prevent and treat the disease. In accordance with this approach, clearing *p16*-positive senescent cells in $Ldlr^{-/-}$ mice led to reduce fatty streaks in early stage and reduced plaque burden in late stages of the disease (63).

C. Bone Disease

1. Osteoarthritis

Osteoarthritis is a disease in which the overall integrity of synovial joints is compromised. The articular cartilage undergoes progressive degeneration characterized by bony projections called osteophytes, thickness of synovial ligaments, and local inflammation. These changes result in chronic pain and movement difficulties for sufferers. Chondrocytes maintain articular cartilage via secretion of various ECM components, but they enter senescence and partially lose this ability in an age-dependent manner (224, 261).

Chondrocytes from osteoarthritic joints display numerous senescence markers including SA-β-gal (261), p16 (365), and expression of various MMPs (30) **(FIGURE 3)**. The transplantation of senescent ear cartilage fibroblasts into the knee joints of mice results in a gain of osteoarthritic symptoms, such as articular cartilage damage and osteocyte formation (116). It is possible that senescence-associated MMP secretion induces local cartilage degradation, but direct evidence is lacking.

Clearing senescent cells may, therefore, be an attractive method to alleviate osteoarthritis. This approach has already been proven to be effective in mice using genetic and pharmacological strategies. Using the p16–3MR model, senescent cells were discovered to accumulate in the synovium and cartilage surface after posttraumatic osteoarthritis induced via anterior cruciate ligament transfection. Clearing senescent cells via administration of GCV or UBX0101 (a compound which selectively kills senescent cells via disruption of the Mdm2/p53 interaction) results in repair of dam-

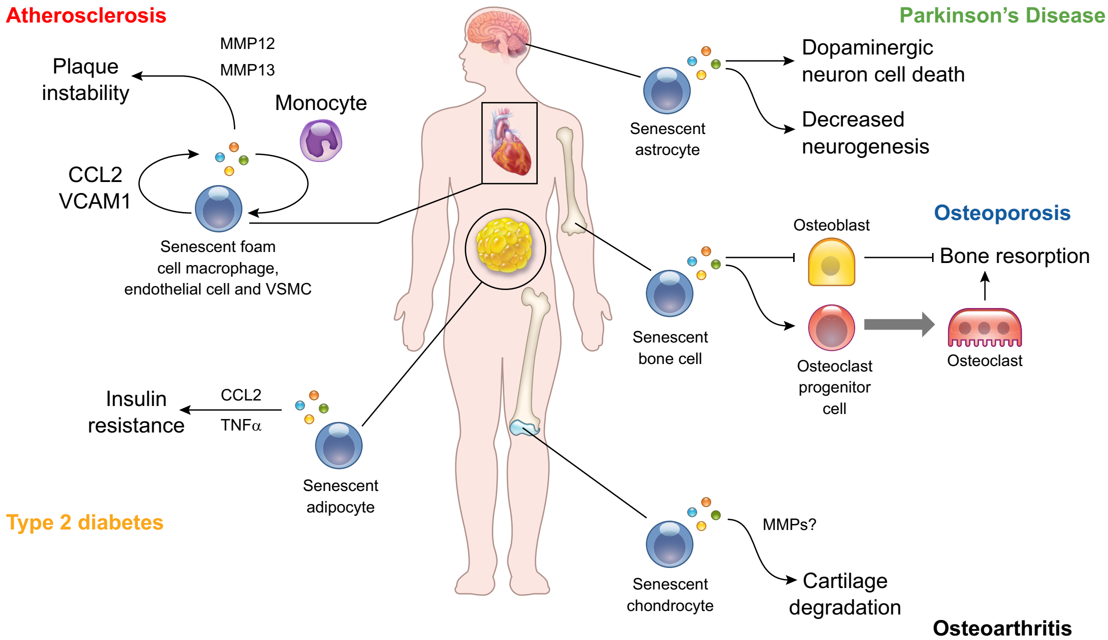


FIGURE 3. Senescent cells in disease. In atherosclerosis, senescent foam cell macrophages secrete CCL2 and VCAM1 to recruit monocytes and trigger their conversion into senescent foam cells. Senescent endothelial and vascular smooth muscle cells secrete MMP12 and MMP13 to promote plaque instability. In osteoarthritis, senescent chondrocytes contribute to cartilage degradation possibly via MMP activity. In osteoporosis, the senescence-associated secretory phenotype (SASP) from senescent bone cells promote osteoclast progenitor survival and inhibit osteoblast activity. Together, these activities contribute to bone resorption. The SASP secreted from senescent astrocytes triggers dopaminergic neuronal cell death and decreased neurogenesis in Parkinson's disease. Senescent adipocytes secrete factors including CCL2 and TNFα, which promote insulin resistance in type 2 diabetes.

aged cartilage, reductions in expression of inflammatory and tissue-modifying SASP factors, and decreased cartilage erosion. Interestingly, when naturally occurring senescent cells were constantly cleared in mice starting at 12 mo of age using the INK/ATTAC model, osteoarthritic symptoms did not manifest. Therefore, therapies which selectively eliminate senescent cells may be effective to both prevent and treat osteoarthritis (167).

2. Osteoporosis

Osteoporosis is a disorder resulting from an imbalance in bone turnover rate, in which bone resorption by osteoclasts occurs in excess of bone formation by osteoblasts. This leads to a reduction in both bone density and bone strength and a resultant increased risk of bone fracture. Advanced age is one of the biggest risk factors for osteoporosis (105), and *p16* expression is significantly upregulated in all cell types found in the bone microenvironment in old mice compared with young mice, although osteocytes and myeloid cells were the only cells types that displayed an upregulated SASP profile (115) **(FIGURE 3)**.

Senescent bone cells were discovered to induce osteoporosis by stimulating an increase in osteoclast progenitor survival and impairing bone synthesis. Both processes are mediated via the SASP and result in an imbalance in bone turnover rate in favor of resorption. Clearing senescent cells in old INK/ATTAC mice or old wild-type mice treated with dasatinib and quercetin (a drug cocktail found to selectively kill senescent cells) improves various measures of bone strength including bone volume density, trabecular number, trabecular thickness, and trabecular spacing in the spine and femur. Ruxolitinib administration was also found to induce improvements in overall bone strength in old mice (116). Ruxolitinib is a Janus kinase inhibitor previously discovered to inhibit production of multiple SASP factors (350). Elimination of senescent cells or interfering with the SASP could, therefore, be useful for osteoporosis treatment.

D. Glaucoma

Glaucoma is currently the leading cause of blindness worldwide and describes the progressive degeneration of the optic nerve, resulting in a reduction in visual sensitivity and eventual sight loss. Approximately 70 million people worldwide suffer from the disease, and 10 million of these individuals are estimated to be bilaterally blind. The most common form of glaucoma is primary open-angle glaucoma (POAG), which accounts for around 80% of cases in the United States. POAG is characterized by an increased intraocular pressure (IOP) owing to increased resistance of aqueous outflow in the trabecular meshwork of the eye. The increased IOP is believed to cause retinal ganglion cell death (341).

Advanced age is one of the leading risk factors for POAG (341). It was recently reported that IOP could induce expression of *SIX6*, a homeobox protein involved in eye development in mice. Interestingly, a risk variant in *SIX6* (His141Asn) was discovered to increase POAG susceptibility by affecting the transcriptional activity of *SIX6*, resulting in increased *p16* transcription and retinal ganglion cell senescence (299). An independent study also reported that the serine/threonine kinase TANK-binding protein 1 (TBK1) is upregulated upon IOP in mice. TBK1 induces *p16* transcription and senescence via an AKT/Bmi1 pathway (205). Thus, there is a potential link between induction of cellular senescence and glaucoma.

However, it is currently unclear how senescent cells could contribute to glaucoma development. The *SIX6* risk variant induces *IL-6* expression, and SA-β-gal positive cells are more predominant in regions of the outflow pathway in POAG patients compared with control donors (208). It is, therefore, plausible that tissue modifying SASP factors can alter the microenvironment to limit aqueous outflow.

E. Neurodegeneration

Neurodegenerative diseases including Alzheimer's and Parkinson's disease place a great economic and social burden on society. Unfortunately, many clinical trials against them have produced disappointing results, and new approaches are desperately needed to develop functional therapies. As the frequencies of these disorders increase with age, cellular senescence may play a critical role (66). In line with this, astrocytes in aged brains express greater levels of p16 than in young brains (29). At this point, there is limited published evidence on whether causal links exist between senescence and neurodegeneration. Inflammatory molecules, including interleukins, are elevated in Alzheimer's and Parkinson's patients **(FIGURE 3)**. This "neuroinflammation" is suggested to contribute to disease pathology (126), and it is possible that senescent cells in affected brains could be the source. Astrocytes in frontal cortices from Alzheimer's patients express greater levels of p16, γ-H2AX, and MMP1 compared with age-matched control samples (29, 126, 238). However, it is unknown how these cells influence disease progression, and future studies of senescence in neurodegenerative disease warrant further investigation. In a mouse model of Parkinson's disease (PD), it has been recently shown that senescent astrocytes affect neurogenesis and contribute to the progression of neurodegeneration. The elimination of senescent cells is sufficient to delay cognitive impairments (65). Interestingly, human PD brains also show an increased expression of senescence markers (65).

F. Type 2 Diabetes

Obesity and aging are two of the major risk factors for type 2 diabetes mellitus (T2DM). Many countries face issues with rapidly aging populations as well as drastic increases in the prevalence of obese individuals. As a result, T2DM represents one of the major worldwide health issues today (252).

Excessive caloric intake in mice induces senescence in adipose tissue via ROS-mediated activation of *p53* and *p21* (230). In contrast, both mice and humans under caloric restriction show lower levels of senescence markers (122).

The induction of senescence due to excessive calorie intake is reported to occur via an upregulation of ROS-scavenging enzymes such as superoxide dismutase 2 and catalase (34). Senescent adipocytes upregulate proinflammatory factors, including *CCL2* and *TNF-α,* and downregulate anti-inflammatory factors such as *Adiponectin*. This can lead mice to develop impairments in insulin sensitivity and glucose tolerance. The *p53*-deficient mice do not display signs of adipocyte senescence in response to excessive calories intake and are rescued from the resultant pathological conditions (230). Adipose senescence is also suggested to play a role in human insulin resistance owing to the same senescence markers being expressed in adipocytes from human diabetic patients (230).

The mechanistic link between inflammatory molecules and insulin resistance is poorly understood. In rat hepatoma cells, TNF-α prevents tyrosine autophosphorylation of the insulin receptor, thereby impairing glucose homeostasis (117). CCL2 is a well-known macrophage recruiter, and macrophage infiltration into white adipose tissue results in the generation of feedback loops where macrophages secrete proinflammatory factors to further exacerbate insulin resistance (349) **(FIGURE 3)**. Adiponectin reduces overall glucose levels in vivo by stimulating phosphorylation of 5′-AMP–activated protein kinase (AMPK) to increase cellular glucose uptake and reduce expression of enzymes involved in gluconeogenesis (352).

Evidence of senescence in pancreatic β cells has also been observed. The *p16* expression is increased in pancreatic islets from old mice and attenuates islet cell proliferation (188, 247). Surprisingly, it has recently been reported that p16 plays a beneficial role in pancreatic β cell function, as the protein can increase glucose-stimulated insulin secretion and improve glucose homeostasis. Islets from old mice also secrete more insulin upon glucose stimulation than in young mice, suggesting that insulin secretion does not necessarily depend upon islet regeneration, and pancreatic β cell senescence may not be a factor in age-associated T2DM (153, 247). Nevertheless, it is still possible that proinflammatory factors are released and act on adipose tissue in a paracrine manner. Pancreatic β cell senescence also arises in mice continuously fed a high-fat diet, and importantly, the insulin secreting function of these cells is compromised (300). Islet senescence could, therefore, play opposite roles in age-associated and diet-induced T2DM.

V. SENESCENT CELLS IN CANCER

Cellular senescence plays important roles in different phases of tumorigenesis such as tumor initiation (OIS), establishment [PTEN loss–induced cellular senescence (PICS), TIS], and escape (258) **(FIGURE 4)**. In this section, we will describe in detail the mechanisms that regulate senescence in cancer cells, the dual role played by the SASP in the tumor microenvironment, and the identification of therapies that target senescent tumor cells for the treatment of cancer patients.

A. Cell-Autonomous Regulation of Senescence in Cancer

1. Oncogene-induced senescence

Oncogene activation in mammalian cells results in proliferative stress and senescence induction that limits tumor growth. Thus, senescence is a physiological tumor-suppressive mechanism that inhibits the progression from benign tumor lesions to malignant tumors. The induction of senescence by oncogene activation is termed OIS **(FIGURE 4)**. The first experimental evidence of OIS came from overexpression experiments of oncogenic HRAS^{G12V} in human fibroblasts resulting in a permanent cell cycle arrest (79). Mutations in the RAS oncogene are common in many human cancers. However, its sole activation is not sufficient to drive transformation and requires the cooperation with other oncogenes and tumor suppressors (91). RAS overexpression in the absence of additional hits drives cells into senescence, and this mechanism works as a barrier to block tumor growth in vivo (37, 79). Interestingly, HRAS^{G12V} overexpression is accompanied by the concomitant upregulation of p19^{ARF}, Pml, p53, retinoblastoma, and p16^{INK4a} (253, 283), and inactivation of these genes results in evasion of HRAS^{G12V}-induced cellular senescence. Similarly, coexpression of oncogenes such as c-MYC, E1A, or DRIL1 bypasses RAS^{G12V}-induced senescence (257). Overexpression of additional oncogenes such as HER2, EGFR, and PI3K can also drive senescence in primary and tumor cells, and their signaling alters the SASP (14, 132). Mutations in BRAF are a common feature in human melanoma patients. However, mutations that lead to constitutive activation of BRAF promote OIS in vitro and result in the formation of melanocytic nevi in vivo, a form of benign skin tumor with senescent cells. In particular, mutated BRAF overexpression initially drives hyperproliferation in melanocytes and then induces p16^{INK4a} expression, which drives arrest of the cell cycle and establishment of senescence (334). As discussed above for RAS, BRAF-induced senescence is also the result of interaction between BRAF itself and other oncogenes and tumor-suppressor genes. In this case, the expression of IGFBP7 is necessary for senescence establishment, and loss of this protein is a critical step in the progression to melanoma (84). Loss of the tumor suppressor PTEN in a BRAF-mutated context promotes tumor progression and metastatic melanoma in vivo (60). On the other hand, inactivation of oncogenes can also induce senescence. MYC inactivation induces cellular senescence and regression in different tumoral specimens such as lymphoma, osteosarcoma, and hepatocellular carcinoma (HCC) (78). These effects are driven by multiple mechanisms, reflecting the implication of MYC in different elements of the tumor microenvironment (26). Importantly, the presence of a proficient immune system is a prerequisite for senescence resulting from MYC inactivation (241). Another mechanism, by which senescence is induced, is represented by the loss or inactivation of TSGs. One of the first descriptions of this phenomenon in vivo is related to the tumor suppressor PTEN, whose loss induces a senescence response named PICS **(FIGURE 4)** (60). Unlike OIS, PICS occurs in the absence of DDR. In PICS, PTEN loss drives p53 activation through activation of mTOR and ARF-mediated inhibition of MDM2. In addition, PTEN loss can induce p16^{INK4A} through upregulation of the transcription factor Ets2 (248) and involves APC/CDH1 (301). In murine models of prostate cancer, ablation of PTEN leads to a benign prostate tumor lesion called prostatic intraepithelial neoplasia, which is characterized by a number of senescent tumor cells (60). However, when combined with p53 inactivation, these lesions progress

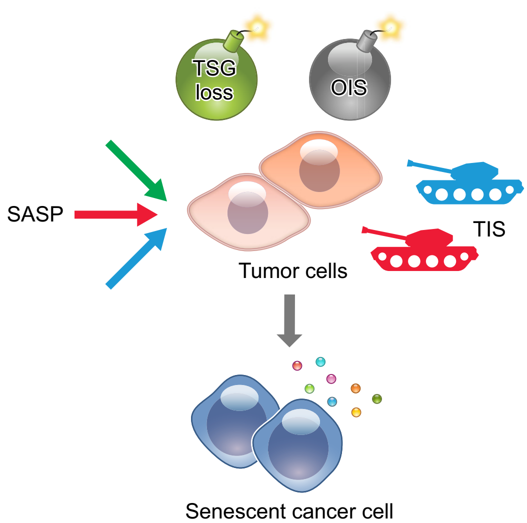


FIGURE 4. Senescence induction in cancer. Senescence initiation in cancer can rely on genetic alterations such as oncogene-induced senescence (OIS) and tumor-suppressor gene (TSG) loss–induced senescence or upon therapeutic interventions [therapy-induced senescence (TIS)]. Senescent cells can induce senescence in neighboring tumor cells by autocrine and paracrine mechanisms through the release of senescence-associated secretory phenotype (SASP), thus restraining cancer cell proliferation.

to invasive prostate cancer because of evasion of PICS (28, 60). Interestingly, in recent years, several regulators of PICS have been identified. For instance, the inhibition of S-phase kinase–associated protein 2 (Skp2) restores senescence in PTEN- and p53-deficent tumors through the upregulation of p27 (351). SMAD4 inactivation or overexpression of COUP-TFII, a SMAD4 inhibitor, also promotes the bypass of PICS by allowing the transcription of cyclin D1 in Pten-null tumors (263). Similarly, because PTEN-deficient prostate cancer cells rely on NOTCH signaling for proliferation, pharmacological inhibition of γ-secretases or inhibition of NOTCH1 enhances senescence in both Pten- and Pten;p53-deficient prostate cancers through induction of p27 expression (268). Casein kinase 2 (CK2) also regulates senescence driven by loss of PTEN through STAT3 activation (176). Preclinical and clinical studies have also shown that HER2 activation in Pten-null tumors leads to PICS escape, causing aggressive prostate cancer (6). Finally, inactivation of the tumor-suppressor inositol polyphosphate-4-phosphatase (INPP4B) in a PTEN-deficient context leads to an increase in cellular senescence driven by p53 upregulation (138).

Mutations or loss of function in the gene neurofibromin 1 (NF1) drive a human disorder called type I neurofibromatosis, characterized by the development of benign tumors in both the peripheral and central nervous system. In these lesions, mutations or inactivation of NF1 lead to activation of the N-RAS pathway and to the induction of senescence characterized by high expression of SA-β-Gal and $p16^{INK4a}$ (68, 284). In addition to this, inactivation of NF-1 has been shown to drive senescence establishment in human melanocytes too (200). Inactivating mutations of TSC2 gene in primary murine embryo fibroblast displayed early senescence associated with overexpression of $p21^{CIP1/WAF1}$ that is rescued by loss of p53 (201). Mutations in von Hippel–Lindau TSG, an E3-ubiquitine ligase, are frequent in human renal cell carcinomas and hemangioblastomas. Studies in murine models clarified that von Hippel–Lindau inactivation induced cellular senescence and benign renal tumors through the upregulation of pRB and p27 in a process dependent on functional p53 and HIF (361). The absence of RB1 in thyroid cells leads to cellular senescence driven by N-RAS, resulting in the formation of benign adenomas, and only upon inactivation of the RAS pathway is there progression to carcinoma (285). Restoration of the TSG p53 in vivo in p53-deficient tumors drives tumor regression in lymphoma and sarcoma models by enhancing senescence (28). Additional studies in a liver cancer model show that p53 reactivation leads to senescence induction and tumor regression through the activation of the innate immune system (166). Further examples of therapies targeting p53 will be provided in sect. V*E*. Thus, not only loss of TSGs can drive senescence, but also upregulation of TSGs can elicit a senescence response.

2. Therapy-induced senescence

Several drugs in clinical use for the management of human cancers can mediate TIS, including docetaxel, bleomycin, cyclophosphamide, doxorubicin, vincristine, etoposide, and cisplatin (114) **(FIGURE 4)**. Ionizing radiation can also induce senescence in different cancer cell lines (11, 120, 260). The mechanisms that force tumor cells into senescence are generally linked to DNA damage enhancement (83). In vitro, evidences of this process were described in tumor cell lines right after the discovery of OIS (54). Analysis of senescence markers in human cancer biopsies from patients previously exposed to neoadjuvant chemotherapy confirmed the occurrence of TIS and its association to treatment outcome (12, 293, 316, 321). Primary murine lymphomas have shown to respond to chemotherapeutic treatment with cyclophosphamide by engaging a senescence program controlled by p53 and $p16^{INK4a}$ (278). Several targeted therapies that inhibit CDKs, NOTCH, CK2, MDM2, JAK2, and SKIP2 can also promote growth arrest and senescence in tumors of different genetic background (268). The CDK4/6 inhibitor palbociclib is currently considered the most relevant prosenescent compound in the clinic. Additional targeted therapies that induce senescence in cancer cells are discussed later (see sect. V*D*). Intriguingly, some clinically available compounds can also block senescence induced by chemotherapy or oncogenic stress, limiting the outcome of the treatment. For instance, rapamycin, a macrolide compound that blocks mTOR, can promote senescence inhibition in tumor cells, allowing the bypass of senescence in specific conditions (12).

B. Noncell-Autonomous Regulation of Senescence in Cancer: Role of SASP

Senescent tumor cells, through the SASP, can educate and shape the tumor microenvironment **(FIGURE 4)**. In the tumor microenvironment, senescent tumor cells are surrounded by stromal cells, nonsenescent (proliferating) tumor cells, and infiltrating immune cells. The main immune cell subset–infiltrating tumors are T cells, natural killer (NK) cells, myeloid-derived suppressor cells (MDSCs), and macrophages that can have either an antitumor activity (canonical or M1-like) or promote tumor growth (alternatively activated or M2-like) (92). The SASP has been defined as a double-edged sword because it can act on neighboring cells and on the recruitment and activation of immune cells, resulting in both antitumorigenic and tumor-promoting effects (318). Via the SASP, senescent cells can induce paracrine senescence in neighboring cells, thus acting as a barrier against tumor growth. For instance, IL-8 and its cognate receptor CXCR2 are needed for the establishment and maintenance of senescence, and inhibitors targeting CXCR2 lead to OIS bypass (4, 5). Similarly, inhibition of IL-6 or IL-6 receptor also promotes senescence evasion in OIS (193). The release of IL-1α by senescent cells transmits senescence to normal and tumor cells, and inhibition of IL-1α signaling bypasses OIS and PICS (92, 94). Oncogenic BRAF promotes senescence by upregulating IGFBP7, and its inhibition promotes melanoma formation (334). The SASP of senescent tumor cells can also induce senescence in normal cells through TGF-β, VEGF, CCL2, and CCL20 (3).

The SASP is composed of a number of chemokines and cytokines that can activate immune surveillance and bring innate and adaptive immune responses to clear senescent and proliferating tumor cells (329), enhancing the tumor suppressive capability of senescence in cancer. Interestingly, Th1 lymphocytes can promote senescence in tumor cells by releasing in the tumor microenvironment "SASPs factors," such as IFN-γ and TNF-α. Such cytokine-induced senescence strictly requires STAT1 and TNFR1 signaling in addition to p16^{INK4A} (38). In addition to this, studies in the Eμ-myc B cell lymphoma model have demonstrated that the secretion of TGF-β by macrophages triggers cellular senescence and limits tumorigenesis, whereas its neutralization abrogates senescence and leads to aggressive disease (266). On the other hand, senescent tumor cells through the SASP can promote tumor progression, boosting cell proliferation and driving tumor vascularization (73), a phenomenon named as maladaptive senescence (73, 193, 196, 272). An informative and striking example of maladaptive senescence is TIS in cancer patients. Although TIS can be initially beneficial in blocking tumor cell proliferation, it also impairs the elimination of senescence tumor cells from the immune system. This leads to the accumulation of senescent cells both in the tumor and in normal tissues of treated mice (217, 243). As a consequence of the inefficient removal of senescent cells, the SASP of tumor cells promotes tumor relapse by sustaining the proliferation of nonsenescent tumor cells, whereas the SASP of normal cells promotes aging-related phenotypes in TIS-treated mice. This is in line with clinical data that demonstrate that chemotherapy can induce premature aging in adults and children treated with high-dose chemotherapy (217, 243). In addition, TIS might generate tumor cells that have an enhanced potential to drive tumor growth by promoting cancer stemness (228). The SASP of senescent fibroblasts can also support cell proliferation of premalignant and malignant, but not normal, epithelial cells (191, 213). Moreover, in PICS prostate tumors, activation of STAT3 results in a SASP with immunosuppressive properties, which attracts tumor-infiltrating MDSCs. MDSCs recruited in the tumors blocked the CD8$^+$ T cell response and blunted the efficacy of chemotherapy-induced senescence (166) by releasing in the tumor microenvironment IL-1 receptor antagonist that block IL-1 signaling in tumor cells (94). Additional examples of non-cell-autonomous regulation of senescence in cancer include sterile inflammation and the gut microbiota. Intriguingly, factors secreted by damaged tumor cells during sterile inflammation can promote OIS bypass, driving pancreatic cancer (142). Finally, senescence regulation by the gut microbiota is a rather new and intriguing field of research that will be discussed in sect. VII. Eggert et al. also went on to provide evidence suggesting that the SASP can either promote or suppress tumor progression. In the early stages of liver tumorigenesis, the induction of senescence acts as a tumor-suppressive mechanism. However, when senescent cells are present in later phases of disease, the SASP inhibits immunosurveillance, thus favoring tumor progression (112). The roles of the SASP in maintaining and propagating senescence on one side, and on bypassing senescence and promoting proliferation on the other side, make it a peculiar target for therapy in a wide range of pathological conditions. In fact, the concept of SASP reprogramming is currently a hotspot in the field, as will be discussed later on in this section. In addition to this, the notion of maladaptive senescence and the controversial role of the SASP have paved the way to an approach defined as senolysis, aimed at the elimination of senescent cells (302).

C. Immune Clearance of Senescent Tumor Cells

The main players in the clearance of senescent cells are M1-like macrophages, NK cells, and T-helper 1 (Th-1) lymphocytes (178, 215) **(FIGURE 5)**. NK cells recognize senescent cells through the expression of NK cell receptor (NKG2D), MICA, and ULBP2, found consistently upregulated upon replicative senescence and OIS (275). NK cells target and mediate the killing of senescent cells via granules production (274). The restoration of p53 in liver carcinoma results in tumor regression because of expression of proinflammatory cytokines and the establishment of senescence (351). The p53-expressing senescent cells release factors that promote macrophage polarization toward antitumor M1 macrophages able to target senescent cells in cultures (215). Macrophages can also participate to the clearance of premalignant senescent cells (178). Kang et al. reported that the presence of this population is indeed required for the correct function of CD4$^+$ T cells and for the killing of premalignant senescent hepatocytes (178). This study demonstrated the importance of the immune surveillance on senescent cells in tumor suppression, showing how the impairment of the immune clearance of premalignant senescent hepatocytes resulted in the development of HCCs (178).

Recently, another study also suggested that during OIS, primary human melanocytes express major histocompatibility complex class II molecules that activate the adaptive immune response (324). Several possible mechanisms have been proposed for the recognition of senescent cells by macrophages. These processes are probably not specific to senescent cells; rather, they are "eat me" mechanisms associated with macrophage recognition in cancer immunosurveillance and apoptotic cell clearance. The oxidized form of membrane-bound vimentin has been reported to be ex-

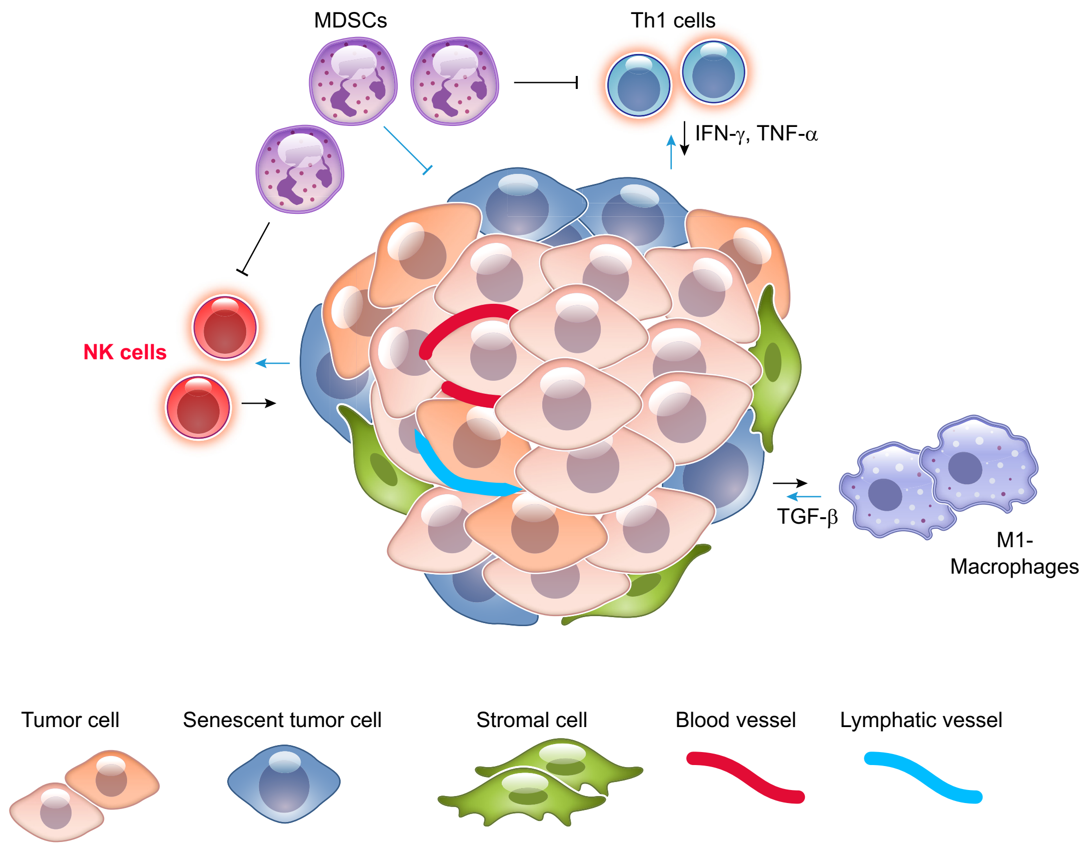


FIGURE 5. Noncell-autonomous modulation of senescence in cancer. Within the tumor microenvironment, senescent tumor cells can promote both the recruitment and the activation of several immune populations including M1 macrophages, natural killer (NK) cells, and Th1 cells through the SASP. Such tumor-infiltrating immune subsets can restrain tumor progression by mediating the clearance of senescent tumor cells and by promoting senescence in the neighboring cells. Conversely, myeloid-derived suppressor cells (MDSCs) are able to block the senescence induction and/or the antitumor immunity.

pressed on the surface of senescent human fibroblasts and acts as eat me signal–leading macrophage phagocytosis (127).

D. Therapies Targeting Senescent Cells

1. Prosenescence therapy for cancer

Because, as discussed above, senescence can limit cancer development acting in autocrine and paracrine manners, our group and others envisioned that targeted therapies aimed at the selective enhancement of senescence in cancer cells could be used to implement anticancer therapeutic regimens. This approach is named "prosenescence" therapy for cancer and differs from chemotherapy-induced senescence that affects both normal and cancer cells in that it specifically aims at senescence induction in cancer cells (241) **(FIGURE 6)**.

A) TELOMERASE INHIBITION. One of the mechanisms by which cancer cells bypass cellular senescence is the increased expression and reactivation of the telomerase complex, a process required for tumor transformation and progression (31). High levels of TERT expression and/or elevated telomerase activity are commonly observed in cancer and usually correlate with a poor prognosis (137). Numerous studies have focused their attention in the identification of compounds or strategies to inhibit telomerase activity in cancer cells with subsequent loss of telomere integrity and induction of senescence (reviewed in Refs. 8, 141). Because of the complexity of the telomerase complex, a wide variety of strategies to inhibit telomerase have been developed. These approaches include: antisense oligonucleotides, targeting RNA component of telomerase (169, 185), chemical inhibitors of telomerase (286), oligonucleotides and nucleoside (144), small molecule pharmaceuticals that target human (h) TERT (24), gene therapy constructs, molecules that target telomere and telomerase-associated proteins, and inhibitors from microbial sources. The first telomerase inhibitor to be reported has been the 3-Azido-2,3-dideoxythymidine (azidothymidine or zidovudine) (144) and results from phase I and II clinical trials of azidothymidine alone or in combination have shown some rate of regression in different solid tumors (170). Among the many small molecules developed to inhibit telomerase activity, BIBR1532 [2-[E]-3-naphthalene-2-yl-but-2-enoylylamino]-benzoic acid] is the best known. BIBR1532 is a noncompetitive inhibitor of TERT and hTR responsible for the reduction of telomere length, inhibition of cell proliferation, and induction of senescence (255). The antisense oligonucleotide imetelstat or GRN163L, a lipid-conjugated, 13-mer oligonucleotide sequence that is complementary to hTR has shown good results in vitro (41, 100, 135, 161, 218) and has been tested in 14 clinical trials. Regarding immunotherapy, different approaches are currently under development. The idea behind this strategy is to sensitize immune cells to tumor cells expressing hTERT

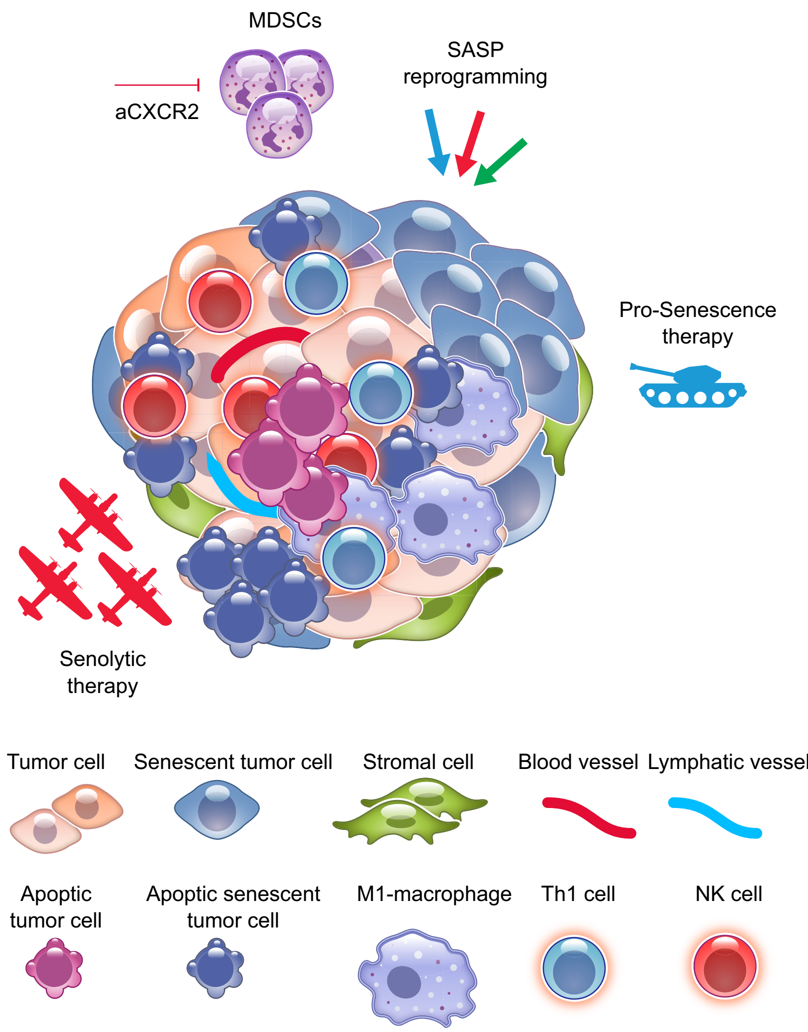


FIGURE 6. The "two-punch" approach. Pharmacological reprogramming of the SASP may increase the antitumor immune response upon treatment with prosenescence therapies. Senolytic therapies may remove senescent tumor cells in tumors where senescence surveillance is impaired to avoid negative effects induced by the SASP. Anti-CXCR2 strategies, limiting MDSC recruitment to the tumor, may favor senescence induction and/or the antitumor immunity.

peptides as surface antigens via the human leukocyte antigen (HLA) class I pathway. Different clinical trials with immunological peptides are ongoing, and among the most promising is the one testing GV1001 (tertomotide). Although at the moment, there are still no telomerase inhibitors used in the clinic, this therapeutic approach represents one of the most promising.

B) THERAPEUTIC MODULATION OF CELL CYCLE MACHINERY. The reprogramming of cell cycle is a fundamental hallmark of the senescence response. Progression to the cell cycle is controlled by a complex machinery composed by a family of protein kinase complexes, wherein each complex is formed by a catalytic subunit, the CDK, and its essential regulatory subunit, the cyclin (165, 294). Each stage of the cycle is controlled by the activity of a unique combination of cyclins and CDKs. The induction of senescence is characterized by an increased expression and the subsequent accumulation of CDKs inhibitors such as p16INK4a, p15, p27, and p21CIP1/WAF1 (207, 283). This observation had brought up the idea that compounds able to enhance the levels of CDK inhibitors or drugs that inhibit CDKs may be used for prosenescence therapy for cancer.

One of the first indications comes from the results obtained with the SKP2 inhibitors. Skp2 is a F-box protein constituting one of the four subunits of the Skp1/Cullin-1/F-Box (SCF) ubiquitin E3 ligase complex that regulates apoptosis, cell cycle progression, and proliferation by promoting the ubiquitination and degradation of p27 (239). Several compounds and small molecules inhibitors of Skp2 or Skp2SCF complex have been identified (reviewed in Refs. 53, 202). Among those, a small molecule called compound A targets Skp2SCF E3 ligase activity toward p27 ubiquitination (59) and the small molecule MLN4924, which affects the formation of the Skp2SCF complex (303). Currently, MNL4924 (pevonedistat) is tested in two different phase I clinical trials for the treatment of lymphoma and multiple myeloma and for nonhematologic malignancies, respectively. The modulation of the p21 activity has shown to have a similar effect in inducing the senescence response. Inhibition of ZNF313, a novel cell cycle activator with an E3 ligase activity for p21WAF1, profoundly delays the cell cycle progression and accelerates p21WAF1-mediated senescence (147).

Other compounds that are currently investigated for the ability to induce senescence by modulating the cell cycle machinery are the CDK inhibitors. These compounds are known to prevent the phosphorylation of retinoblastoma, thus arresting the cell cycle (50, 203) and determining a state of quiescence. However, recent findings have demonstrated that some of these CDK4/6 inhibitors, such as palbociclib, ribociclib, and amebaciclib, are able to induce senescence. Even if the mechanisms responsible for the induction of the senescence response over quiescence are not fully clarified yet (314, 359), these compounds are currently under clinical evaluation. In particular, PD0332991 (palbociclib), LEE011 (ribociclib), and LY2835219 (amebaciclib) are in phase I–II clinical trial and they have been tested alone or in combination with chemotherapy.

The inhibition of Cdk2 could represent another strategy to induce senescence. A recent study has indeed demonstrated that the pharmacological inhibition of Cdk2 induces Myc-dependent senescence in various cell types (45). Therefore, Cdk2 may be regarded as a potentially therapeutic target for cancer therapy, and the several Cdk2 inhibitors that are currently in clinical development (282) may represent a valid class of prosenescence compounds for cancer therapy.

C) P53 AND MYC TARGETING. Because of the impact of p53 in the senescence process, targeting p53 either directly or indirectly may represent a potential approach in the prosenescence therapy. Compounds and small molecules that activate p53 and/or its pathway are currently under development. In tumors that retain wild-type p53, one of the approaches that are being tested is to inhibit the MDM2/p53 interaction, enhancing p53 function. The discovery of nutlin, a specific inhibitor of the p53/MDM2 interaction [Vassilev et al. (325)] has inspired other researchers to design new MDM2 inhibitors with higher selectivity and potency and an improved pharmacokinetics. This has led to the discovery of RG7112 (RO5045337) (332), the first MDM2 inhibitor to be advanced into phase I human clinical trials (13, 332) and others such as RG7388 (99), MI-77301 (338), MI-888 (364), AMG-232 (307), AM-7209, DS-3032b (18), Serdemetan (JNJ-26854165), and the Nutlin family members RO5503781, RO5045337, RO6839921, RO683992 (33), and UBX0101 (167).

Another strategy that is currently under investigation is to target SIRT1, a deacetylase involved in the regulation of p53 activity. Indeed, SIRT1, by deacetylating p53, leads to its ubiquitination and degradation, thus suppressing its function (216, 326). Several SIRT1 inhibitors such as sirtinol (250), suramin (280), tenovins (197), 3,2′,3′,4′-tetrahydroxychalcone (174), EX-527 (240), and cambinol (154) have been demonstrated to induce senescence in preclinical tumor models (323). In tumors with mutant p53, the use of small molecules that restore wild-type activity, such as CP-31398 (124, 206), PRIMA-1 (262), MIRA-1, and APR-246 (PRIMA-1 analog) (43, 231) have been shown to promote cellular senescence. APR-246 is now in clinical trial in combination with carboplatin in ovarian cancer.

In tumor cells lacking p53, the use of adenoviral vector of p53 have been shown to induce senescence (295). The first attempt to perform p53 gene therapy in humans was made by Jack Roth in 1996. Since then, several patients have received p53-based gene therapies in clinical trials mostly in the United States and in China, but although the use of this strategy is now widespread in China (295), it has not been approved yet in the United States (56). Gendicine and H101, two recombinant adenoviruses engineered to express wild-type/p53, have been approved in China for the treatment of head and neck squamous cell carcinoma in combination with chemotherapy, but they have not received the approval of the Food and Drug Administration.

Among other compounds showing p53-mediated senescence is Dasatinib, a Src and c-Kit kinase inhibitor that is currently used in the clinic (103).

Another transcriptional factor well known for its role in regulating cellular proliferation, growth, differentiation, and survival and is often found deregulated in cancer is c-Myc. Myc is viewed as an antisenescence oncogene, and different strategies targeting Myc have been shown to induce a senescence response (357). Small molecules such as 10058-F4 (164) and its derivatives (164) as well as RNA interference (RNAi) technologies (69, 256) are currently tested at the preclinical level.

A promising class of compounds found to suppress MYC transcription, thereby enhancing senescence, are represented by BET protein bromodomain inhibitors such as JQ1 or CPI-0610 and are currently tested in clinical trials in different cancer patients (87, 118).

D) IMMUNOTHERAPY. Immunotherapies have been also linked to senescence induction in cancer. The presence of myeloid cells in the tumor bed promotes prostate tumor progression by opposing senescence in vivo (94). In addition, myeloid cells suppress the recruitment and activation of cytotoxic T cells (CTLs) and so are bona fide MDSCs (318, 319). MDSCs are a phenotypically heterogeneous cell population that has common biological activity in the suppression of the anticancer immune response, particularly T cells. Myeloid cells differentiate in the BM and are recruited to the tumor bed by cytokines and chemokines, which could also promote the suppressive phenotype (131). MDSCs mediate senescence evasion in prostate cancer through the release of IL-1 receptor antagonist (IL-1RA) into the tumor microenvironment. IL-1 receptor signaling that is essential for the establishment of PICS, and its block determines senescence evasion. Interestingly, patients with high IL-1RA tumor levels did not respond to chemotherapy-induced senescence (docetaxel) and showed a short disease-free survival compared with patients with normal IL-1RA levels. Taken together, these findings demonstrate that senescence in cancer can be antagonized in a noncell-autonomous manner by a subset of tumor-infiltrating immune cells. Importantly, interfering with MDSC recruitment in the tumor bed with CXCR2 antagonist potentiates senescence induced by docetaxel (94). An intriguing aspect of the role of MDSCs in cancer is that their abundance in biopsies has prognostic relevance in cancer patients (92). Several studies demonstrate that the number of circulating MDSCs correlates with poor prognosis in patients affected by head and neck, melanoma, breast, lung, and prostate cancers (92, 333, 342). Moreover, as anticipated, MDSCs can affect tumorigenesis not only by blocking senescence induction in cancer cells, but also by additional mechanisms that involve immunosuppression in the tumor microenvironment. Their suppressive activity is mediated by a variety of mechanisms, mostly involving arginase, inducible nitric oxide synthase, ROS, TGF-β, IL-10, and prostaglandin E_2. As a result of this suppressive activity, CTLs can be tolerized and thus lose their effector function (40, 131). Finally, MDSCs are also involved in a whole array of nonimmunological functions, such as the promotion of angiogenesis, tumor local invasion, and metastases. Indeed, MDSCs produce MMPs that can support tumor cell invasion by directly promoting tumor angiogenesis and lymphangiogenesis (95, 237, 297, 354). Several chemotherapies can suppress MDSC count, and it is postulated that this may be critical to benefit from such treatments (184, 306). However, following anticancer treatments, the frequency of MDSCs does not decline to the level seen in tumor-free mice and healthy human subjects. Moreover, tumor recurrence after several treatments correlates with re-expansion of MDSCs (81). Therefore, immunotherapies that decrease the trafficking or function of myeloid cells in the tumors may not only enhance the efficacy of prosenescence therapies but also limit the additional protumorigenic features of MDSCs **(FIGURE 6)**. Many cancer immunotherapies based on vaccination or T cells reactivation in cancer do not cause cytotoxic cancer elimination but arrest cancer growth or induce slow cancer regression. Of note, a recent paper demonstrates that autologous infusion of tumor antigen–specific CD4 Th1 cell that produces IFN-γ, and TNF-α induces senescence in RIP1/tumor antigen 2 pancreatic cancers. This arrest occurs in the absence of significant T cell infiltration and is independent of either CTL (38). Although data in human cancer patients still do not exist, this paper suggests that autologous infusion of T cells and the chimeric antigen receptor T cell therapy may work by inducing senescence in cancer. Another recent report demonstrates that T cell–activating therapies based on CD137 antibodies enhance the efficacy of prosenescence compounds in a xenograft model of melanoma (330). Finally, senescent tumor cells can be employed as an antitumor vaccine. Indeed, injection of senescent tumor cells into tumor-bearing mice induces an antitumor CTL response, which potentiated the effects of radiation, resulting in elimination of established tumors (226). Thus, treatments that combine different immunotherapies with prosenescence compounds and novel trials are ongoing to validate the relevance of these findings in patients affected by different tumors.

E) SASP REPROGRAMMING. As discussed above, SASP has profound effects on the tumor microenvironment, and it represents a promising target for cancer therapy. Several groups have demonstrated that therapies reprogramming the SASP can enhance the tumor suppressive role of senescence in cancer and restrain the negative effects of the SASP. For instance, we discussed previously in this review that Stat3 regulates the SASP of PICS, promoting an immunosuppressive tumor microenvironment (sect. V*D1*B). Pharmacological inhibition of Jak2 in this context induces SASP reprogramming, leading to the reactivation of the senescence immune surveillance (319) **(FIGURE 6)**. mTOR is a critical regulator of the SASP, and its inhibition with rapamycin suppresses the SASP by regulating the translation of the MK2 kinase through 4EBP1 (158). Although mTOR inhibition prevented the protumorigenic effects of the SASP in vivo, it also interfered with the induction of paracrine senescence and senescence surveillance, two important tumor suppressive arms of senescence (158). Moreover, the mTOR inhibitor rapamycin through reduction of IL-1α translation and NF-κB signaling reduces SASP (IL-6) and impairs the ability of senescent fibroblast to support tumor growth in vivo (196).

BRD4, a member of the BET family, is a chromatin reader whose role in the activation of the SASP and in the subsequent immune clearance is well documented, and its inhibition with JQ1 or analogs impairs both processes in a model of NRAS driven OIS in vitro and in vivo (315). However, as previously underlined, BET inhibition with JQ1 is also a driver of cellular senescence (87, 118), underlining, once again, the dual role of the SASP and senescence itself in cancer and the need of coupling prosenescence and senolytic approaches. The HMG-coA reductase inhibitor simvastatin is known to attenuate inflammation and retard tumor growth, and this effect is mediated by downregulation of the SASP. In fact, simvastatin suppresses breast cancer cell proliferation induced by senescent cells (209).

Finally, antagonists of CXCR2 **(FIGURE 6)**, which acts as a receptor for a number of SASP cytokines, result in the reshaping of the tumor infiltrating immune cells impacting on cellular senescence and TIS, as discussed in section V*D1*B.

Altogether, these studies highlight the importance of strategies aiming at the SASP reprogramming as potential cancer therapies. Thus, identification of compounds that can attenuate the "dark side" of the SASP without affecting its tumor suppressive function could be used in the clinic to enhance the therapeutic efficacy of prosenescence compounds.

2. Selective elimination of senescent cells

As already discussed in this review, senescence can act as a double-edged sword in different physiological contexts. SASP can indeed have anti- as well as protumorigenic effects, and therefore, selective killing of senescent tumor cells has been proposed to prevent the relapse of tumors treated with chemo- or radiotherapy and to reduce the risk of metastasis (302). Moreover, in mouse, the accumulation of senescent cells in normal tissue induced by the chemotherapy has been recently linked to a premature aging phenotype and to cancer-related fatigue, a syndrome commonly experienced by patients treated with chemoradiotherapy (88). Notably, a recent study demonstrates that the clearance of senescent cells in doxorubicin-treated mice not only decreases the incidence of tumor recurrence and metastasis, but also reduces several short- and long-term effects of the drug, such as BM suppression, cardiac dysfunction, and cancer-related fatigue (88). Thus, the use of senolytics may have positive effects for chemotherapy-treated patients in terms of reduction of tumor relapse and amelioration of the side effects due to the drug (183) **(FIGURE 6)**. Nowadays, there are few examples of effective senolytic compounds.

A) BCL-2 PROTEIN FAMILY INHIBITORS. Senescent cells are resistant to apoptotic stimuli and this feature may contribute to their accumulation in aged tissues and following chemotherapy. Bcl-2 protein family, which includes Bcl-2, Bcl-W, Bcl-XL, and Mcl-1, plays a central role in the regulation of cell death–related processes including autophagy and apoptosis and are found upregulated in senescent cells (44); therefore, compounds that target these proteins are intensively studied as senolytic drugs. In particular, inhibitors of Bcl-2 family have shown the potential to alleviate age-related diseases, such as in the case of atherosclerosis, and enhance radioprotection and rejuvenation of the hematopoietic system in mice (55, 63, 88). One of the first molecules to be identified as inhibitor of Bcl-W and Bcl-XL proteins, the ABT-737, induced apoptosis preferentially in senescent cells both in vivo and in vivo (358) and opened the door to new strategies for the treatment of age-related pathologies. However, because of the poor oral availability of ABT-737, an orally available analog, ABT-263 (navitoclax), was identified (368), which paved the way for clinical trials of the first generation of Bcl-2/Bcl-XL inhibitors (317). However, these drugs have been associated to severe toxicities in cancer patients (271, 344), and for this reason, new compounds, such as A1331852 and A1155463, have been currently tested in the hope to find better candidates for translation into clinical applications (367). However, the efficacy of these compounds is variable, and it depends on the genetic background of the senescent tumor cells being effective in some types of senescence but not in others. ABT-737 exerted activity in different tumor context as single agent or in combination with chemo- or radiotherapy (317).

B) DASATINIB AND QUERCETIN. Dasatinib is a Food and Drug Administration–approved anticancer drug known for its ability to induce apoptosis. Interestingly, Dasatinib can work as senolytic compound when combined with Quercetin (a flavonol) (143). It has been shown to be effective in killing senescent preadipocytes, endothelial cells, and mouse embryonic fibroblasts (MEF) in vitro (368). These findings demonstrate the efficacy of senolytics to alleviate age-related symptoms, including dystonia, loss of grip strength, and urinary incontinence (369), and to prevent osteoporosis progression (115).

C) FOXO4 INHIBITORS. A recent paper has identified Forkhead box protein O4 (FOXO4) as an alternative regulator of viability in senescent cells. FOXO are a class of transcription factors activated downstream of IGF-1. FOXO4 interacting with p53 plays a central role in senescent cell viability (143). The design of a FOXO4 peptide (FOXO4-DRI) that perturbs the FOXO4 interaction with p53 caused p53 nuclear exclusion and then senolysis. The FOXO4-DRI peptide neutralized doxorubicin-induced accelerated aging and restored fitness, fur density, and renal function in treated mice (19). However, therapeutic peptides have some significant drawbacks related to their stability and short half-life (123). Moreover, it is currently unknown whether this outcome would also occur with repeated senolytic administrations, and studies that aim to measure this would be warranted.

D) OTHER SENOLYTIC COMPOUNDS. Other compounds that were recently shown to have senolytic properties are piperlongumine, nicotinamide riboside, danazol, fisetin, and HSP90 inhibitors. Piperlongumine is a natural product that has been shown to induce caspase-mediated apoptosis in senescent cells (363). Nicotinamide riboside, a precursor of nicotinamide adenine dinucleotide (NAD^+), drives an increase in cellular levels NAD^+. Aged mice treated with nicotinamide riboside showed increased lifespan and rejuvenation of muscle stem cells (363). Danazol is a synthetic steroid molecule that has telomere-elongating capacity and has been used to antagonize accelerated telomere attrition (320). Fisetin is a plant polyphenol that reduces cognitive deficits in old mice restoring impaired synaptic function, stress, and inflammation related to aging (367). Finally, HSP90 inhibitors have recently be shown to be effective in delaying the onset of aging related symptoms in a mouse model of progeria (129).

VI. SENESCENCE OF THE IMMUNE SYSTEM

Cellular senescence can occur also in immune cells, and through this mechanism, the immune system, can guide immune cell function and fate decision. Immunosenescence refers to a series of changes in the development and function in both the humoral and cell-mediated immune branches of the immune system that contribute to an increased susceptibility to disease in the elderly.

Characteristics of innate immune senescence are a reduction in the antigen processing and presentation capacity associated with a decreased response to stimuli but keeping a chronic activation state. Adaptive immune senescence is associated with loss of T or B cell receptor repertoire diversity and impaired immunological memory formation. This phenomenon is the cause of an inefficient control of infections and tissue damage with age, as well as of an impaired tumor immunosurveillance that leads to an increased risk of tumorigenesis in old individuals. However, the senescence in the immune system can also occur independently by the age. This aspect and more details about the features of immunosenescence in innate and adaptive immune response are discussed in section V, *A* and *B*.

A. Innate Immune Response

1. Macrophages

Macrophages are key immune cells in the protection of our body from pathogens, and the most abundant immune cell type in tumor microenvironment of several cancers (for review, see Ref. 246). The existence of senescent macrophages in vivo is still under debate. Cudejko et al. and Fuentes et al. (128) reported the expression of senescence markers, such as p16^{INK4a} and p14/p19ARF, in murine BM-derived macrophages and in human adipose tissue macrophages. Interestingly, gene expression analysis of p16^{INK4a}-deficient BM-derived macrophages showed a dramatic downregulation of genes associated with proinflammatory macrophages and upregulation of genes associated with anti-inflammatory macrophages (128). Accordingly, primary macrophages that can become senescent after 2 wk of in vitro expansion, or upon ectopic p16^{INK4a} expression, revealed an anti-inflammatory polarization (236) in mouse and human. However, a recent publication reports that expression of p16^{Ink4a} and positivity to β-galactosidase in macrophages is acquired as part of the physiological response to immune stimulation and not sign of cellular senescence (145). Altogether, these findings point out an unexpected role of p16^{INK4a} in myeloid cells and suggest his potential involvement in the differentiation and polarization of the myeloid lineage.

2. NK cells

NK cells are lymphocytes that participate in the immunosurveillance thanks to their cytotoxic activity and specific cytokine profile (52). A less known role of NK cells is during embryo implantation and through the first trimester of pregnancy. Their role is still elusive. It has been reported that during these phases NK cells, acquire senescence features by the upregulation of p21$^{Cip1/Waf1}$ and pHP1-γ. The activation of NK cells through CD158d, by a soluble nonclassical major histocompatibility complex molecule secreted by fetal trophoblasts, induces permanent cell cycle arrest, DNA damage accumulation, and chromatin remodeling. Then, senescent NK cells start to produce a specific SASP that dictates the neoangiogenesis during embryo implantation (264).

B. Adaptive Immune Response

1. T and B lymphocytes

T and B lymphocytes are the mediators of adaptive immune response. During aging, they are endangered to replicative senescence because of their innate highly proliferative capacity. In vitro, T and B cells, upon stimulation, progressively undergo a series of cell division and they can become exhausted or exhibit features of cellular senescence (110, 163). Exhausted lymphocytes have short telomeres, cannot proliferate even in the presence of costimulatory molecules, are resistant to apoptosis, but not metabolically active, whereas senescent lymphocytes are still metabolically active and express high levels of senescence immunological markers (80, 110, 343). Indeed, although senescent lymphocytes are completely anergic, they are still active and abundantly produce proinflammatory cytokines and active mediators for NK cells (16).

Senescent T lymphocytes harbor, together with the higher expression of p16 and p21, a specific secretome characterized by IL-6/IL-8/IL-10/TGF-β/IFN-γ/TNF-α production, downregulation of surface markers such as CD28 and CD27 and upregulation of PD1 (329). Interestingly, senescence in T lymphocytes can also be triggered in a paracrine manner by a deregulated inflammatory environment. TNF-α or IFN-γ, typical inflammatory cytokines, can induce premature senescence of CD8 T lymphocytes through the activation of p38MAPK and downregulation of the expression of telomerase (93, 198). Persistence of proinflammatory cytokines and antigen stimulation can also drive immune senescence in T lymphocytes by the loss of CD28 expression. CD28 is a costimulatory molecule expressed by T lymphocytes that regulate their activation and proliferation. In cancer and aging, as well as in chronic immune degenerative disorders, such as juvenile idiopathic arthritis, myelodysplastic syndromes, or rheumatoid arthritis, the persistent stimulation of lymphocytes leads to loss of CD28 that can encompass senescent and skewing to cells with regulatory functions such as T regulatory cells (Tregs) (106, 110, 111, 279, 346). The expression of CD28 in human T cells is mediated by the downregulation of the p53β, a splicing variant of p53 (233). Growing literature suggests that induction of senescence in the immune compartment is also a mechanism used by the immune system to regulate the immune response. For instance, Tregs, known to be crucial for the maintenance of the immune self-tolerance and homeostasis (for review, see Ref. 171), induce senescence in effector T cells, limiting their proliferation by the activation of the p38MAPK and p53 signaling pathways that control both the cell cycle inhibitors p16INK4a and p21WAF1 (355, 356). In vivo, p53 protein levels increase in $CD4^+$ T cells upon TCR activation, and several p53 binding sites are present on the promoter of FoxP3, the transcription factor of Tregs (181). Recently, it has been reported that human Tregs mediate functional changes and induce senescence in responder T cells by the regulation of STAT1/STAT3, ERK1/2, and p38 signaling and by metabolic competition during cross-talk (210).

This finding reveals the complex interplay between senescence and immune cell fate. Targeting factors that induce T cell senescence is a checkpoint for immunotherapy against cancer and other associated diseases.

VII. MICROBIOTA AND SENESCENCE

The microbiota is the ensemble of the microorganisms living in symbiosis with the host (109) and plays fundamental roles in many homeostatic processes. The host and its microbiota can be referred to as a new entity, the "superorganism," which is endowed with enlarged genetic and metabolic potential (102). The microbiota and its associated metabolism are fundamental to a number of physiological functions and imbalances in the bacterial community, termed dysbiosis, have been described and correlated to a number of pathological situations, including cancer (134). Microbial dysbiosis can affect the tumor physiology through direct and indirect mechanisms, such as a direct effect on tumor cell proliferation and apoptosis, an effect mediated by the immune system or acting at the level of the host metabolism (134). Some examples of this are the evidence that *Fusobacterium nucleatum*, enriched in human colorectal cancer (CRC), exacerbates intestinal tumorigenesis in vivo by inducing a proinflammatory signature in MDSC (187). Moreover, increasing evidence shows that the microbiota is important for the efficacy of both classical chemotherapy (328) and of immune checkpoint inhibitors (anti–PD-L1, anti–CTLA-4, and anti-PD1) (298, 327). An effect of the microbiota in the modulation of senescence has been proved for the first time by Yoshimoto and colleagues (360). The intestinal microbiota plays a fundamental role in the metabolism of bile acids because bacteria can mediate the conversion of primary bile acids in secondary bile acids, which are reabsorbed and enter the enterohepatic circulation, or their deconjugation that leads to their excretion (39). Yoshimoto and colleagues showed that in obesity-associated HCC there is a gut dysbiosis characterized by an expansion of members of the *Clostridium* cluster. This leads to increased systemic levels of deoxycholic acid. This bile acid induced DNA damage in HSC, driving the establishment of senescence and, consequently, the overexpression of proinflammatory cytokines, namely IL-6, GROα, and CXCL9. These SASP components favored HCC progression in mice treated with a chemical carcinogen that causes oncogenic Ras mutations (360). Genetic ablation of IL-1β as well as microbiota depletion through antibiotic treatment significantly suppressed SASP of HSC and growth of HCC. These results identify in the microbiota/deoxycholic acid/SASP axis the responsible events in driving HCC progression. In addition to this, colibactin-producing *Escherichia coli* are frequently associated with CRC. Cougnoux and colleagues have shown that this bacterium promotes CRC growth in vitro in the AOM/DSS mouse model of colon carcinogenesis and in specimens of human colon cancer biopsy through the induction of a SASP rich in growth factors (76). Colibactin-producing *E. coli* sustains, through c-Myc expression, the upregulation of miR-20a-5p in intestinal epithelial cells. This miRNA negatively regulates the translation of SENP1, an inhibitor of p53 SUMOylation. As a result, senescence is established, and the SASP, rich in hepatocyte growth factor, sustains CRC growth (76).

BOX 1. Call-out Box for Clinicians

- Cellular senescence is defined as a stable state of cell cycle arrest that can occur in many different setting.
- Senescent cells are characterized by morphological changes, altered gene expression, and secretion of a plethora of factors referred to as senescence-associated secretory phenotype, which is responsible for the paracrine effects of senescent cells.
- Senescence is observed in physiological, as well as in pathological, processes. It plays key roles in embryogenesis, tissue repair, and tissue remodeling, and its involvement has been reported in fibrosis.

Senescent cells are known to accumulate during aging and to participate in age-related pathologies, among them atherosclerosis, osteoarthritis and osteoporosis, glaucoma, diabetes, and neurodegeneration diseases. Moreover, senescence has been shown to have a double effect in cancer by suppressing tumor development in early stage and contributing to tumor development in later stage and tumor relapse after chemotherapy.

- Therapies focusing on the modulation of the senescence response are currently in preclinical or clinical phase. In particular, approaches aimed at eliminating selectively the senescent population (senolytic therapies) or at inhibiting the senescence-associated secretory phenotype (SASP) could be used to prevent or treat age-related disease, whereas the enhancement of the senescence (prosenescence therapies) has been proposed as new cancer treatment. In the case of cancer treatment, the sequential use of prosenescence and senolytic therapies could represent winning strategies by avoiding the negative side effects of SASP in tumor.

A recent work shows that the gut microbiota affects the BM niche and how this interaction is altered by systemic chronic hypoxia in patients with cyanotic congenital heart disease. In these patients, chronic hypoxia results in a dysbiotic gut microbiota with a reduction in *Lactobacilli* and a concomitant accumulation of D-galactose in the BM. This metabolite, together with the hypoxic microenvironment, drives senescence in BM mesenchymal stem cells, thus compromising its fundamental role in the self-renewal of the stem cell compartment of the BM. Administration of *Lactobacilli* in chronic hypoxic rats reduced both D-galactose and BM mesenchymal stem cell senescence (347). This influence of the gut microbiota on the BM niche could be relevant for the hematopoietic stem cell transplantation field.

These few works suggest how the microbiota could play a role in driving the senescence response and could be considered a possible modulator of this process, opening new and fascinating perspectives in the senescence field.

VIII. FUTURE DIRECTIONS

As largely discussed in this review, both cell-autonomous and noncell-autonomous mechanisms can account for senescence evasion. Thus, identification of new treatments that elicit senescence induction in cell that bypass senescence may be of fundamental importance to limit tumor progression. Several prosenescence compounds are currently in the clinic (175). In the past, several in vivo evidences have demonstrated that senescence works as a potent tumor suppressive mechanism. However, recent findings have highlighted an unexpected dark side of the senescence induction in cancer may promote relapse upon chemo-radio or targeted therapies by demonstrating that the persistence of senescent cells in tumors (88). A step forward to reconcile this controversy is represented by the use of a multiple targeted therapies that simultaneously or sequentially induce senescence in tumor cells and eliminate them by either activating the tumor immune response or by inducing apoptosis in a cell-autonomous manner (**FIGURE 6**). In this regard, the use of senolytic therapies may enhance the efficacy of prosenescence therapies by removing senescence cells from the tumor. Even a single dose of this therapy could be administered concomitantly or after prosenescence compounds treatment (337, 351). Therefore, the identification of therapeutic targets specifically expressed by senescent cells and absent in proliferating cells would be highly desirable. Laboratories all over the world are working to identify senescence-associated membrane markers that can be targeted by antibodies. As reported previously, NK cells target and kill senescent cells via NKG2D ligands (275). Because many tumor cells also express NKG2D ligands, such ligands have been suggested to be a good target for humoral-mediated therapeutic approaches in cancer (304) and, therefore, adapted for senescent cell clearance to obtain a win/win result. On the same topic, the identification of specific membrane targets for senescent cells could lead to the generation of chimeric antigen receptor T cells with a great potential as an anticancer therapy. These findings highlight a potential immunotherapeutic strategy for targeting tumor senescent cells. In conclusion, we believe that a prosenescence therapy associated with a senolytic treatment could represent a promising therapeutic strategy to treat cancer and that, in the near future, novel therapies based on this combination could become the new standard of care. Moreover, the involvement of senescent cells during aging and age-related diseases suggests that the use of senolytic compounds might play a major role to extend health span. However, long-term interventions against senescent cells, necessary in the case of aging, should be designed to avoid unnecessary side effects relative to interfering with beneficial cellular senescence, as during tissue repair and remodeling. Cautious approaches aiming at interfering with only subsets of senescence, for example, in specific age-related pathologies, should be paving the way for the use of senolytics as antiaging strategies.

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J. Kohli, E. Zagato, and L. Pellegrini contributed equally to this work.

Address for reprint requests and other correspondence: M. Demaria (e-mail: m.demaria@umcg.nl) and A. Alimonti (e-mail: andrea.alimonti@ior.iosi.ch).

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