Mechanisms and functions of cellular senescence

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Cellular senescence is a highly stable cell cycle arrest that is elicited in response to different stresses. By imposing a growth arrest, senescence limits the replication of old or damaged cells. Besides exiting the cell cycle, senescent cells undergo many other phenotypic alterations such as metabolic reprogramming, chromatin rearrangement, or autophagy modulation. In addition, senescent cells produce and secrete a complex combination of factors, collectively referred as the senescence-associated secretory phenotype, that mediate most of their non–cell-autonomous effects. Because senescent cells influence the outcome of a variety of physiological and pathological processes, including cancer and age-related diseases, pro-senescent and anti-senescent therapies are actively being explored. In this Review, we discuss the mechanisms regulating different aspects of the senescence phenotype and their functional implications. This knowledge is essential to improve the identification and characterization of senescent cells in vivo and will help to develop rational strategies to modulate the senescence program for therapeutic benefit.

Introduction

Cellular senescence was originally identified as a stable exit from the cell cycle caused by the finite proliferative capacity of cultured human fibroblasts (1, 2). Currently, senescence is considered a stress response that can be induced by a wide range of intrinsic and extrinsic insults, including oncogenic activation, oxidative and genotoxic stress, mitochondrial dysfunction, irradiation, or chemotherapeutic agents (3).

While the defining characteristic of senescence is the establishment of a stable growth arrest that limits the replication of damaged and old cells, many other phenotypic alterations associated with the senescent program are relevant to understanding the pathophysiological functions of senescent cells (4). For example, senescent cells undergo morphology changes, chromatin remodeling, and metabolic reprogramming, and secrete a complex mix of mostly proinflammatory factors termed the senescence-associated secretory phenotype (SASP) (Figure 1). Here, we review the molecular mechanisms controlling cellular senescence with a special focus on their translational relevance and suitability for identifying and characterizing senescent cells in vivo.

Physiological roles of senescence

Cellular senescence was initially dismissed as a tissue culture artifact. However, a wealth of data has demonstrated that senescent cells can influence disease and aging, as well as normal tissue homeostasis (5). Indeed, senescence can be engaged during development (6, 7) and is also necessary for tissue remodeling. For instance, transient induction of senescent cells is observed during wound healing and contributes to wound resolution (8, 9). Senescence can also be a protective stress response. In fact, senescence

is best known as a potent anticancer mechanism that prevents malignancies by limiting the replication of preneoplastic cells (10).

However, the accumulation of senescent cells also drives aging and age-related diseases (11, 12). The connection between senescence and aging was initially grounded on observations of the accumulation of senescent cells in aged tissues (13). It was suggested that, during aging, senescence of stem and progenitor cells could hinder tissue homeostasis by interfering with the capacity of tissues to repair and regenerate. In the last 10 years, our understanding of senescence's detrimental consequences in aging and age-related pathologies has significantly expanded. Two lines of research have facilitated this awareness. First, the use of transgenic models that allow for the detection of senescent cells has enabled a systematic identification of these cells in many age-related pathologies (5). Second, the development of genetic and drug strategies to selectively eliminate senescent cells, spearheaded by the van Deursen laboratory, has demonstrated that senescent cells can indeed play a causal role in aging and related pathologies (11).

The confirmation that selectively killing senescent cells significantly improves the health span of mice in the context of normal aging and ameliorates the consequences of age-related disease or cancer therapy (14–19) has ignited interest in the identification of compounds that can clear senescent cells. These so-called senolytic therapies, however, still face important caveats. In addition to their potential side effects, the evaluation of senolytic compounds is compromised by limitations such as the lack of universal senescence biomarkers and the heterogeneity of senescent phenotypes in vivo (20). Ongoing research into the pathways that initiate and maintain senescence will provide insights to identify biomarkers and potential therapies to target senescent cells.

Senescence as a dynamic program

Senescence has been traditionally considered as a defined, static cell fate. However, it is now recognized that senescence is a dynamic multistep process (11). A simplified model (Figure 1)
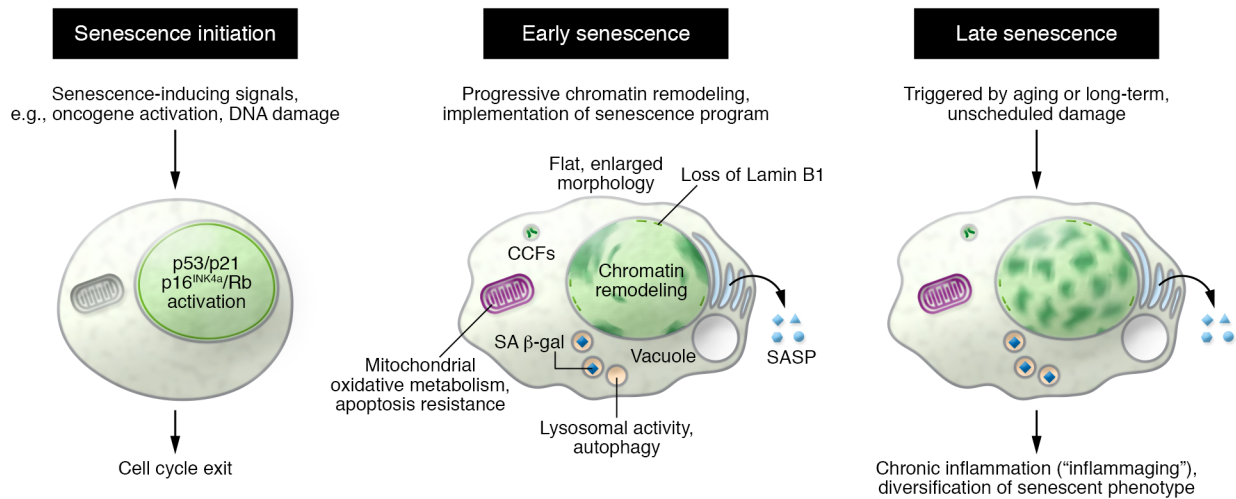
Figure 1. Phenotypic characteristics of senescent cells. Diagram depicting some of the phenotypic alterations associated with senescence initiation, early senescence, and late phases of senescence.

suggests that although the initial senescence-inducing signals are sufficient to initiate cell cycle exit, this merely constitutes an early step in the senescence process. Senescent cells progressively remodel their chromatin and start to sequentially implement other aspects of the senescence program, such as the SASP, to enter into a second step of "full senescence." If these senescent cells persist for extended periods of time, they continue evolving and can be categorized as entering into a third step of "late senescence," which can involve adaptation and diversification of the senescent phenotype. It is tempting to suggest that the concept of senescence progression may help account for the heterogeneity of senescent cells and their associated phenotypes in vivo. Indeed, the senescent responses occurring in vivo can be categorized into two types. Acute senescence seems to be a programmed process that is triggered in response to discrete stressors, is established with fast kinetics, and normally contributes to tissue homeostasis. In contrast, chronic senescence may result from long-term unscheduled damage, and it is often associated with detrimental processes such as aging. Only chronic senescence is associated with the presence of cells in a state of late, deep senescence. Nevertheless, there is no conclusive evidence proving an irrefutable relationship between the different stages of senescence identified in cultured cells and the physiopathological functions associated with senescent cells. In fact, recent evidence suggests that the functionality of senescent cells in vivo strongly relies on the SASP's effect on the surrounding environment and the associated immune responses. However, the effects of the SASP may be difficult to predict. On one hand, the senescent secretome is a heterogeneous mix of proteins whose composition depends not only on the stage of senescence progression but also on the affected cell type and the nature of the inducing stressor(s). The efficiency and kinetics of the clearance of senescent cells may vary depending on the organ in which they accumulate or the general ability to mount an effective immune response. For example, while cells undergoing oncogene-induced senescence (OIS) in the liver are efficiently cleared by the immune system (21), senescent cells in melanocytic nevi often manage to evade immune clearance and persist (22).

Overall, therapeutic approaches aiming to modulate senescent phenotypes will benefit from a better understanding of the steps driving and defining the evolution of senescent cells in vitro.

Cell cycle arrest

One of the defining features of senescent cells is their stable cell cycle arrest. This cell cycle exit is controlled by activation of the p53/p21^{CIP1} and p16^{INK4a}/Rb tumor suppressor pathways (Figure 2). Unlike quiescent cells, senescent cells are nonresponsive to mitogenic or growth factor stimuli; thus, they are unable to reenter the cell cycle even in advantageous growth conditions. Senescent cells are also distinct from terminally differentiated cells, which are also irreversibly withdrawn from cell cycle. While terminal differentiation is the result of a defined developmental program, which turns undifferentiated precursors into specialized effector cells, senescence is mainly implemented as a cellular stress response. However, terminally differentiated cells such as neurons, adipocytes, and hepatocytes can also undergo senescence, or at least show senescence-like features, during aging or in response to oncogenic activation or DNA damage (21, 23–25). This indicates that the onset of senescence can occur independently of an active cell cycle arrest.

DNA damage response

The senescence growth arrest is often triggered by a persistent DNA damage response (DDR) caused by either intrinsic (oxidative damage, telomere attrition, hyperproliferation) or external insults (ultraviolet, γ-irradiation, chemotherapeutic drugs) (26). For instance, during replicative senescence of human fibroblasts, progressive telomere shortening ultimately exposes an uncapped, double-stranded chromosome free end, which is sensed as a double-strand break by the DDR machinery (27). In cells undergoing OIS, the initial hyperproliferative phase that follows oncogene activation induces this DDR. In response to mitotic signals, an increase in usage of DNA replication origins leads to accumulation of genomic damage and activation of a DDR because of stalled replication forks (9, 28, 29). Senescence is associated with a persistent DDR
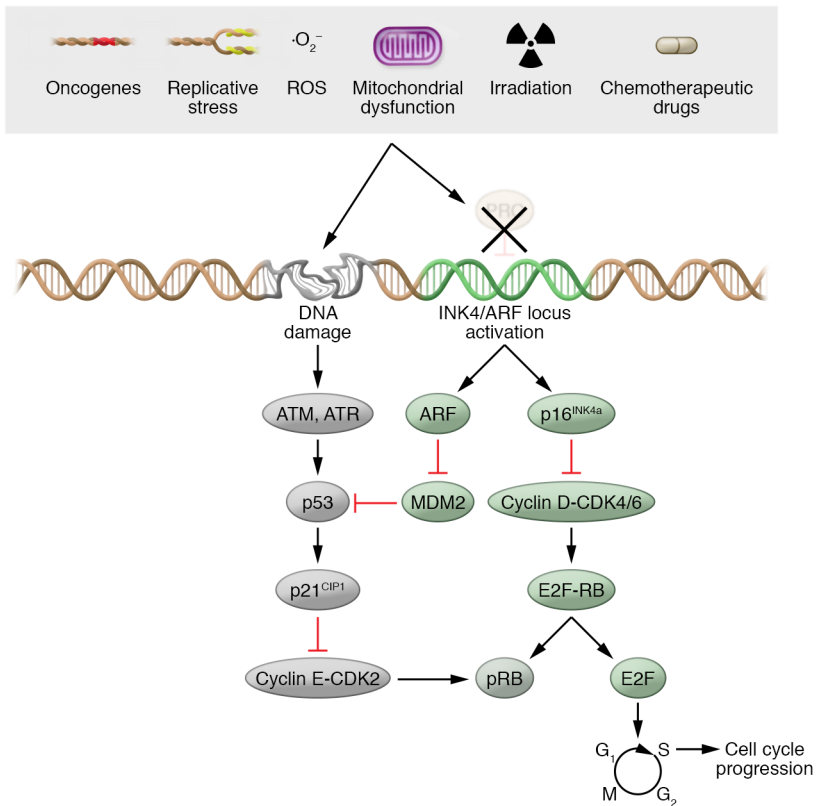
Figure 2. Molecular pathways controlling growth arrest during senescence. A variety of stressors induce senescence-associated growth arrest. Cell cycle exit is regulated by induction of the p16INK4a/Rb and p53/p21CIP1 pathways. Figure reproduced with permission from McHugh and Gil (126).

kinase inhibitor (CDKi) p21CIP1, which in turn blocks CDK2 activity, resulting in hypophosphorylated Rb and cell cycle exit (26). In agreement with this, inactivation of p53 signaling by different means interferes with the onset of cellular senescence (36–39).

Importantly, if the stress that triggers senescence is transient, p53 induction can enact a quiescent state and activate DNA repair processes. Upon resolution of the stress, cells can resume cycling (12). Persistent stress or additional signals can activate p16INK4a, an inhibitor of CDK4 and CDK6, contributing to a long-lasting arrest (40). It has been suggested that the role of p21CIP1 may be limited to the onset of senescence, whereas p16INK4a maintains a durable growth arrest, possibly signifying the existence of differently regulated phases of senescence. Indeed, although the induction of p21CIP1 is important for senescence initiation, its expression does not necessarily persist in senescent cells (20). In agreement with this, upregulation of p21CIP1 (but not p53 or p16INK4a) is the driver of developmental senescence: a short-term "programmed" type of cellular senescence that occurs during mammalian embryonic development (6, 7). Although p21CIP1 can be a valuable marker of senescence in some settings, it is also induced during transient cell cycle arrest or in response to DNA damage, and it should be used as a senescence marker only in combination with others.

Activation of the *INK4/ARF* locus

The *INK4/ARF* locus encodes for three tumor suppressors: ARF (p14ARF in human and p19Arf in mouse) and p16INK4a — each encoded by the *CDKN2A* gene — and p15INK4b, encoded by *CDKN2B* (41, 42). Both p16INK4a and p19ARF have key roles in maintaining the senescent growth arrest. p16INK4a selectively inhibits CDK4 and CDK6, and p19ARF prevents p53 degradation. Indeed, loss-of-function mutations in p16INK4a are among the most frequent in human malignant cancers (43), suggesting that loss of p16INK4a enables senescence bypass and tumor progression.

The functional importance of the *INK4/ARF* locus has driven strong efforts to understand its regulation. In normal cells, the *INK4/ARF* locus is silenced by the Polycomb repressive complexes PRC1 and PRC2. Thus, disruption of PRC1 or PRC2 components, such as CBX7, BMI1, or EZH2, and the consequent loss of the trimethyl histone H3K27 repressive mark and/or PRC1 from the *INK4/ARF* locus are sufficient to activate p16INK4a and induce senescence (44–47). Epigenetic regulation of the *INK4/ARF* locus, however, goes beyond Polycomb proteins. Other epigenetic regulators such as MLL1, JMJD3, or ZRF1 are involved in the regulation of the locus (41, 48–51). How the recruitment and displacement of PRCs and other epigenetic components to the *INK4/ARF* locus are controlled is still not completely understood. The suggested model is that interaction with transcription factors (e.g., TWIST1 or transcription factors of the homeobox family) and long intergenic noncoding RNAs (e.g., ANRIL) can dictate the recruitment

that results in irreparable DNA damage (30). The DDR associated with replicative senescence is telomere-dependent: it correlates with telomere uncapping and an overall loss of telomeric length (27). During OIS, DDR occurs independently of telomeric length, but it is still associated with telomeric dysfunction (31). Regardless of which mechanism drives the damage, the DDR is characterized by increased deposition of γ-H2Ax (the histone H2AX phosphorylated at Ser139) and p53-binding protein 1 (53BP1) in the chromatin as well as activation of a kinase cascade involving first the serine/threonine-nonspecific kinases ATM and ATR and later the checkpoint serine/threonine kinases CHK1 and CHK2, which eventually leads to activation of the p53/p21CIP1 axis (26, 27).

Despite DDR's role in initiating senescence, DDR markers have limited utility for identifying senescence in vivo. In fact, activation of p53 and/or p21CIP1 during senescence can occur in a DDR-independent manner (6, 7, 32, 33). Moreover, the majority of cells expressing markers of DNA damage in vivo, especially in a nonpathological setting, are not in fact senescent; rather, they are responding to a transient reparable damage.

Induction of the p53/p21CIP1 pathway

The senescent growth arrest is implemented by activation of the p53 and p16INK4a/Rb tumor suppressor networks (34). Once activated, p53 regulates a complex antiproliferative transcriptional program (35). The most relevant function of p53 in senescence is to induce the transcription of the cyclin-dependent
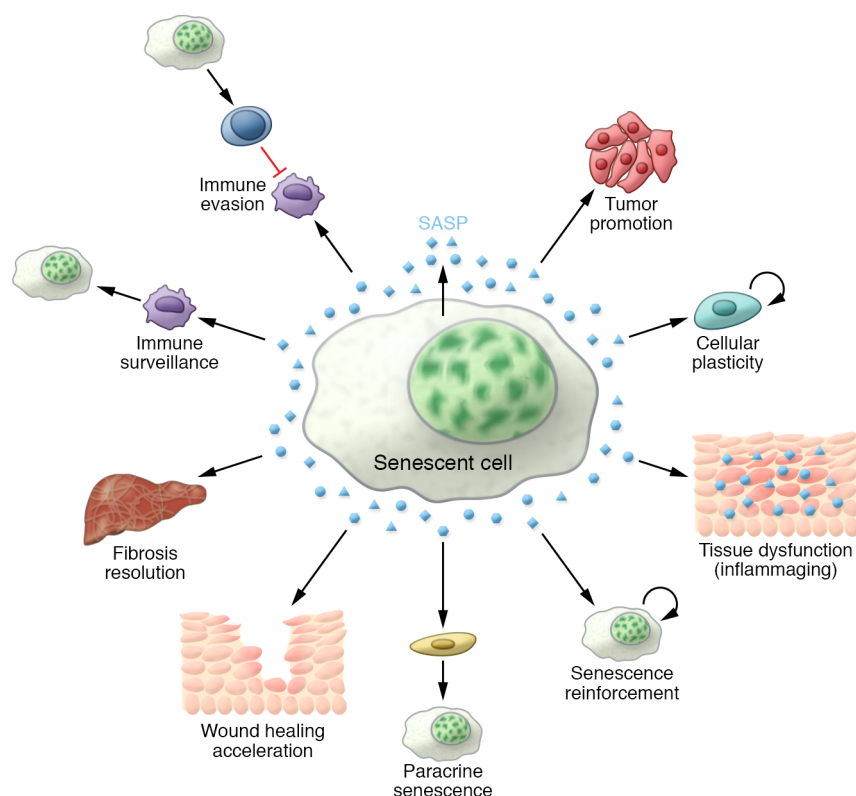
Figure 3. The SASP is an important mediator of the pathophysiological functions of senescent cells. Scheme summarizing some of the functions associated with the SASP.

cancer cells with inactivated Rb), and it is unclear whether p16^{INK4a} expression in macrophages or lymphocytes reflects a senescence phenotype or cell fates such as differentiation or exhaustion (20). Finally, the currently available antibodies are rather poor at detecting p16^{INK4a} in murine tissues.

Non–cell-autonomous effects of senescence

Cellular senescence was initially considered to be a cell-intrinsic program. Increasing evidence, however, has shown that senescent cells have the ability to signal and influence their surrounding environment. Senescent cells produce a complex mixture of soluble and insoluble factors that are collectively termed senescence-associated secretory phenotype (SASP) or senescence-messaging secretome (58, 59). SASP is the general term given to the combination of cytokines, chemokines, extracellular matrix proteases, growth factors, and other signaling molecules secreted by senescent cells. Importantly, its specific composition varies depending on the cell type and the senescence inducer. Likewise, the functions attributed to the SASP, or at least some of its members, are also very diverse and depend not only on the nature of the SASP, but on the surrounding environment and the genetic context of the cells being exposed to the senescent secretome. The SASP is the best-studied mechanism by which senescent cells influence their neighbors, but is not the only one. For example, senescent cells can signal and influence adjacent cells through juxtacrine NOTCH/JAG1 signaling (60) or ROS production (61), or by cargo transfer, which occurs via formation of cytoplasmic bridges (62) or release of exosomes (63).

Functions of the SASP. The SASP can have beneficial or detrimental effects (Figure 3). It is important to recognize, however, that the SASP's effects in specific contexts are pleiotropic. The SASP reinforces the senescence growth arrest in vitro by implementing an autocrine positive-feedback loop. Indeed, knockdown of IL-6R, insulin-like growth factor–binding protein 7 (IGFBP7), or CXCR2, a receptor for IL-8 and related chemokines, prevents senescence (64–66). This autocrine loop contributes to the tumor-suppressive function of senescence. Interestingly, the SASP can also induce nonmalignant proliferating neighbor cells to undergo senescence (termed paracrine senescence) (61, 67, 68). This suggests that senescent cells could also amplify the antitumoral response by limiting the proliferation of nearby cells exposed to similar stressors.

Conversely, the senescent secretome can also promote tumorigenesis. In fact, the SASP has an important proinflammatory nature, and inflammatory mediators are powerful drivers of tumor progression. Early work showed that the SASP of senescent fibroblasts can promote proliferation and metastatic features in premalignant epithelial cells or increase tumor vascularization in xeno-

specificity (52–55). On the other hand, senescence-dependent transcriptional regulation of some of these chromatin modifiers, such as JMJD3 or EZH2, may drive the activation of the locus.

Further highlighting its functional relevance, p16^{INK4a} stands out as one of most specific markers of senescence in vivo (13). While p16^{INK4a} expression is almost undetectable in young healthy organisms, it markedly increases during tumorigenesis and aging. This evidence is the combined result of expression studies and the use of mouse models reporting for p16^{INK4} (56, 57). For instance, the Sharpless group (57) demonstrated an exponential increase in p16^{INK4a} expression during aging. In these studies, while p16^{INK4a} activity did not correlate with mortality, it did predict cancer initiation with higher sensitivity than glucose uptake (measured by fluorodeoxyglucose-PET).

More recently, mouse models have been generated in which p16^{INK4a}-positive cells can be selectively eliminated based on the expression of inducible suicide genes under the control of p16^{INK4a} regulatory elements (9, 15). These models have served to unequivocally show the causal roles of senescent cells in aging, age-related diseases, wound healing, and cancer therapy. The findings reported in these studies will be described in more detail in the other Reviews of this series.

Despite the clear benefits of exploiting p16^{INK4a} activation as a tool to understand the role of senescence in pathophysiology, its use as an in vivo biomarker of senescence has limitations. First, forms of p16^{INK4a}-independent senescence can occur in vitro. More importantly, p16^{INK4a} can be expressed in nonsenescent cells (e.g.,

graft transplants (69, 70). More recently, the protumorigenic role of the SASP has been examined using models that better resemble human cancer. For example, the SASP of senescent hepatic stellate cells (HSCs) promotes the proliferation and malignancy of the surrounding hepatocytes in obese mice treated with chemical carcinogens (71). Interestingly, the senescent secretome also mediates the harmful effects of senescent cells that accumulate upon chemotherapy treatment in vivo. In fact, elimination of senescent cells in this context prevents tumor relapse (17).

The interplay between the SASP and the immune response is also complex. On one hand, it is believed that the SASP might have initially evolved as a way to recruit the immune system to eliminate senescent cells. Indeed, during cancer initiation, SASP-dependent recruitment of Th1 cells, NK cells, and macrophages is essential to clear incipient preneoplastic cells and prevent the progression of hepatocellular carcinoma (HCC) (21, 72). On the other hand, the SASP can have immunosuppressive properties (73–75). For instance, Eggert and colleagues (75) showed that when premalignant senescent hepatocytes coexist with liver cancer cells, the SASP-dependent recruitment of immature myeloid cells may promote HCC progression by impairing the function of NK cells, suggesting a multifaceted interaction between the SASP, immune cells, and cancer.

The SASP has also been strongly linked to aging and age-related diseases. Low-level chronic inflammation (also referred to as "sterile inflammation" or "inflammaging"; ref. 76) underlies many age-related pathologies. It seems that the SASP could explain, at least in part, this local inflammation within tissues. Indeed, the elimination of senescent cells reduces levels of proinflammatory cytokines such as IL-6, IL-1α, and TNF-α in fat, kidneys, and skeletal muscle of aged mice (14, 15, 18). However, the relevance of this decreased inflammation in the improvement of age-related diseases after senolysis is not well understood. Further hinting at the SASP's relevance as a mediator of the detrimental effects of senescent cells in aging is the observation that often only a small percentage of cells in aged tissues are senescent (77). Given the positive impact of eliminating these senescent cells, it is tempting to speculate that SASP suppression may underlie many of the beneficial effects of senolysis.

Altogether, recent discoveries seem to indicate that the deleterious effects of the SASP may outweigh its beneficial properties. Still, the SASP explains important physiological effects exerted by senescent cells. For instance, senescent fibroblasts are induced in response to a cutaneous wound and accelerate wound closure, in part thanks to secreting factors such as the extracellular matrix–associated signaling protein CCN1 and platelet-derived growth factor AA (PDGF-AA), reinforcing the idea that acute, nonpersistent senescence can be beneficial (8, 9). Senescence also limits fibrotic responses (78). For example, senescence acts as a break in the progression of liver fibrosis that is induced by acute liver damage. Dying hepatocytes trigger the proliferation of HSCs, which secrete fibrogenic compounds to form a fibrotic scar. Eventually, these HSCs enter into senescence, and the SASP contributes to fibrotic scar degradation, clearance of the senescent cells, and restoration of tissue homeostasis (79). Finally, in response to tissue damage, factors secreted by senescent cells can also promote stemness (80). The processes that determine the balance between senescence, regeneration, and repair in response to damage remain to be further established.

Irrespective of their functional relevance, components of the SASP have limited utility as biomarkers, as they are not specific to senescence. Only reliable costaining or single-cell profiling will allow us to pinpoint which SASP components are driving the functions of the senescent secretome in specific contexts in vivo.

Regulation of the SASP. The SASP is regulated at multiple levels, including transcription, translation, mRNA stability, and secretion. The SASP also depends on autocrine and paracrine positive-feedback loops that enhance robust signal amplification. Multiple signaling pathways, including the DDR (81, 82), p38 MAP kinase (33), and cGAS/STING (83–85), have been linked with the regulation of the SASP. Most of these cascades seem to converge on the activation of NF-κB and CCAAT/enhancer-binding protein-β (C/EBPβ).

NF-κB and C/EBPβ are activated and enriched in the chromatin fraction in senescent cells (64, 65, 86) and regulate SASP components by directly controlling transcription of key regulators of the inflammatory SASP such as IL-8 or IL-6. In turn, IL-6 and IL-8 act in an autocrine feed-forward loop to enhance the activity of C/EBPβ and NF-κB and amplify SASP signaling (64, 65). IL-1α also behaves as a master regulator of the SASP (87). Indeed, IL-1α signaling is sufficient to partially recapitulate the inflammatory SASP (68). Moreover, inhibition of the NLRP3 inflammasome, necessary to process and activate IL-1β, can blunt the SASP (68).

The SASP can also be regulated at an epigenetic level. For example, MLL1 inhibition blunts the SASP via a decrease in γ-H2Ax at SASP genes (88), and the histone variant macroH2A1 is a critical component of the positive-feedback loop that maintains SASP expression (89). Perhaps more relevant, SASP induction requires the recruitment of BRD4 to senescence-activated superenhancers that lie adjacent to SASP genes (90).

The mTOR pathway is an important node in SASP regulation. It has been described that mTOR-mediated phosphorylation of the translation repressor protein 4EBP regulates the translation of IL-1α (91) and MAP kinase–activated protein kinase 2 (MAPKAPK2) (92) to control the SASP. MAPKAPK2 inhibits ZFP36L1, an mRNA-binding protein that specifically targets proinflammatory SASP components for mRNA degradation. Thus, mTOR can control the senescent secretome by indirectly regulating the stability of SASP mRNAs (92). Moreover, cells undergoing OIS boost the production of SASP components by coordinating protein synthesis and autophagy in the TOR-autophagy spatial coupling compartment, often abbreviated as TASCC (93). However, the regulation of the SASP by autophagy is complex, as GATA4 controls the SASP by inhibiting autophagy (94).

Most of the regulatory mechanisms reviewed here have a global effect on SASP regulation or preferentially control a proinflammatory SASP subset. In fact, this particular proinflammatory arm is highly conserved among the most studied forms of senescence, including replicative, irradiation-induced, and oncogene-induced senescence. The heterogeneous and variable composition of the SASP, however, suggests the existence of different SASP subsets. For example, a distinct senescent secretome has been described in the context of mitochondrial dysfunction (95), and NOTCH signaling enables a switch between a TGF-β and an inflammatory secretome (60).

SA β-galactosidase activity

The most widely used senescence marker is senescence-associated β-galactosidase (SA β-gal) activity. This enzymatic activity, which is found in many normal cells under physiological conditions (pH 4.0–4.5), is significantly amplified in senescent cells as a result of increased lysosomal content (96, 97). Because of this, histochemical detection of β-gal activity at pH 6.0 (suboptimal for normal cells) allows specific identification of senescent cells (98). Since SA β-gal activity is detected in most senescent settings, both in vitro and in vivo, it is considered a de facto hallmark of senescence. However, cells deficient in *GLB1* (the gene encoding for lysosomal β-gal) do not exhibit impairments in the functional aspects of senescence (97). Another lysosomal enzyme, α-fucosidase, has been proposed as an alternative senescence biomarker (99). The increased lysosomal content of senescent cells, which might reflect increased autophagy (100), is also a feature of cell types such as active macrophages, Kupffer cells, and osteoclasts (101, 102). This highlights the need to use additional markers in combination with SA β-gal activity to characterize senescence. Recently, lipofuscin, an aggregate of oxidized proteins, lipids, and metals that are known to accumulate in aged tissues, was reported to colocalize with SA β-gal activity in senescent cells (103). Although the specificity of lipofuscin accumulation during senescence has not been broadly studied, its potential as a biomarker is appealing, especially since, unlike SA β-gal activity, lipofuscin staining can be performed in paraffin-embedded archival material.

Chromatin reorganization

Senescence is associated with large-scale chromatin rearrangements (104). Besides the already described DDR (26) and the formation of PML bodies (a type of matrix-associated nuclear domain) (105), the most striking chromatin change observed in senescent cells is the formation of senescence-associated heterochromatic foci (SAHFs), which are more prominent in human cells undergoing OIS (106). These foci can be identified by DAPI staining and are characterized by enrichment of repressive marks such trimethylated H3K9 and heterochromatic protein 1 (HP1), accumulation of high-mobility group HMGA proteins, and loss of linker histone H1 (4). Therefore, it was hypothesized that SAHFs may represent a senescence-specific heterochromatic compartment. The SAHFs are the result of the spatial repositioning of preexisting repressive marks rather than being caused by global changes in histone methylation (107). Interestingly, genomic regions contained in the SAHFs are found in lamina-associated domains (LADs) in proliferating cells (108). Upon induction of senescence and loss of lamin B1 (LMNB1), these LADs detach from the nuclear periphery and cluster within the nuclei. Strikingly, Hi-C analysis (a high-throughput variant of chromosome conformation capture) suggests that there is a loss of internal structure and possible decompaction of the DNA enriched in the SAHF core (109), thus calling into question the concept of SAHFs as silencing compartments. Another consequence of the autophagy-mediated degradation of LMNB1 observed in senescence (110) is compromised integrity of the nuclear envelope, which may lead to the observed blebbing of cytoplasmic chromatin fragments (CCFs) (111). These CCFs, via activation of the cGAS/STING pathway, are crucial to initiate a proinflammatory response in senescence and cancer. Indeed,

mice deficient in STING show impaired immunosurveillance and decreased tissue inflammation due to reduced SASP (83–85).

Although the diverse global chromatin changes occurring in senescence have clear functional consequences, these changes are not always observed macroscopically or conserved among different cell types or senescence inducers (112). Therefore, it is not straightforward to rely on these global chromatin alterations to identify senescent cells in vivo.

Metabolism, autophagy, and mitochondrial function in senescent cells

Senescent cells display metabolic changes such as increases in glycolysis, mitochondrial metabolism, and autophagy. A clear illustration of this came when Dörr and colleagues showed that the high production of components of the SASP relies on enhanced ATP production mediated by mitochondrial metabolism (i.e., the TCA cycle) and glycolysis (113). The authors suggested that the increased SASP production and secretion lead to a proteotoxic stress that can be attenuated by activation of autophagy. Previous evidence highlighting the role of autophagy in the sustainability of the SASP had reported that the amino acid supply required to maintain the rapid protein turnover that the SASP demands relies on coupling autophagy (autolysosomes) with protein synthesis (93).

The role of mitochondrial metabolism and autophagy in senescence, however, remains controversial. Some studies have shown that inhibition of autophagy facilitates senescence (94, 114). Also, senescent cells display decreased levels of mitophagy (mitochondrial autophagy), which results in a defective mitochondrial network that may contribute to metabolic dysfunction in aging (115). In agreement with this, Garcia-Prat et al. showed that mitophagy contributes to the maintenance of muscle function during aging by preventing senescence in satellite cells (116). Defective mitophagy leads to accumulation of dysfunctional mitochondria and ROS-induced senescence. Similar results were observed in a cell model of parkin-mediated mitophagy (117). Interestingly, depletion of mitochondria impaired senescence by disrupting a positive-feedback loop involving ROS production and a DDR rather than as a result of insufficient energy levels. Indeed, senescent cells lacking mitochondria displayed higher ATP levels due to increased glycolysis. Therefore, it seems that at least in some contexts, the execution of the senescence program is compromised not by insufficient energy levels, but rather by the status of mitochondrial oxidative metabolism. In work related to this concept, Kaplon and colleagues (118) argued that senescent cells must reprogram metabolism in order to support their metabolic demands. They showed that an increase of mitochondrial oxidative respiration through activation of pyruvate dehydrogenase was required for the execution of OIS.

Morphological changes associated with senescence

In cell culture, senescence is normally accompanied by significant morphological changes. Senescent cells become flat, enlarged, and vacuolized, and sometimes appear with multiple or enlarged nuclei. Changes in shape rely on the status of the scaffolding protein caveolin 1 and the Rho GTPases Rac1 and CDC42 (119), and vacuolation has been associated with ER stress caused by the unfolded protein response (120). Senescent cells also form cyto-

plasmic bridges that allow them to signal to neighboring cells via direct intercellular protein transfer (62). Beyond these examples, the functional significance of most morphological changes associated with senescence is unclear. In vivo, senescent cells appear to preserve the morphology dictated by the architecture of the tissue. However, recent studies have discovered that SA β-gal+ cells in aged mice increase in size (77).

Resistance to apoptosis

Senescence and apoptosis are alternative cell fates that often can be triggered by the same stressors. While we do not have a full understanding of what makes the cell decide between one and another program, mechanisms must be in place to lock those decisions. In this regard, senescent cells are resistant to extrinsic and intrinsic apoptosis (121, 122). Recent studies have suggested that this is a result of the upregulation of BCL-2 family proteins such as BCL-W and BCL-XL (123). This is of extraordinary practical relevance since inhibiting BCL-2 family proteins induces apoptosis on senescent cells (19, 123, 124).

Concluding remarks

Senescence is emerging as a therapeutic target relevant for a wide range of pathologies. Pro-senescent and anti-senescent (senolytic) therapies are showing promising results in preclinical mouse models, and human clinical trials are in progress. One of the most widely used senolytics are BCL-2 family inhibitors that target apoptotic resistance of senescent cells (19, 123, 124). Conversely, drugs that induce senescence (such as palbociclib and other CDK4/6 inhibitors) have shown significant benefits as anticancer agents (125). In agreement with the observations that inhibition of CDK4/6 can induce senescence in certain tumor cell lines in vitro, clinical trials have shown that combining palbociclib with current breast cancer therapies significantly increases median progression-free survival. Interestingly, in order to avoid the possible side effects mediated by the resulting senescent tumoral cells, the pro-senescence therapy with CDK4/6 inhibitors could be combined with senolytics, especially if the immune system is compromised and cannot effectively clear senescent cells.

Overall, it is becoming apparent that it is essential to characterize the mechanisms and functions of senescence cells in each context to design specific, optimal, nontoxic senescence therapies. This will require better characterization of the various subtypes of senescence in vivo and establishing which senescence features can be targeted or enhanced without negatively affecting normal cells. Although emphasis is currently shifting to practical aspects and viability of such therapies, a better understanding of the molecular mechanisms of senescence is essential. The accurate identification of senescent cells in vivo is also important. As indicated in this Review, all in vivo biomarkers for senescence, including SA β-gal activity or p16INK4a expression, may be unreliable depending on the context or owing to technical limitations. Because it seems unlikely that a new universal biomarker of senescence will appear, especially considering the heterogeneity of senescent phenotypes in vivo, single-cell transcriptome and proteome profiling of tissues will be key to understand the nature of these senescent cells, investigate their specific liabilities, and eventually implement therapeutic approaches.

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