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Senescent cells as a source of inflammatory factors for tumor progression

Albert R. Davalos · Jean-Philippe Coppe · Judith Campisi · Pierre-Yves Desprez

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Abstract Cellular senescence, which is associated with aging, is a process by which cells enter a state of permanent cell cycle arrest, therefore constituting a potent tumor suppressive mechanism. Recent studies show that, despite the beneficial effects of cellular senescence, senescent cells can also exert harmful effects on the tissue microenvironment. The most significant of these effects is the acquisition of a senescent-associated secretory phenotype (SASP), which entails a striking increase in the secretion of pro-inflammatory cytokines. Here, we summarize our knowledge of the SASP and the impact it has on tissue microenvironments and ability to stimulate tumor progression.

Keywords Aging · Senescence · Cancer · Inflammation · Cytokines · Interleukins · Proliferation · Invasion · Migration

1 Introduction

Organisms with renewable tissues are at risk for developing hyperproliferative diseases, the most deadly of which is cancer. This risk is mitigated by tumor suppressor mechanisms, which suppress cancer for much of the life span. However, because these mechanisms are imperfect, cancer still poses a major challenge to the longevity of such organisms [1, 2]. Some tumor suppressor mechanisms act by curtailing the acquisition of mutations, either by preventing DNA damage or optimizing DNA repair. Others act by preventing damaged or mutant cells from developing into a tumor by eliminating them entirely (apoptosis) or permanently arresting their proliferation (cellular senescence). Several lines of evidence suggest that these tumor suppressor mechanisms can be doubled-edged swords: whereas they undoubtedly suppress the development of cancer early in life, they can also result in altered tissue structure, organization, and homeostasis. These tissue changes can drive phenotypes and pathologies associated with aging, including, ironically, late-life cancer.

In support of a role in tumor suppression, cellular senescence is induced by many potentially oncogenic stimuli [3–5]. In addition, the senescence response depends on two potent tumor suppressor pathways: that governed by the p53 protein and that governed by the pRB and $p16^{INK4a}$ proteins. Indeed, germline mutations in p53 or $p16^{INK4A}$ allow cells to ignore certain senescence-inducing signals and greatly increase cancer susceptibility [6, 7]. Further, virtually all cancers harbor mutations in the p53 and/or p16/pRB pathways, which, among other activities, are crucial for the senescence response [8, 9]. Finally, senescent cells are found in pre-malignant lesions in mice and humans, and in mouse models, the senescence response prevents malignant progression [10]. Interestingly, in culture and *in vivo*, some tumor cells retain the ability to senesce and do so in response to DNA damaging chemotherapy; in mouse models, this response is associated with arrested tumor progression and eventual regression [11–13].

Consistent with a role in aging, senescent cells accumulate with age in many rodent, non-human primate, and human tissues [14, 15]. Moreover, they are found at sites of

A. R. Davalos · J.-P. Coppe · J. Campisi · P.-Y. Desprez
Buck Institute for Age Research,
8001 Redwood Boulevard,
Novato, CA 94945, USA

P.-Y. Desprez (✉)
California Pacific Medical Center, Research Institute,
475 Brannan Street,
San Francisco, CA 94107, USA
e-mail: pydesprez@cpmcri.org

age-related pathology, including degenerative disorders such as osteoarthritis and atherosclerosis [14] and hyperproliferative lesions such as benign prostatic hyperplasia [16] and melanocytic naevi [17]. A limited number of cell culture and mouse xenograft studies support the idea that senescent cells secrete factors that can disrupt tissue structure and function and promote cancer progression [18–21]. Recent studies on the senescence-associated secretory phenotype (SASP) of human and mouse fibroblasts show it is conserved across cell types and species and that specific secreted factors are strong candidates for stimulating malignant phenotypes in neighboring cells [22, 23].

The idea that a biological process such as cellular senescence can be both beneficial (tumor suppressive) and deleterious (pro-tumorigenic) is consistent with a major evolutionary theory of aging termed antagonistic pleiotropy [24]. The SASP may be the major reason for the deleterious side of the senescence response and is the focus of this review. We particularly emphasize the potential effects of the SASP [25] on cell behavior in the context of tumor progression.

2 Cellular senescence as a stress response

Cellular senescence was first identified as a process that limits the proliferation of human cells in culture [26]. These early experiments showed that cultured human fibroblasts gradually lose proliferative capacity until all cells in the culture cease division. Much of this growth arrest is known to occur because most human cells do not express telomerase. Consequently, with each cell cycle, telomeres shorten and eventually fail, generating a persistent DNA damage signal that permanently arrests growth [27]. It is now known, however, that cellular senescence occurs in culture and *in vivo* as a response to excessive intracellular or extracellular stress (see Fig. 1). The senescence program locks the cell into a cell cycle arrest that prevents the damage from affecting the next cell generation, thereby preventing potential malignant transformation [24]. Senescent cells have been shown to accumulate over the life span of rodents, non-human primates, and humans and are found primarily in renewable tissues [15, 28].

Many types of stresses can provoke cellular senescence [5, 29] (see Fig. 1). These stresses include dysfunctional telomeres resulting from repeated cell division (replicative senescence) or other telomeric damage. They also include oxidative stress resulting from mitochondrial deterioration or other causes, severe or irreparable DNA damage from external sources and disrupted chromatin organization due to DNA replication or damage (genotoxic stress), and the expression of certain oncogenes (oncogene-induced senescence) [10, 14, 30–35]. Stresses that cause cellular senescence can be induced by external or internal chemical or physical insults encountered during the course of the life span, during therapeutic interventions (for example, X-irradiation or chemotherapy), or as a consequence of endogenous processes such as oxidative respiration or mitogenic signals. External mitogenic signals, for example growth-related oncogene alpha (GROα) secretion by tumor cells in close to normal cells [36] or circulating angiotensin II [37, 38], have also been shown to induce cellular senescence. All somatic cells that have the ability to divide can potentially undergo senescence. Regardless of the disparate mechanisms of senescent-inducing stresses, the senescence program is activated once a cell has sensed a critical level of damage or dysfunction. Senescent cells are detected in culture and *in vivo* by a variety of markers, including the senescence-associated β-galactosidase (SA-βgal) [28], $p16^{INK4a}$ [39], telomere-associated DNA damage foci [40], senescence-associated heterochromatin foci [41], and several other molecules; all of these markers have some limitations, and thus must be used in combination with each other, as well as proliferation markers (absent in senescent cells) [42].

3 The secretory phenotype of senescent cells

Cellular senescence is accompanied by a striking increase in the secreted levels of >40 factors involved in intercellular signaling [22, 25, 43]. This phenotype has been termed the "senescence-associated secretory phenotype", or SASP [22]. The SASP has many of the paracrine effects one would expect from a pro-inflammatory stimulus, which can be deleterious if left unchecked (see Fig. 2). For example, senescent cells promote the proliferation and tumorigenesis of epithelial cells [18], stimulate angiogenesis [44], trigger an epithelial to mesenchymal transition, accelerate the invasion of transformed cells [22], and increase the growth of xenograft tumors *in vivo* [19]. Further, the SASP has been shown to occur after treatment of cancer patients with DNA damaging chemotherapy [22].

The SASP includes several families of soluble and insoluble factors. These factors can affect surrounding cells by activating various cell surface receptors and corresponding signal transduction pathways that may lead to multiple pathologies, including cancer. SASP factors can globally be divided into the following major categories: soluble signaling factors (interleukins, chemokines, and growth factors), secreted proteases, and secreted insoluble components. SASP proteases can have three major effects: shedding of membrane-associated proteins resulting in soluble versions of membrane-bound receptors, cleavage/degradation of signaling molecules, and degradation or

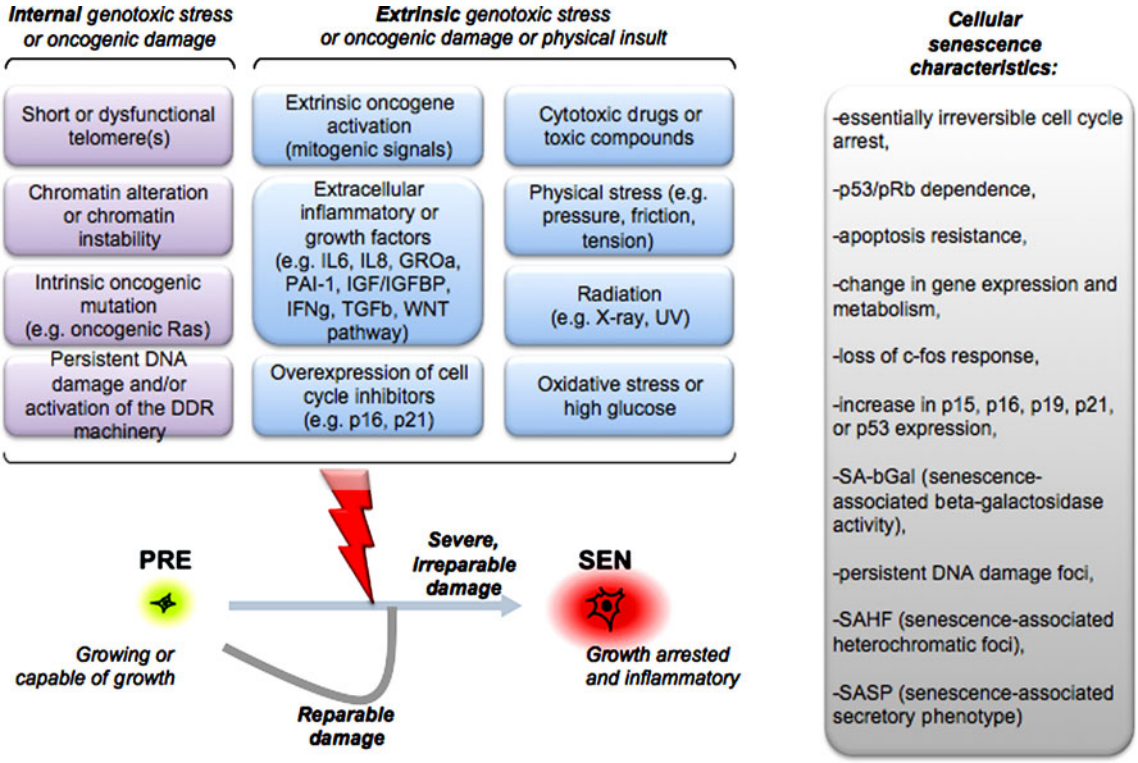

Fig. 1 Stimuli that trigger cellular senescence. DNA damage or mitogenic signals of sufficient magnitude, as well as other stresses, can cause cells to permanently arrest and senesce. Most of these senescence inducers lead to the acquisition of multiple senescence markers (*right panel*), including the senescence secretory phenotype (SASP factors). *PRE* pre-senescent, *SEN* senescent

processing of the extracellular matrix. These activities provide potent mechanisms by which senescent cells can modify the tissue microenvironment. In the following sections, we discuss the SASP subsets and some of their known paracrine effects on nearby cells with an emphasis on their ability to facilitate cancer progression.

3.1 Soluble factors

The most prominent cytokine of the SASP is interleukin (IL)-6, a pleiotropic pro-inflammatory cytokine. IL-6 secretion has been shown to increase markedly after DNA damage- and oncogene-induced senescence of mouse and human keratinocytes, melanocytes, monocytes, fibroblasts, and epithelial cells [22, 45–47]. Another interleukin signaling pathway that is upregulated by senescent cells is IL-1 [48, 49]. Both IL-1α and β are overexpressed and secreted by senescent endothelial cells [50], fibroblasts [51, 52], and chemotherapy-induced senescent epithelial cells [53]. As discussed below, IL-6 and IL-8 expressions depend on the expression and secretion of IL-1α, indicating a hierarchy on how SASP components are regulated.

Most senescent cells overexpress IL-8 (CXCL-8), along with GROα and GROβ (CXCL-1, -2; the murine CXCL-1

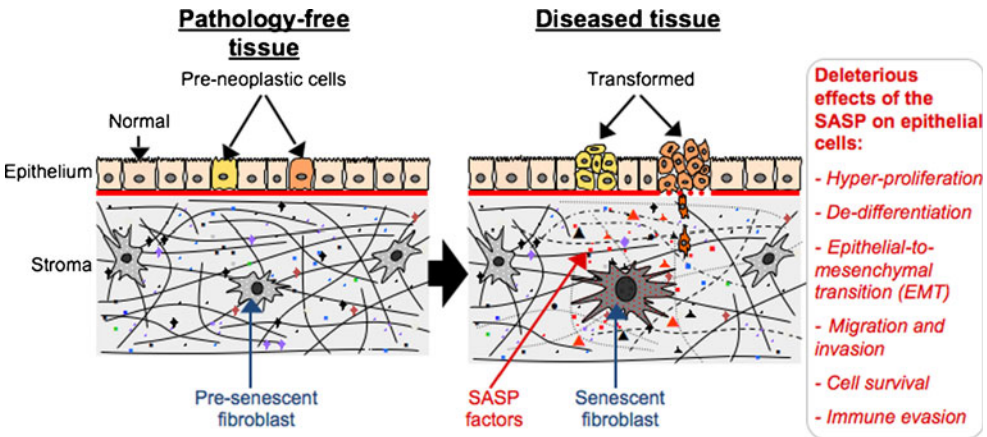

Fig. 2 Pro-tumorigenic paracrine effects of senescent cells. Senescent stromal fibroblasts can promote various facets of cancer progression (*right panel*). Pre-neopastic or transformed epithelial cells are shown in *dark color*; senescent cells cells are represented in *dark gray*. Pre-senescent and senesent fibroblasts secrete SASP factors that can promote cancer progression and aggressiveness

is named KC) [16, 46, 54]. Among CCL family members that are generally upregulated in senescent cells are: MCP-2, -4, -1 (CCL-8, -13, -2), HCC-4 (CCL-16), eotaxin-3 (CCL-26), MIP-3α, and -1α (CCL-20,-3). MCP-3 (CCL-7) is overexpressed by senescent liver stellate cells and by prostate and skin fibroblasts. Fibroblasts induced to senesce by oncogenic RAS secrete high levels of MCP-3, as well as I-309 (CCL-1).

The insulin-like growth factor (IGF)/IGFR network may also contribute to the effect senescent cells exert on their microenvironment. Senescent endothelial, epithelial, and fibroblast cells express high levels of almost all the IGF-binding proteins (IGFBPs) including IGFBP-2, -3, -4, -5, and -6 [22, 55, 56] and their regulators, IGFBP-rP1 and IGFBP-rP2 (connective tissue growth factor—CTGF) [57, 58]. There are additional soluble factors associated with the SASP. For example, inflammatory cytokines such as colony stimulating factors (CSFs including granulocyte-macrophage (GM)-CSF and G-CSF) are secreted at high levels by senescent fibroblasts [22].

3.2 Secreted proteases

Aside from soluble signaling cytokines and growth factors, senescent cells also secrete increased levels of some matrix metalloproteinases (MMPs). The MMP family members that are consistently upregulated in human and mouse fibroblasts undergoing replicative or stress-induced senescence are stromelysin-1 and -2 (respectively, MMP-3 and -10) and collagenase-1 (MMP-1) [19, 21, 59–61]. In some instances, the MMP-1 and -3 produced by senescent cells [62] can also regulate the activity of the soluble factors present in SASP. For example, these MMPs can cleave MCP-1, -2, and -4 and IL-8 [63].

Another family of proteases involved in carcinogenesis and present in the SASP are serine proteases and regulators of the plasminogen activation pathway: urokinase- or tissue-type plasminogen activators (uPA or tPA), the uPA receptor (uPAR), and inhibitors of these serine proteases (PAI-1 and -2) [64].

3.3 Non-protein secretions

Due to senescence-induced changes in cellular metabolism, senescent cells may exert influences on tissue microenvironments due to the secretion of molecules other than proteins. These molecules include reactive oxygen species (ROS) and transported ions. For example, senescent cells have been shown to release nitric oxide and ROS due to alterations in inducible nitric oxide synthase, endothelial nitric oxide synthase, and superoxide-dismutase activities [65–69]. These molecules can enhance cancer cell aggressiveness, as well as promote aging and age-related degeneration [70, 71].

4 Regulation of the inflammatory component of the SASP

Overall, the gene expression profiles (mRNA) of senescent cells determined by transcript analyses resemble the profiles of secreted proteins determined by antibody arrays [22, 23]. This finding suggests that the secretory phenotype of senescent cells is at least in part regulated at the transcriptional level. However, because the changes in gene expression that occurs at senescence are so widespread, the transcriptional control may well be at the level of chromatin organization, rather than changes in specific transcription factors. In fact, dramatic chromatin alterations are known to occur at senescence [72–75]. Support for the idea that gene expression specific to the senescence program may be partially attributed to larger changes in chromatin conformation is suggested by the physical clustering of genes that comprise the SASP [23].

The expression of many SASP components depends on the transcription factors NF-κB and C/EBPβ, which have increased activity at senescence [47, 76] (A. Freund and J. Campisi, unpublished data). Knockdown of C/EBPβ diminishes the expression of both IL-6 and IL-8, which are among the most upregulated cytokines at senescence [47], and NF-κB knockdown significantly decreases the levels of numerous (75%) SASP factors (A. Freund and J. Campisi, unpublished data).

A surprising recent finding was that the DNA damage response (DDR) is required for the increased secretion of a subset of SASP factors, including IL-6 and IL-8 [77]. The DDR is a signal amplification cascade that senses DNA damage, inducing cell cycle arrest and initiating DNA damage repair. If the extent of DNA damage is irreparable, the cell undergoes cellular senescence but maintains a chronic, low level DDR [78]. It is this persistent DDR that is necessary for a robust SASP; depletion of upstream components of the DDR cascade, specifically ATM, NBS1, or CHK2, prevents an increase in secreted protein levels of IL-6, IL-8, and the GRO family, among others.

However, the DDR cannot be the sole regulator of the SASP because the DDR is activated immediately after damage, whereas the SASP, like other aspects of the senescence phenotype such as SA-βgal activity, takes several days to develop. Additionally, a transient DDR—for example, caused by a low level of ionizing radiation that does not induce senescence—does not induce a SASP [77]. Thus, while the DDR is important, it is not sufficient; there must be other, slower events that cooperate with the DDR to induce the SASP. One such event is p38MAPK

activation, which increases slowly after DNA damage, reaching peak levels after several days (A. Freund and J. Campisi, unpublished data).

Like many cytokine networks, the SASP also has an important positive-feedback component. IL-1α, a cytokine that regulates its own synthesis in an autocrine, receptor-mediated, positive-feedback loop via NF-κB [79, 80], is a key positive regulator of IL-6 and IL-8 expression at senescence [81]. IL-1α depletion by RNAi in senescent cells markedly reduced the extracellular protein levels of IL-6 and IL-8. Similar results were achieved using an IL-1 receptor (IL-1R) antagonist or neutralizing antibodies, demonstrating that sustained IL-1R stimulation by surface-bound IL-1α is required to maintain senescence-associated IL-6 and IL-8 extracellular levels [81].

MicroRNAs also play a role in SASP regulation. Thus far, two microRNAs, miR-146a and miR-146b (miR-146a/b), have been demonstrated to negatively regulate the senescence-associated secretion of IL-6 and IL-8 [82]. Senescent human fibroblasts with a strong SASP upregulate these microRNAs late in senescence, tuning down the secretion of inflammatory cytokines. IRAK1 is an established target of miR-146a/b and is indeed targeted in senescent cells, suggesting that these microRNAs downregulate the SASP by reducing NF-κB activity [83]. When miR-146a/b are overexpressed in human fibroblasts, IRAK1 levels decline, along with the enhanced secretion of senescence-associated IL-6/IL-8. In addition, blockage of IL-1 receptor signaling prevented upregulation of miR-146a/b, consistent with these microRNAs being part of an NF-κB feedback loop [83]. These data once again highlight the important role of NF-κB, and place miR146a/b as central players of IL-6 and IL-8 secretion within the SASP.

Although the SASP is at least partly regulated by the activation of transcription factors, the general gene expression profile acquired at senescence may be more attributable to the larger changes in chromatin conformation that senescent cells develop [72–74, 84]. Consistent with this idea is the physical clustering of SASP genes such as matrix metalloproteinases (MMP-1, MMP-3, MMP-10, and MMP-12) as well as CXCL and CCL family members [23]. Moreover, recent work indicates the key role for the High Mobility Group Box 1 protein (HMGB1) in senescent cells.

5 HMGB1 regulates senescence-associated inflammatory cytokine secretion

HMGB1 is a member of the non-histone, chromatin-binding high mobility group family of proteins [85]. Unlike histones, HMGB1 loosely binds the minor grove of DNA, stabilizing nucleosome formation, thereby influencing the expression of certain genes [86–88]. HMGB1 was shown to directly interact with p53, and recombinant HMGB1 protein enhanced p53 DNA binding *in vitro* [89].

The discovery that necrotic cells, but not apoptotic cells, passively release HMGB1 focused recent studies on the role of extracellular HMGB1 [90]. Initial studies suggested that extracellular recombinant HMGB1 promoted secretion of inflammatory cytokines (TNFα, IL-1β, and IL-6) by macrophages [91]. However, later work revealed that recombinant HMGB1 protein alone exhibited only weak activity, but synergized with the ability of bacterial components like lipopolysaccharides to stimulate inflammatory cytokine secretion [92, 93]. Further, HMGB1 can enhance the pro-inflammatory activity of cytokines [94]. These findings appear in conflict with those showing that anti-HMGB1 antibodies or antagonist reduced inflammation in arthritis models [95]. However, the general consensus now is that HMGB1 alone may not promote inflammation, but rather augment the inflammatory response in certain biological contexts.

While it was not observed that cells cultured with recombinant HMGB1 stimulated cytokine secretion, extracellular HMGB1 may enhance of the effect of the SASP (A. Davalos and J. Campisi, unpublished data). Similar to recent data showing that apoptotic cells release "find-me signals", which recruit innate immune to clear the dying cells, senescent cells also secrete HMGB1, known to recruit and activate cells from the innate immune system [96]. This notion is consistent with two studies that suggest senescent cells may undergo clearance *in vivo* [13, 97].

Despite the obvious benefit of recruiting and activating innate immune cells to clear senescence cells, extracellular HMGB1 protein may promote cancer and metastatic progression. Experiments in cell culture showed that HMGB1 stimulated the migration of neuroblastoma cells, which was attenuated by an antibody against HMGB1 [98]. Further, in an *in vitro* angiogenesis assay, HMGB1 stimulated sprouting of endothelial [99]. When immunocompromised mice were injected with rat glioma cells, followed by injection with anti-RAGE (a HMGB1 receptor) or anti-HMGB1 antibodies, both antibodies individually reduced tumor volume, but maximal reduction occurred with both antibodies [100]. Thus, extracellular HMGB1 is able to provoke both beneficial and deleterious biological consequences. It may act to alert and identify senescent cells that require clearance. Failure to clear all senescent cells could leave sites of persistent secretion of inflammatory molecules.

6 Senescent cells create a permissive microenvironment allowing cancer cells to thrive

Inflammation has been shown to initiate or enhance several age-related diseases, including atherosclerosis, Alzheimer's,

osteoarthritis, and cancer [101–103]. Factors secreted by senescent cells can promote tumor development *in vivo* and malignant phenotypes such as proliferation and invasiveness in cell culture models. These effects have been observed with a number of cell types, including cells derived from breast [18, 19, 21, 22, 104], skin [105], prostate [22, 106], pancreas [107], and oro-pharyngial mucosa [108]. The effects of the complex SASP is, of course, dependent on the tissue context. In the following sections, we present the various behavioral changes cells can undergo when residing in the proximity of senescent cells and discuss how the senescent tissue microenvironment can facilitate tumor initiation and progression (see Fig. 2).

6.1 Effects on cell proliferation

One of the most direct, pro-tumorigenic effects of the SASP is to promote the proliferation of epithelial cells. In the case of breast epithelial cells, senescent human fibroblasts can stimulate the growth of pre-malignant and malignant mammary epithelial cells [18, 21, 22]. Irradiated stromal cells, which are presumed to be senescent, have been shown to perturb the mammary epithelial microenvironment and fuel inappropriate epithelial cell growth in the mammary gland [104, 109]. Furthermore, MMPs secreted by senescent fibroblasts have been shown to be responsible for the higher tumorigenicity of breast epithelial cell xenografts in mice, most likely by allowing mitogenic and chemotactic signals greater access to breast cancer cells [19, 104]. In addition to secreted soluble factors, there is evidence that the matrix laid down by senescent cells can also stimulate mammary epithelial cell growth [18].

Fibroblasts from the human prostate gland that undergo senescence in culture have been shown to create a local tissue environment that favors prostate epithelial cell hyperproliferation, in part owing to amphiregulin secretion [20]. Furthermore, senescent fibroblasts increase the expression of CTGF (or IGFBPr2) [58]. CTGF was shown to promote prostate tumor progression in xenografts and is also expressed by the cancer-associated reactive stroma [110]. Moreover, it was recently determined that senescence induced by irradiation in prostate cancer patients was associated with a significantly increased release of exosome-like microvesicles [111]. This novel secretory phenotype was dependent on the activation of p53.

In the skin, unidentified factors secreted by human fibroblasts were shown to be capable of inducing clonal expansion of keratinocytes [112]. In addition, senescent endometrial fibroblasts promoted anchorage-independent epithelial cell growth, due primarily to IL-1 oversecretion [52]. In the oro-bucal cavity, tobacco-driven senescence of supportive stromal cells was shown to stimulate the hyperplastic growth of epithelial cells and was associated with the loss of E-cadherin, ZO-1, and involucrin as well as with the loss of epithelial integrity [108].

Melanocytic naevi (moles) are often composed of senescent melanocytes, which were induced to senesce by oncogenic mutations in BRAF (V600E mutations) [17]. Only rare cell variants in naevi can evolve into melanoma. Malignant melanocytes express high levels of the CXCR2 receptor [113] and can be stimulated to grow by its ligands GROα [114] or IL-8 [115]. Given that both GROα and IL-8 are part of the core SASP, the senescent microenvironment may therefore stimulate the proliferation of rare pre-malignant cells in naevi and thus drive the development of melanoma.

Endothelial cells can undergo proliferation during angiogenesis, which is stimulated by vascular endothelial growth factor (VEGF), IL-8, I-309, and eotaxin [44, 116, 117], all of which are SASP components. Indeed, senescent cells can stimulate endothelial cell migration in culture and angiogenesis *in vivo* in mouse xenografts of breast cancer cells. The blood vessel density was significantly higher when tumors developed in the presence of senescent, but not pre-senescent, fibroblasts [44]. RAS-driven tumors are also known to contain significant numbers of senescent cells [118]. These tumors are also highly vascularized [119]. Many of the SASP factors can also affect leukocyte proliferation during the course of cancer development. For example, IL-7 directly promotes lymphocyte proliferation in peripheral tissues, and GM-CSF stimulates myeloid suppressor cells, which are known to have important immunosuppressive functions that affect cancer progression [120].

6.2 Effects on cell migration and invasion

Senescent cells oversecrete an array of chemokines that can create a gradient to promote cell migration and invasion. In pancreatic cancer, hepatocyte growth factor (HGF), and to a lesser degree bFGF, promote cancer cell invasion in culture and potentially can drive cancer dissemination *in vivo* [107, 121]. In breast cancer, high levels of IL-6 and -8 secreted by senescent fibroblasts are responsible for enhancing the invasiveness of a panel of cancer cell lines in cell culture models [22]. Moreover, the secretion of MMP-2 and -3 by senescent cells can also promote the invasion of multiple epithelial cell types [19, 21, 104, 122, 123]. Other proteases, such as uPA and its regulator (PAI1), are likewise implicated in cancer cell invasion. Senescent stromal cells may promote an epithelial-to-mesenchymal transition (EMT) [22], which is an important phenotypic switch that enables cancer cells to migrate and invade [108]. Thus, senescent cells and the SASP can induce phenotypes in nearby human epithelial

cells that are common during the acquisition of an aggressive phenotype in tumors.

In cell culture models, endothelial cells are induced to migrate by factors secreted by senescent fibroblasts [44]. This is in part due to VEGF secretion and chemokine gradients set up by senescent cells [124]. Neo-angiogenesis, which is dependent on endothelial cell motility and invasion, is enhanced in xenograft models containing senescent fibroblasts [44]. Further, it is known that IL-1, which is a SASP component, activates the endothelium and consequently increases the adhesive potential of cancer cells to vessel walls [125]. Thus, senescent cells might promote extravasation of cancer cells to secondary metastatic sites. However, the effects of senescent cells on angiogenesis might be cell-type dependent. For example, senescent keratinocytes oversecrete maspin, which acts as a dominant inhibitor of endothelial cell migration and invasion [126].

Senescent fibroblasts may promote leukocyte recruitment, since they chronically release chemokines [127]. In p53-deficient RAS-driven tumors induced to senesce by reestablishing p53 function [13], innate immune cells were shown to migrate into the vicinity of the senescent tumor area. CSF-1, CXCL-1, or MCP-1 and ICAM-1 transcripts were found to be higher in these senescent tumor masses and may be responsible for the immune response. For example, neutrophils express CXCR-1, -2, and -4 to sense their microenvironment and invade tissues; eosinophils use the broad spectrum receptor CCR-3 to fulfill their function; monocytes use CCR-1, -2, -5, CXCR-4, and CX_3CR1 to extravasate and enter peripheral sites where they differentiate; natural killer cells express CCR-2, -5, CXCR-4, CX_3CR1, and XCR1; and immature myeloid dendritic cells display CCR-1, -2, -5, -6, and CXCR-4, which facilitate their transport, migration, and function [127–130].

6.3 Effects on cell differentiation

Senescent human and mouse fibroblasts disrupt the differentiation of mammary epithelial cells and inhibit the expression of differentiation markers [21, 104]. This activity is due in large measure to the secretion of MMP-3 by the senescent cells. Furthermore, weakly tumorigenic pancreatic [107] and mammary [22] epithelial cells undergo morphologic changes in culture resembling an EMT in the presence of senescent conditioned medium. The effect on mammary epithelial cells is attributable to IL-6 and IL-8 [22], as well as HGF, uPAR, and MMPs [21], which are all capable of disrupting epithelial cell clusters and stimulating de-differentiation in culture and *in vivo* [131–133].

Strikingly, no angiostatic factors have been reported among SASP constituents (for example, IFNγ, TSP-1, MIG, PF4, IP-10, IL-4, IL-12, and endostatin). This contrasts with the largely pro-angiogenic profile of the SASP (IL-8, MCP-1 and -2, GROs, PGE2, VEGF, EGF, CSFs, uPA/tPA, MMPs, FN, and laminin) [134]. Furthermore, there may be an amplifying activation loop since senescent stromal cells secrete MCPs, CSFs, MIPs, GROs, and CXCLs, which in turn recruit inflammatory and immune cells that also secrete pro-angiogenic factors (VEGFs, IL-8, and MMPs). Thus, senescent cells are well poised to support the differentiation of a new vasculature around and within a progressing tumor.

6.4 Effects on tumor immunology

No anti-inflammatory factors (e.g., IFNα, IFNγ, IL-3, IL-5) are significantly secreted by senescent fibroblasts, and some of these factors are even downregulated upon senescence (e.g., IL-2, IL-12). Nonetheless, some reports show that massive amounts of MCP-1 or IL-8, which are prominent components of the SASP, lead to tumor destruction [135, 136]. Senescent fibroblasts might influence the macrophage balance in the tumor environment. Molecules that are implicated in the recruitment and differentiation of circulating monocytes to tumor sites also happen to be overexpressed by senescent fibroblasts [137]. These molecules can lead to an inadequate immune response within the close proximity of senescent cells. Senescent fibroblasts might affect lymphocytic populations infiltrating the tumor. Specific T-cell populations associated with tumor progression (i.e., Th2 and T-reg) respond to inflammatory cytokines that are commonly present in the SASP of fibroblasts.

7 Conclusions

From the above discussion, the SASP may play an important role in senescence, especially in the days immediately after senescence induction, by helping the growth arrest to occur efficiently and the immune system to target senescent cells for clearance. However, senescent cells appear to accumulate *in vivo* despite clearance, suggesting that either the clearance is not 100% efficient, or the rate of senescence increases with age, outpacing the rate of clearance. Consequently, the SASP's deleterious, chronic inflammatory effects may only become apparent with time. It is therefore important to understand the pathways that regulate the SASP, to determine how it can be modulated and whether the potentially deleterious effects of the SASP can be inhibited without sacrificing the beneficial effects of senescence.

Most insoluble components of the extracellular matrix (ECM) are enzymatic targets of secreted proteases. There-

fore, the senescence-associated changes in proteolytic activities could affect the physical properties of the tissue structure around cells. In particular, the accumulation of senescent cells could lessen the supportive role of the ECM, globally diminishing tissue tension and elasticity. In addition, the relaxed tissue structure and higher levels of MMPs might help tumor cells migrate and invade through the ECM, thus enabling metastasis. In fact, the panel of proteases secreted by senescent cells extensively overlaps with those found in malignant tumors.

Overall, senescence is a molecular program with a unique phenotypic outcome. How its extracellular molecular signature is activated and maintained, and the extent to which it influences the tissue milieu in healthy tissues, aged tissues, and diseased tissues, are some of the many questions that remain unanswered. However, even with our current limited knowledge of the senescent secretory phenotype and its potential effects on carcinogenesis, promising new strategies for cancer therapies emerge. For example, restoring the activity of tumor suppressor genes is an attractive, potentially powerful therapeutic approach. Taking into account our current understanding of the cell's non-autonomous effects of these tumor suppressor genes, small chemicals that can pharmacologically restore their normal function would help reestablish the proper tissue and cell signals, thereby stimulating cancer regression [138–141]. Such approaches would limit inflammation and thus possibly allow proper tissue repair, and they would directly promote the immune-mediated clearance of cells that drive cancer progression.

Chronic inflammation, once established, often takes on a momentum of its own due to the feedback loops of the immune system: cytokines activate leukocytes, which produce more cytokines [142]. Therefore, even a small pro-inflammatory stimulus, such as a population of senescent cells scattered throughout organs and tissues, could be the seed for a more systemic chronic inflammatory response over time, leading to age-related diseases such as cancer.

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