Two-Step Senescence-Focused Cancer Therapies

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Damaged cells at risk of neoplastic transformation can be neutralized by apoptosis or engagement of the senescence program, which induces permanent cell-cycle arrest and a bioactive secretome that is implicated in tumor immunosurveillance. While from an evolutionary perspective senescence is beneficial in that it protects against malignancies, the accumulation of senescent cells in tissues and organs with aging and at sites of various pathologies is largely detrimental. Because induction of senescence in cancer cells is emerging as a therapeutic concept, it will be important to consider these detrimental effects, including tumor-promoting properties that may drive the formation of secondary tumors or cancer relapse. In this review we discuss the complex relationship between senescence and cancer, and highlight important considerations for therapeutics.

Senescent Cells: Modulators of Aging and Cancer

Advanced age is the leading risk factor for numerous chronic diseases including various types of cancer [1]. Although the causes and mechanisms of aging remain poorly understood, senescent cells have emerged as a central contributor to premature and natural aging [2] and to age-related diseases [3–5]. Various studies in mice demonstrate that senescent cells represent a druggable target to extend healthy lifespan and ameliorate various chronic diseases [2–4,6]. These findings have prompted collective interest in the fundamental biology of senescent cells, not only in cell culture but also in tissues and organs across species, with the ultimate goal of identifying molecular vulnerabilities for therapeutic purposes [7] (Box 1).

Rational targeting of senescent cells, particularly in the context of cancer, requires a comprehensive understanding of the molecular and physiological properties of senescent cells, their different phenotypic variations, and their complex association with cancer, which can be both beneficial and detrimental. Acutely generated forms of senescent cells (acute senescent cells; see Glossary), that arise during wound healing or embryogenesis for example, are thought to enhance organismal fitness by inhibiting neoplastic transformation [8] or by recruiting immune cells [9]. However, chronically existing senescent cells during aging and chronic diseases can be deleterious for the organism, for instance by creating a microenvironment that promotes neoplastic growth [10], metastasis [11], or immunosuppression [12]. In the following sections we discuss the various forms of cancer-associated senescent cells in human and mouse tissues as well as their therapeutic implications. We propose that senescent cell removal, senotherapy, is not only a viable therapeutic option for aging and age-related diseases but also for combination, two-stage cancer treatment – pro-senescent chemotherapy followed by senotherapy. This approach could maximize chemotherapy efficiency, preventing cancer relapse and maintaining an anti-tumor tissue microenvironment.

Highlights

Senescent cells are a cell cycle-arrested but highly bioactive cell type. Although the proportion of senescent cells in tissues is relatively low, these cells are causally implicated in aging and in an ever-expanding list of diseases including cancer.

Cancer-associated senescent cells can modulate all stages of tumor development, with their contributions being either detrimental or beneficial towards tumor initiation, growth, metastasis, or cancer relapse.

Although highly context-dependent, the senescence-associated secretory phenotype (SASP) serves many functions in the tumor microenvironment, including mitogenic induction, immune surveillance, or immune deterrence.

A two-step anticancer therapeutic concept, senescence-inducing chemotherapy followed by senotherapy, may represent a viable option to maximize therapeutic efficiency and patient outcome.
Senescent Cell Types Implicated in Cancer

Senescent Neoplastic Cells

Historically, cellular senescence has been described as a tumor-protective mechanism that inhibits the uncontrolled proliferation of cancer-prone cells. Activation of particular oncogenes or the loss of particular tumor-suppressor genes induces the senescence program to establish a durable cell-cycle arrest [8] (Figure 1A, Key Figure). This mechanism is described in a plethora of cellular systems with multiple oncogenes in vitro, as well as in murine tissues, including but not limited to liver (RAS activation [9]), lymphocytes (RAS activation [13]), skin (BRAF activation [14]), thyroid gland (BRAF or RAS activation [11,15]), mammary gland (RAS activation [16]), prostate (Pten or Slek2 loss [17,18]), colon (Csnk1a1 loss [19]), and pituitary gland (Pttg1 loss [20]). Evidence for ‘oncogene-induced senescence’ (OIS) in human primary tumors has also been reported. For instance, melanocytes with oncogenic BRAF mutations undergo senescence and remain benign in melanocyte nevi [21,22]. Likewise, senescence markers have been identified in early-stage prostate tumors [17] as well as colon adenomas [10], astrocytomas [23], and neurofibromas [24].

Inactivation of senescence pathways in mice, for instance through inactivation of the Cdkn2a encoded cell-cycle inhibitors p16INK4a and p19ARF (human p14ARF), leads to early death from tumors [16,25], illustrating why natural selection has favored the senescence program. Furthermore, alteration of CDKN2A in humans, either genetically or epigenetically, is one of the most frequent events in neoplastic lesions [26,27], indicating that disruption of the senescence program is a major event during human tumor development. p16 can also be predictive of tumor subtype because high p16 levels distinguish early-stage small cell lung cancer from lung adenocarcinoma [28,29], and early-stage papillary thyroid microcarcinoma from papillary thyroid carcinoma [30]. Tumor subtypes often show distinct therapeutic response profiles, suggesting that p16 levels could predict therapeutic efficacy [28]. In prostate oropharynx cancer, elevated p16 levels correlate with a superior response to radiation therapy [31]. On the other hand, it must be taken into consideration that p16 levels may increase outside the context of senescence, for example owing to loss of Rb1 [32], another key cell-cycle regulator with frequent loss-of-function mutations in human tumors [26]. Overall, senescent cells are found in both benign and premalignant tumors, suggesting that cellular senescence is an evolutionary cancer-protective mechanism designed to enhance organismal fitness.

Therapy-Induced Senescent Cells

Albeit metabolically active, senescent cells are cell cycle-arrested, and therefore cellular senescence has been viewed as a desirable outcome during cancer treatment (Figure 1B). To this end, senescence-inducing compounds have been developed, including CDK4/6 inhibitors such as abemaciclib, palbociclib, and ribociclib. Because this class of drugs has
apoptosis in antigen-specific T cells while simultaneously suppressing apoptosis in regulatory T cells. **Signal transducer and activator of transcription 3 (STAT3):** a transcription factor that is phosphorylated by Janus kinases (JAKs) in response to cytokines and growth factors, triggering translocation to the nucleus where it acts as a transcriptional activator and mediates cell growth and apoptosis. **SRC homology phosphatase 2 (SHP2):** also known as PTPN11 (tyrosine-protein phosphatase non-receptor type 11), SHP2 is an enzyme and signaling molecule that regulates cell growth, mitotic cell cycle, differentiation, and oncogenic transformation.

**Key Figure**

Cancer-Associated Senescent Cells Affect Tumors in Multiple Ways

**Figure 1.** Acute senescent cells that arise due to oncogene activation (A) (oncogenic RAS for example) or chemotherapy (B) show tumor-suppressing properties, including cell-cycle arrest and SASP production that may promote immuno-surveillance. However, prolonged presence of these cells, in addition to tumor-induced or paracrine senescence in the stroma (C,D) or age-related senescence (E), can promote several hallmarks of cancer. Stromal senescent cells may arise from paracrine signals originating from tumor cells (C) (grey and white secreted factors) or other senescent cells (D) (colored SASP factors). Age-related senescent cells are hypothesized to promote both neoplastic transformation of adjacent cells and proliferation of tumor cells (E). Immunosenescence (F) is a complex process, but largely renders immune cells (especially T cells) unresponsive to activating signals and also promotes a SASP with protumorigenic capacities. Abbreviation: SASP, senescence-associated secretory phenotype.
shown promise in treating several cancers in preclinical and clinical studies [33–35], high-throughput screens have been employed to find additional drug targets that trigger senescence in cancer cells [36]. Studies in mice support the beneficial effects of senescence induction in tumors because this not only leads to tumor stalling but also activates a SASP-mediated immune response (Box 1) that can result in elimination of the senescent tumor cells, as well as neighboring neoplastic cells, ultimately leading to tumor regression [9,37,38].

Conversely, accumulating evidence indicates that senescent tumor cells promote tumor relapse, aggressiveness, and metastasis (Figure 1B). It has been reported that p53-mediated senescence in mammary tumors can hinder chemotherapeutic efficiency and promote rapid cancer relapse, compared to slowly relapsing tumors in p53 mutant mice that fail to arrest but undergo apoptosis due to mitotic catastrophe [39]. Similarly, p16-positive patient tumors are associated with cancer recurrence [40,41]. Strikingly, a recent study shows that therapy-induced senescence is associated with stem cell and self-renewing features, and can promote both cancer initiation and aggressiveness, in several mouse tumor models including B cell lymphoma and T cell acute lymphoblastic leukemia [42]. In addition to cancer recurrence, senescent cells within thyroid tumors have also been linked to invasion, suggesting that cancer metastasis is promoted by senescent cell non-autonomous features [11]. Importantly, while chronic senescent cells induced by radiation therapy or chemotherapeutic drugs contribute to local and systemic inflammation, targeted removal of these cells in transgenic mice attenuates cancer recurrence and detrimental side effects including bone marrow suppression and cardiac dysfunction [6,43]. Therefore, although senescence induction in cancer cells is a viable therapeutic option to reduce initial tumor growth, chronically persisting senescent cells need to be removed to minimize regression risk and avoid deleterious side effects.

### Senescence Induction in Tissue Adjacent to Tumors

The presence of senescent cells within tissues can promote proliferation of neighboring cells, including preneoplastic cells [10]. This property of senescent cells has been well studied in vivo using xenograft models and coinjection of cancer cells and either senescent or non-senescent fibroblasts [12,44–46]. In vitro studies show that senescent cell non-autonomous effects, via secretion of SASP factors (further detailed in a later section), induce growth, angiogenesis, and invasive properties in neighboring cells [10,47,48]. Established tumors or neoplastic cells can also induce cellular senescence in neighboring cells (Figure 1C). Indeed, senescent cells have been identified in the stroma of hepatocellular carcinoma [49] and ovarian cancer [50], and using a p16-luciferase mouse model, one group showed that injection of tumor cells induced senescence in the stroma surrounding tumors [51].

Stromal senescent cells drive tumor growth in several studies, and the gene expression profiles of cancer-associated fibroblasts and senescent cells are similar, suggesting that senescent cells drive neoplastic cell proliferation through similar paracrine mechanisms [52,53]. In fact, increased p16 levels in the stroma surrounding human mammary ductal carcinoma in situ lesions predict disease recurrence independently of other typical histological markers [54]. Recent studies have also shown that senescent cells can promote tumor growth by establishing an immunosuppressive microenvironment via secreting cytokines that recruit myeloid-derived suppressor cells, which inhibit T lymphocyte-mediated targeting of tumor cells [55]. Overall, senescent cells are induced by neighboring neoplastic cells or tumors, and support a protumorigenic microenvironment and increased risk of relapse.

In addition, senescent cells can also potentiate their own effects by inducing senescence in neighboring cells through paracrine mechanisms (bystander effect) via the SASP or gap
junction-mediated cell–cell contact (Figure 1D) [56,57]. Indeed, several studies have demonstrated this effect in vitro using senescent cell conditioned media, and have shown that numerous SASP factors or signaling pathways, including TGFβ1 [58,59], reactive oxygen species (ROS)-activated NF-kB signaling [60], IL-8 and CXCL1 [61], and cGAS–STING signaling [62], can mediate the induction of paracrine senescence. Further, another group showed that short-term exposure of normal cells to SASP from senescent cells induces expression of stem cell markers conferring regenerative capacity; however, prolonged exposure induces senescence [63], suggesting that only short-term exposure may be beneficial. Induction of senescence in neighboring cells has also been demonstrated in vivo, in pituitary stem cell clusters in mouse models of pediatric craniopharyngioma [64] and in ischemic retinal cells in a mouse model of ischemic retinopathy [65]. Senescent cells clusters have also been identified in the thymus of aged mice [66], hepatocytes from mouse livers [56], and intervertebral discs of patients suffering from intervertebral disc degeneration [67]. Together, paracrine senescence induction by neighboring senescent cells represents a mechanism for senescent cells to potentiate their effects, and may amplify negative impacts on cancer (Figure 1D), aging, and other age-related diseases.

Aging-Related Senescent Cells
Aging is a major risk factor for cancer, and most tumors are diagnosed in aged patients [68]. In addition, 5 year survival for many cancer types dramatically declines with age [68,69]. Epidemiological studies show that familial factors correspond to both reduced cancer and longevity, and most genetic and dietary modifications in mice that impact on aging also have an impact on cancer [69,70]. Further, several progeroid syndromes (Hutchinson–Gilford progeria syndrome, Werner syndrome, Bloom syndrome, xeroderma pigmentosum, ataxia telangiectasia, and mosaic variegated aneuploidy syndrome) are also associated with the development of cancer [71].

Although historically cancer aggressiveness has been thought to decrease with age, several tumor types including acute myeloid leukemia and ovarian cancer have a worse prognosis with increasing age [72,73]. Experimental evidence for a relation between aging and cancer from animal models is variable, and appears to be tumor or cell type-dependent [69]. In prostate cancer and melanoma xenograft experiments, no change in growth or faster growth in young mice was observed, respectively [74,75]. However, in these studies 12-month-old mice were used as ‘aged’ mice, but the severity of age-related tissue deterioration or presence of senescent cells at this age may be limited. However, implantation of neoplastic liver epithelial cells into livers of young and old rats resulted in reduced proliferation and more apoptosis in young rats [76]. This suggests that differences between tumor type, cell type, and/or site of implantation may explain the variation in results.

In other genetic approaches, continued senescent cell removal in naturally aging mice (INK-ATTACtransgenic mice) throughout adulthood was found to extend lifespan and delay tumor latency [2], suggesting a detrimental role for age-related senescent cells in tumor progression. This result is further supported by timed somatic p53 deletion in young and old mice, where reduced tumor latency was observed in aged mice [77]. Further, using an inducible conditional mouse model expressing the cell cycle inhibitor p27kip1 to mimic skin aging, other researchers discovered the presence of stromal senescent cells and increased recruitment of suppressive myeloid cells which inhibit tumor immune surveillance and promote tumor formation [12]. Collectively, these studies show that accumulation of senescent cells in tissues with aging promotes tumor formation and growth (Figure 1E), and highlights these cells as optimal therapeutic targets not only for the amelioration of age-related deterioration but also for cancer prevention and treatment.
Cancer and the Aging Immune System

Both the adaptive and innate immune systems are capable of infiltrating and clearing tumor cells. While T cells (CD4+ helper and CD8+ cytotoxic), tumor-associated macrophages, and natural killer (NK) cells prevent tumor growth by targeting antigenic tumor cells, regulatory T cells that secrete immunosuppressive cytokines as well as myeloid and stromal cells suppress T cell responses in lesions that have lost immunogenicity [9,78,79]. Interestingly, these same immune cell types are effective in eliminating senescent cells [9,37,38,80,81]. The immune system undergoes profound changes with aging as reflected by increased susceptibility to infection, autoimmunity, impaired response to vaccination, and cancer development [82,83]. With increasing age, both the ability of the adaptive immune system to mount T cell-mediated responses and the regulation of the innate immune system decline, which may impact on both senescent and tumor cell clearance [84,85].

Interestingly, the accumulation of aged immune cells, referred to as immunosenescence, increases with age in both B and T cell populations (Figure 1F) [86]. We focus here on T cell immunosenescence because T cells function in immunosurveillance of tumors and senescent cells. T cell immunosenescence can be induced by multiple mechanisms including, but not limited to, repeated or chronic T cell stimulation (viruses, pathogens, tumor antigens, or immunogenic self-antigens) and a deregulated inflammatory environment [86,87]. Senescent T cells are nonresponsive to stimulation, but are metabolically active and produce cytokines including IL-6 and TNF-α [86]. Senescent T cells can be protumorigenic through their ability to suppress proliferation of responder T cells [88], but can also modulate macrophage cell fate and contribute to antitumoral functions [89].

One of the hallmarks of cancer is the ability of tumor cells to escape from immune surveillance [90]. Several recent studies have shown that immunosurveillance of tumor and senescent stromal cells is an important tumor protection mechanism. It has been shown that oncoene-induced senescent hepatocytes secret chemokines, which facilitate clearance by the adaptive immune system (CD4+ T cell-mediated), whereas impaired immune surveillance resulted in the development of hepatocellular carcinomas [9]. This suggests that decreased immune surveillance, as observed with age, may drive tumor formation. Indeed, in a mouse model of squamous cell carcinoma, conditional induction of mutant HRAS in keratinocytes resulted in dysplastic changes and 50% tumor incidence in aged mice only, which showed increased cellular senescence in dermal immune cells [91]. By contrast, two studies demonstrated that senescent cells within tumors facilitated NK cell recruitment and tumor elimination, suggesting that senescent cells may provide beneficial immune attraction properties [37,80]. Together, these results suggest that presence of senescent cells may be a benefit or detriment to neoplastic cells/tumors by averting or attracting immune cells.

In addition, the immunosuppressive nature of the tumor microenvironment limits the ability of immune cells to infiltrate and target tumor cells [92]. Senescent cells within tumor stroma, for example, may deter immune cell infiltration and drive tumorigenesis. One study showed that myeloid-derived suppressor cells (MDSCs) promoted an age-related increase in lung cancer growth in mice, and that these cells increase with age in the circulation of humans and in the spleens of mice [93]. Further, in a model of skin aging, senescent stromal cells were sufficient to recruit and increase MDSCs, which inhibit T cell responses and promote tumor growth [12]. Overall, the aging process increases the senescent cell burden and impairs immune function, which in turn escalates senescent cell accumulation and inferior neoplastic surveillance, establishing a protumorigenic environment (Figure 1F).
The SASP and Cancer

Senescent cells restrict and contribute to cancer via both cell-autonomous (restriction of cell proliferation or transformation) and cell non-autonomous mechanisms (SASP) that can result in extracellular matrix remodeling, growth stimulation, or suppression of adjacent cells and signaling to the immune system. Senescence-associated paracrine signaling seems to be context-dependent, with the type of senescence stimulus and cell type having dramatic consequences on the SASP profile [47,94].

The establishment and regulation of the SASP can be orchestrated, at least in vitro, by multiple signaling pathways and transduction networks, including NF-κB signaling [95], the p38 MAPK pathway [96], the cGAS–STING pathway [62,97], inflammosome activation [57], TGF-β signaling [98], JAK–STAT signaling [55], PI3K–AKT–mTOR signaling [99], GATA4 activation [100], and C/EBP-β activation [10] (Figure 2). Which of these signaling pathways and networks are active seems to be dependent on senescent cell maturation [63,98] and origin [42]. Extensive crosstalk among pathways and networks has been observed [99,101]. Each SASP signaling pathway may drive the expression, translation, or protein stability of numerous SASP factors. However, only a few of these factors have been mechanistically linked to physiological events in tissues or diseases, and the mechanistic action of single components is still largely based on studies performed in cultured cells. We describe below select SASP factors to illustrate their context/potential in impacting cancer-associated processes (Figure 2).

IL-1α is an important SASP initiator and is activated in therapy-induced [99,101], oncogene-induced [102], and age-related senescent cells [2]. IL-1α drives autocrine proinflammatory signaling including NF-κB activation and the expression of key cytokines such as IL-6 and IL-8 [99]. IL-1α can act locally as a membrane-bound cytokine that may recruit hematopoietic cells or be cleaved by extracellular proteases and promote systemic inflammation. IL-1α signaling may therefore not only contribute to senescent cell immunosurveillance but also to tissue inflammaging. Studies to determine the role of senescent cell-derived IL-1α in tumor growth have yet to be conducted [103].

IL-6 and IL-8 are two of the most investigated proinflammatory SASP factors, and have been linked to oncogene-induced senescent cells, senescent stroma [10–12,15,104], and murine senescent cells during natural aging, progeria, and disease [2,4,105]. In addition to promoting an inflammatory response and immunosurveillance to control liver tumor progression [80], CXCR2 receptor activation via IL-6 and IL-8 reinforces senescence and cell-cycle arrest through elevated ROS production and activation of the DNA damage response [61,101]. In some instances, however, stromal cell-derived IL-6 can act in immunosuppression [12]. Although functions ascribed to IL-6 and IL-8 such as profibrotic signaling [106] or proproliferative signaling [107] are unexplored in the context of senescence, investigating these characteristics may be of integral importance in the context of cancer and cancer-associated senescence.

Chemokines such as CXCL1/GROα are broadly expressed in several senescence contexts. CXCL1 is not only highly expressed in oncogene-induced senescent cells in vitro and in mice [50,104] but also in human ovarian cancer samples [50]. Secretion of CXCL12 by cancer-inherent, likely oncogene-induced, senescent cells promotes thyroid tumor invasion and metastasis in mice [11], and the cytokine CCL2/MCP-1 has been linked to OIS in the liver and immune surveillance of premalignant hepatocytes [38] or senescent liver tumor cells [37]. However, in the context of established hepatocellular carcinoma, CCL2 among others restricts NK cell function through the recruitment of immunosuppressive myeloid cells, and facilitates the
establishment of advanced disease [38]. On the other hand, CXCL1 can also be secreted by tumor cells and confers paracrine stromal senescence that, in turn, could promote tumor growth [50]. While these studies illustrate that senescent cell-derived chemokines are integral SASP components, their physiological contributions to cancer are of a complex and context-dependent nature.

Growth factors or extracellular vesicles with growth-stimulatory properties are secreted by senescent cells and may contribute to tumor initiation, growth, and angiogenesis [47,108]. Vascular endothelial cell proliferation may be mediated by senescent cells of various origins.
through secretion of pro-angiogenic VEGF, resulting in tumor vascularization [45]. Osteopontin (OPN), a secreted glycoprotein, is highly produced by senescent stromal cells in murine skin papillomas, and coinjection of OPN-deficient senescent cells restricts tumor growth compared to OPN-expressing senescent cells [109]. Conversely, hepatocyte growth factor derived from tumor cells or ascitic fluid of an ovarian cancer-patient can also induce senescence in mesothelial cells, and this may modulate ovarian cancer development and potentially metastasis [110,111].

Matrix metalloproteinases (MMPs), secreted enzymes that process and degrade extracellular matrix (ECM) components, contribute to tissue remodeling and are often released by senescent cells. This class of SASP factors is well described in multiple tissues with age [2,112] and age-related diseases [3,4], as well as in thyroid tumors associated with senescent cells [11]. While destruction of the ECM barrier per se may facilitate tumor growth and cell invasion, growth factors and cytokines that are sequestered by ECM components can also be liberated by MMP activity [113]. Indeed, senescent cell-derived MMPs were shown to support tumor growth [44] and promote VEGF-stimulated tumor vascularization of murine xenografts [114]. Further, stromal cell-derived MMP1 can cleave protease-activated receptors on tumor cells to enable migration and tumor cell invasion [115]; however, whether this mechanism applies to senescent cell-derived MMPs remains to be explored. Therefore, while tumor-inherent senescent cells could render tumor tissue permissive to cancer growth, vascularization, or cell invasion, age-associated senescent cells may render target tissue permissive to metastases.

Overall, the SASP of age-related senescent cells and of senescent cells in established tumors appears to be mostly detrimental because it catalyzes several hallmarks of cancer, and removal of senescent cells during natural aging delays tumor latency [2]. One key feature is the interplay between tumor cells, cancer-associated senescent cells, and the immune system orchestrating immune responses. Although only few studies address this relationship, it is apparent that senescent cell and tumor immune surveillance is complex and often context-dependent. Molecular mechanistic insights into the implicated events, proteins, and kinetics will be necessary to understand and predict therapeutic outcome. Although dissecting the identity and origin of donor and recipient cells during paracrine signaling is technically challenging, recent advances in single-cell sequencing techniques and single-cell proteomics will aid these efforts and address the notion that targeting of senescent cells or their secretome in cancer patients may represent a viable therapeutic option that should be considered as a supplement to chemotherapy.

**Senotherapy as an Anticancer Strategy**

Although the central objective of chemotherapeutic and radiation therapies is to prevent the proliferation of cancer cells through the induction of cellular senescence or cell death [116], the persistence of therapy-induced senescent cells after treatment is detrimental. The use of senotherapy in combination with currently used cancer therapies should be taken into consideration to control this problem [43]. Several cancer types are discussed here which represent suitable candidates for consideration of combination cancer and senotherapies (Table 1).

Indeed, treatment with the CDK4/6 inhibitor palbociclib is initially effective in inhibiting melanoma tumor growth; however, prolonged treatment induced senescence and SASP production in stromal cells, which became tumor-promoting [117]. In addition, inhibition of **SRC homology phosphatase 2** (SHP2) prevented and arrested mammary tumor growth in mice through the induction of senescence; however, the activation of **signal transducer and activator of transcription 3** (STAT3) and SASP secretion suppressed immune surveillance...
### Table 1. Senescence-Associated Cancer Types and Therapeutic Potential

<table>
<thead>
<tr>
<th>Tissue/tumor type</th>
<th>Model</th>
<th>Senescent cell type</th>
<th>Potential senotherapeutic outcome (aspect)</th>
<th>Refs</th>
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<tbody>
<tr>
<td><strong>Brain</strong></td>
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<tr>
<td>Adamantinomatous craniopharyngioma</td>
<td>Mouse, human</td>
<td>Oncogene-induced, age-related</td>
<td>Beneficial (initiation)</td>
<td>[64]</td>
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<td><strong>Breast</strong></td>
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<tr>
<td>Mammary tumors</td>
<td>Mouse</td>
<td>Therapy-induced</td>
<td>Beneficial (recurrence)</td>
<td>[39]</td>
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<tr>
<td>Xenograft (breast cancer)</td>
<td>Mouse, coinjection (tumor and senescent cells)</td>
<td>Therapy-induced</td>
<td>Beneficial (growth)</td>
<td>[44]</td>
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<tr>
<td>Xenograft (mammary epithelial cancer)</td>
<td>Mouse, coinjection (tumor and senescent cells)</td>
<td>Replicative, oncogene-induced, and p16 overexpression</td>
<td>Beneficial (vascularization)</td>
<td>[45]</td>
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<tr>
<td>Mammary ductal carcinoma</td>
<td>Human</td>
<td>Tumor-induced</td>
<td>Beneficial (recurrence)</td>
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<td><strong>Liver</strong></td>
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<td>Hepatocellular carcinoma</td>
<td>Mouse</td>
<td>Oncogene-induced (and others?)</td>
<td>Detrimental, early stages (immunosurveillance)</td>
<td>[9,37,38]</td>
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<td>Beneficial, late stages (immunosurveillance)</td>
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<td>Mouse</td>
<td>Genetic p53 reactivation</td>
<td>Detrimental (immunosurveillance)</td>
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<td>Hepatocellular carcinoma</td>
<td>Human</td>
<td>Age-related or tumor-induced?</td>
<td>Unclear</td>
<td>[49]</td>
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<td>Mouse, tumor cell injection</td>
<td>Age-related</td>
<td>Beneficial (growth)</td>
<td>[76]</td>
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<td>Lung cancer</td>
<td>Mouse, tumor cell injection</td>
<td>Age-related</td>
<td>Beneficial (growth)</td>
<td>[93]</td>
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<td>B cell lymphoma</td>
<td>Mouse, tumor cell injection</td>
<td>Therapy-induced</td>
<td>Beneficial (initiation, growth)</td>
<td>[42]</td>
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<td>T cell acute lymphoblastic leukemia</td>
<td>Mouse, tumor cell injection</td>
<td>Therapy-induced</td>
<td>Beneficial (initiation, growth)</td>
<td>[42]</td>
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<td>Age-related</td>
<td>Beneficial (growth, initiation?)</td>
<td>[2,77]</td>
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<tr>
<td>(age-related cancer in mice)</td>
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<td>Xenograft (breast, pancreatic, endometrial, and lung cancer)</td>
<td>Mouse, tumor cell injection</td>
<td>Tumor-induced</td>
<td>Unclear</td>
<td>[51]</td>
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<td>Xenograft (human epidermal keratinocytes, immortalized mouse mammary epithelial cells, human breast cancer)</td>
<td>Mouse, coinjection (tumor and senescent cells)</td>
<td>Oncogene-induced, replicative</td>
<td>Beneficial (initiation, growth)</td>
<td>[46]</td>
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<td>Tumor-induced</td>
<td>Beneficial (growth)</td>
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<td>Beneficial (recurrence)</td>
<td>[40,41]</td>
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<td>Prostate cancer</td>
<td>Mouse</td>
<td>Pten deletion</td>
<td>Beneficial (immunosurveillance, growth, and chemoresistance)</td>
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<td>Mouse</td>
<td>Tumor-induced</td>
<td>Beneficial (growth)</td>
<td>[109]</td>
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<td>Skin (squamous cell carcinoma)</td>
<td>Mouse</td>
<td>Age-related</td>
<td>Beneficial (initiation, growth?)</td>
<td>[91]</td>
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<td>Skin (squamous cell carcinoma)</td>
<td>Mouse, coinjection (tumor and senescent cells)</td>
<td>Genetic p27 overexpression</td>
<td>Beneficial (immunosurveillance, growth)</td>
<td>[12]</td>
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<td><strong>Thyroid</strong></td>
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<tr>
<td>Papillary thyroid carcinoma</td>
<td>Human</td>
<td>Unclear, potentially oncogene-induced</td>
<td>Beneficial (metastasis)</td>
<td>[11]</td>
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</table>
Further, two mouse studies showed that removal of senescent cells after cancer therapy alleviated their detrimental effects, including reduced bone marrow suppression, cardiac dysfunction, cancer recurrence, and improved physical activity and strength [6,43]. Together, these results underpin the relevance and potential benefit of senotherapy following cancer therapeutics.

Three principle categories can be considered for senotherapy: permanent removal of senescent cells (senolyisis), immune-mediated senescent cell clearance, and SASP neutralization [7]. Although senescent cells have been eliminated without negative consequences during aging and disease [2,4,105], acutely generated senescent cells in adults exhibit some beneficial effects in wound healing [2,119] and tissue regeneration [63]. Senolytic drugs which target the antiapoptotic response in senescent cells, such as signaling through BCL-2 family members (navitoclax/ABT-263 or ABT-737), have proved to be effective in inducing cell death in senescent cells; however, these compounds are unlikely to meet required safety standards owing to the risks of thrombocytopenia and neutropenia [7,120,121]. However, these risks can be minimized by short-term treatment, and potentially by localized delivery, where applicable. Effective targeting of senescent tumor cells has been achieved using inhibition of lysosomal ATPases, thereby exploiting the high metabolic activity of cyclophosphamide- or adriamycin-induced senescent lymphoma cells in mice [122].

Treatment to enhance immune activity could also be harnessed to improve the antitumor activity of senescent cell-recruited immune cells [123]. This could be achieved through the use of ipilimumab, an antibody that enhances activation of cytotoxic CTLA-4 receptor, or with antibodies against the PD1 immune checkpoint, both of which are in clinical use for the treatment of melanoma [124]. SASP modulation may also be employed, and can be achieved by blocking pro-SASP signaling or inhibiting individual SASP components [7]. Blocking pro-SASP signaling may be complex because perturbation of these pathways is tumorigenic in some cases, for example IL-6 is necessary to maintain the senescent cell state [101]. In addition, SASP reduction through NF-κB inhibition in a lymphoma mouse model disrupts immnosurveillance following therapy-induced senescence, and leads to treatment resistance and relapse [95]. Similarly, inhibition of mTORC1, a component of the PI3K–AKT–mTOR pathway, with rapamycin diminishes p53 translation in Pten-deficient senescent cells and promotes murine prostate tumorigenesis [125]. On the other hand, STAT3 inhibition had beneficial effects in alleviating detrimental SASP effects, and resulted in reduced secretion of immunosuppressive cytokines, triggering a strong CD8+ T lymphocyte response and prostate tumor regression [55]. Together, this suggests that inhibition of pro-SASP signaling is pathway-dependent, and further investigation into the efficiency and risk of these strategies is required. Inhibition of selected SASP components can also be beneficial because of reduced off-target effects. Perhaps the most prominent SASP factors, for which approved drugs are available, include IL-1α (IL-1 receptor drug anakinra, currently used for treatment of rheumatoid arthritis), and IL-6 (IL-6 antibody siltuximab, currently used for treatment of Castleman disease; IL-6 receptor inhibitor tocilizumab, currently used for treatment of rheumatoid arthritis) [126–128]. These strategies have not yet been tested in preclinical models of cancer or aging, but represent promising targets for future study. Together, several suitable approaches are available for targeting senescent cells in combination with chemotherapy or in the context of aging to promote effective therapy, minimize relapse, and delay or prevent cancer onset; however, further testing of these strategies in cancer is required.

In addition, careful consideration of the timeline for senotherapy in combination with cancer therapy should be taken into consideration because senescent cells have both beneficial and
detrimental effects on tumor initiation, growth, and relapse in a cell/tumor type-dependent fashion. With current knowledge, incorporation of senotherapy may be beneficial (i) before cancer therapy to increase therapeutic efficacy by removing existing senescent cells, (ii) following cycles of cancer therapy to improve therapeutic outcome, and (iii) after final treatment to reduce risk of recurrence and alleviate the negative impacts of indirect senescence induction during therapy. In all cases, senescent cell removal by senolyis or improving immune targeting would be most efficacious; however, if modulation of particular SASP factors can prove beneficial, with minimal off-target effects, this may also be a viable option. In all instances, however, additional study using preclinical animal models will be necessary to determine the safety and efficiency of these strategies.

Concluding Remarks and Future Directions

Cellular senescence is a feature of cancer that can be induced by multiple mechanisms in and around tumors, and can have both beneficial and detrimental effects on tumor initiation, growth, therapeutic efficacy, and tumor recurrence. However, the features of these different senescent cell types, as well as the mechanisms for their phenotypic impact on neoplastic cells, remain incompletely understood, and in-depth *in vivo* analysis is currently lacking (see Outstanding Questions). Although these studies are technically challenging, it is difficult to translate *in vitro* findings. Further, given the complex and important role of immune surveillance in tumorigenesis and cellular senescence, experimentation in immunocompetent animal models is required. In addition, the role of senescent cells in different tumor types appears to be relatively variable, and furthering our understanding of these differences is an important consideration for both cancer and senotherapy. With current knowledge, it seems that the detrimental effects of senescent cells in cancer appear to outweigh the beneficial effects that are observed in some instances. Nevertheless, increasing our understanding of the differences between the SASP of senescent cells derived from multiple mechanisms, and how these components contribute to immune attraction and deterrence, will be crucial for consideration of combination cancer and senotherapy. Although additional studies will be necessary to determine the safety and efficiency of combination cancer and senotherapy, this concept shows great promise in improving current cancer therapeutics and overall the health and outcomes of cancer patients.

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Outstanding Questions

To what extent can combination cancer therapy and senotherapy be employed to improve therapeutic efficacy, reduce risk of recurrence, and ultimately improve patient outcome?

Can removal of age-related senescent cells in humans reduce cancer risk?

Do different cell/tumor types have different dependencies on senescent cells, in other words more or less beneficial or detrimental roles, within their niche?

What is the mechanism for senescent cell induction of regenerative capacity in neighboring cells with short-term exposure, and can this contribute to the protumorigenic properties of senescent cells?

Which properties of senescent cells determine their role in immune attraction or deterrence, and how can these be differentially mediated in senescent cells induced by similar mechanisms?

Does immune efficiency underlie these differences?

Do beneficial tumor-suppressing senescent cells modulate immunosurveillance differently from detrimental, cancer-promoting senescent cells?

How do senescent cell features and SASP from senescent cells induced by different mechanisms (oncogene-induced, therapy-induced, tumor-induced, age-related, and bystander-induced) differ *in vivo*, and how does this impact the tumor microenvironment and immune surveillance?

Which SASP components are involved in driving growth and bystander senescence in neighboring cells, immune attraction, and immune deterrence in *in vivo*?

How do tumors/neoplastic cells induce senescence in neighboring cells/tumor stroma?


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