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Cell Press
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Review

Senescent Cells in Cancer Therapy: Friends or Foes?

Boshi Wang,[1,2] Jaskaren Kohli,[1,2] and Marco Demaria[1,*]

Several cancer interventions induce DNA damage and promote senescence in cancer and nonmalignant cells. Senescent cells secrete a collection of proinflammatory factors collectively termed the senescence-associated secretory phenotype (SASP). SASP factors are able to potentiate various aspects of tumorigenesis, including proliferation, metastasis, and immunosuppression. Moreover, the accumulation and persistence of therapy-induced senescent cells can promote tissue dysfunction and the early onset of various age-related symptoms in treated cancer patients. Here, we review in detail the mechanisms by which cellular senescence contributes to cancer development and the side effects of cancer therapies. We also review how pharmacological interventions to eliminate senescent cells or inhibit SASP production can mitigate these negative effects and propose therapeutic strategies based on the age of the patient.

Highlights

Cellular senescence is a common outcome of various anticancer interventions.

Senescence-associated secretory phenotypes (SASPs) have pro-tumorigenic functions.

Evidence exists of increased cellular senescence in patients treated for various types of cancer.

Therapy-induced senescence can cause cancer metastasis and relapse and several adverse reactions to cancer treatments.

Pharmacological interference with detrimental senescence might be considered to improve the efficacy of cancer treatments and improve the life quality of treated patients.

Cellular Senescence

Cellular senescence is a multifaceted and highly heterogeneous state characterized by generally irreversible growth arrest, elevated lysosomal activity, resistance to apoptotic stimuli, deregulated metabolism, persistent DNA damage, and elevated secretion of chemokines, cytokines and growth factors [1]. The stability of the cell cycle arrest relies on the cyclin-dependent kinase (CDK) inhibitors p16 and p21 – often regulated by the master tumor suppressor protein p53 – and their elevated expression serves as a marker for senescence detection [2]. Another important feature of virtually all senescent cells is increased activation of senescence-associated (SA)-β-galactosidase, a lysosomal enzyme whose activity is dispensable for the senescence phenotype [3]. Especially in cancer studies, SA-β-galactosidase activity is often used as a unique surrogate marker to define a senescent state. However, SA-β-galactosidase activity can be observed in some non-senescent contexts such as hair follicles and sebaceous glands in the skin [4]. It can also be detected in activated macrophages, including those infiltrating tumors, which may cause difficulties in the precise identification of senescent cancer cells in a tumor microenvironment *in vivo* [5,6]. Recent studies have demonstrated that senescent cells are able to resist proapoptotic stresses via the upregulation of antiapoptotic mechanisms. Specific regulators of this function seem highly cell-type and stress dependent, making their use as markers for senescence challenging [7,8]. On the same line, while metabolism is perturbed in senescent cells, no clear common metabolic mechanism that can be exploited for senescence identification has been demonstrated. A common trigger for senescence induction is the activation of a persistent DNA damage response (DDR), making the DDR markers γ-H2AX and 53BP1 focus staining widely used markers for senescent cells. These proteins are also components of DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS) [9]. Moreover, senescence-associated heterochromatin foci (SAHFs), which are regions of condensed chromatin containing heterochromatin protein 1 and the histone H2A variant macroH2A and HMGA proteins, are observed in human, but not mouse, senescent cells [10]. One of the most variable senescence-associated phenotypes is the SASP, a transcriptional program for genes encoding secreted proteins that is a consequence of persistent DDR [11] and mediated by the NF-κB

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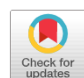

[12], p38 MAPK [13], and C/EBPβ pathways [14]. The SASP is enriched in proinflammatory factors, such as IL-6, IL-8, CXCL1, CCL2, CCL5, and matrix metalloproteinase (MMP) 3 [15], which can be used as markers for DNA damage-induced senescence. The senescence-associated growth arrest is seen as a potent strategy to interfere with cancer progression. Moreover, SASP factors can stimulate immunosurveillance mechanisms and potentiate the tumor suppressive function of senescent cells by guiding the immune system to mount anticancer responses. For this reason, features of senescent cells serve as common biomarkers to evaluate the efficacy of anticancer treatments. While cellular senescence is an oncosuppressive mechanism and a potent anticancer therapeutic strategy (for more information see [16,17]), several issues exist with the generation and persistence of therapy-induced senescent cells. First, the SASP can play detrimental roles by protecting tumors from immune clearance, providing growth factors, and enhancing angiogenesis. Second, because most anticancer treatments are administered via systemic routes, many senescent cells are generated in nontumor areas [18]. This excessive accumulation can have substantial side effects and accelerate the onset and progression of chronic diseases such as cardiovascular, fibrotic, and neurodegenerative diseases, which normally are observed at advanced age [19]. Third, because cancer cells are genomically unstable, they can bypass the senescence growth arrest and restore aggressive and uncontrolled proliferation. This review analyzes these points and discusses the potential strategies to limit the detrimental effects of therapy-induced senescent cells.

Cancer Interventions and Induction of Senescence

A significant number of commonly used cancer interventions have been associated with the induction of cellular senescence in either tumor or nontumor cells and tissues (Table 1, Key Table). These senescence-inducing interventions can be categorized into five families: chemotherapy, radiotherapy, CDK4/6 inhibitors, epigenetic modulators, and immunotherapy.

Chemotherapy

Chemotherapy is defined as the use of cytotoxic compounds to inflict unrepairable DNA damage and impair mitosis in cancer cells. It originated in the 1940s, when nitrogen mustard was used to treat non-Hodgkin's lymphoma; later work found this was due to its ability to alkylate DNA (alkylation is described in more detail later) [20]. Over time, other types of cytotoxic agents have been developed and adopted. Numerous studies demonstrated that chemotherapeutics can induce senescence in both malignant and nonmalignant cells, but in many reports the only evidence of senescence is elevated SA-β-galactosidase activity [21–25]. Few analyses have also measured the level of SASP expression, a phenotype that could provide information on the nonautonomous effect of senescent cancer and noncancer cells.

Topoisomerase inhibitors block topoisomerase enzymes from re-ligating DNA strands after supercoil unwinding, thereby preventing replication [26]. Non-sealed DNA is recognized as a break and activates DDR signaling. Doxorubicin is the most common topoisomerase inhibitor and is used to treat a variety of cancers including lung, breast, lymphoma, and acute lymphocytic leukemia [26]. Doxorubicin is capable of inducing senescence in various cancer cell types *in vitro* (Table 1). Additionally, doxorubicin is able to induce senescence in nonmalignant cells such as vascular smooth muscle cells, prostate stromal cells, and human and mouse dermal fibroblasts as well as tissues of cancer-free mice (Table 1). Etoposide and camptothecin are topoisomerase inhibitors that are also used to treat a range of cancers similar to doxorubicin. These drugs have also been found to induce senescence of colon, breast, ovarian adenocarcinoma, hepatocarcinoma, and osteosarcoma cells *in vitro*. Etoposide also induces senescence in primary fibroblasts (Table 1).

Key Table

Table 1. Summary of Senescence-Inducing Cancer Therapy

Category	Drug	Cell type	Marker	Treatment	Refs
Chemotherapy	Doxorubicin	Fibrosarcoma (HT1080)	(i) Elevated SA-β-galactosidase activities (ii) G1 cell cycle arrest (iii) Increased micronuclei formation	40 nM for 4 days	[38]
		Breast cancer (MCF7)	(i) Elevated SA-β-galactosidase activities (ii) Increased micronuclei formation	50 nM for 2 days	
		Breast cancer (MDA-MB-231)	(i) Increased micronuclei formation	50 nM for 2 days	
		Colon cancer (HCT116)	(i) Elevated SA-β-galactosidase activities (ii) G1 cell cycle arrest (iii) Increased micronuclei formation	50 nM for 4 days	
		Prostate cancer (PC3)	(i) G1 cell cycle arrest (ii) Increased micronuclei formation	100 nM for 4 days	
		Prostate cancer (LNCaP)	(i) Elevated SA-β-galactosidase activities (ii) G1 cell cycle arrest (iii) Increased micronuclei formation	100–500 nM for 4 days	
		Ovarian cancer (A2780)	(i) Elevated SA-β-galactosidase activities (ii) G1 cell cycle arrest (iii) Increased micronuclei formation	20–50 nM for 4 days	
		Glioma (U251)	(i) Elevated SA-β-galactosidase activities (ii) Increased micronuclei formation	200 nM for 4 days	
		Leukemia (K562)	(i) Elevated SA-β-galactosidase activities	50 nM for 4 days	[22]
		Melanoma (SK-MEL-103)	(i) Elevated SA-β-galactosidase activities	1 μM for 7 days	[32]
		Vascular smooth muscle cells	(i) Elevated SA-β-galactosidase activities (ii) Morphology (enlarged) (iii) G1 cell cycle arrest (iv) p53/p21 upregulation (v) p16 upregulation (vi) SASP induction (vii) Increased DDR	100 nM for 24 h	[135]
		Prostate stromal cells (PSC27)	(i) Elevated SA-β-galactosidase activities (ii) Reduced BrdU incorporation	10 μM for 24 h	[136]
		Rat alveolar epithelial cells (L2)	(i) Elevated SA-β-galactosidase activities (ii) p53/p21 upregulation	50 nM for 24 h	[137]
		Human fibroblasts (IMR90)	(i) Elevated SA-β-galactosidase activities (ii) p16 upregulation (iii) SASP induction	100 nM for two times 24 h 24 h drug holiday in between	[138]
		Human fibroblasts (WI38)	(i) Elevated SA-β-galactosidase activities (ii) p53 upregulation	100 or 500 ng/ml for 4 days	[139]
		Mouse dermal fibroblast	(i) Elevated SA-β-galactosidase activities (ii) Reduced EdU incorporation (iii) p21 upregulation (iv) p16 upregulation (v) *LMNB1* reduction (vi) SASP induction (vii) DDR	250 nM for 24 h	[30]
		Reporter p16-3MR mice	(i) p16 upregulation in tissues (ii) Increased bioluminescence (iii) DDR markers (iv) SASP in serum (v) p21 staining in the heart and skin	10 mg/kg once	
	Etoposide	Fibrosarcoma (HT1080)	(i) Elevated SA-β-galactosidase activities (ii) G1 cell cycle arrest (iii) Increased micronuclei formation	900 nM once	[38]

Table 1. (continued)

Category	Drug	Cell type	Marker	Treatment	Refs
		Fibroblasts (IMR90)	(i) Elevated SA-β-galactosidase activities (ii) p53 upregulation	100 μM once	[12]
	Camptothecin	Colon cancer (HCT116)	(i) Elevated SA-β-galactosidase activities (ii) Reduced colony formation capacity (iii) p53/p21 upregulation	20 nM for 96 h	[140]
		Lung cancer (H1299)	(i) Elevated SA-β-galactosidase activities	30 nM for 24 h or 48 h	[23]
	Cisplatin	Nasopharyngeal carcinoma (CNE1)	(i) Elevated SA-β-galactosidase activities	0.5 or 2 μg/ml for 24 or 48 h	[21]
		Fibrosarcoma (HT1080)	(i) Elevated SA-β-galactosidase activities (ii) G1 cell cycle arrest (iii) Increased micronuclei formation	2.2 μM	[38]
		Immortalized skin fibroblasts (hTERT-BJ)	(i) Elevated SA-β-galactosidase activities (ii) p53 upregulation (iii) IL-6 upregulation	100 μM for 24–72 h	[141]
		Reporter p16-3MR mice	(i) Increased bioluminescence (ii) p16 upregulation in skin and lung	2.3 mg/kg for 3 consecutive days	[30]
	Paclitaxel	Breast cancer (MCF7)	(i) Reduced proliferation (ii) Increased DDR (iii) Elevated SA-β-galactosidase activities	0.03, 0.3, 1 μM for 5 days	[29]
		Endothelial cell (HUVEC)	(i) Elevated SA-β-galactosidase activities	2.5 or 5 nM for 24 h	[24]
		Immortalized skin fibroblasts (hTERT-BJ)	(i) Elevated SA-β-galactosidase activities (ii) p53 upregulation (iii) IL-6 upregulation	100 nM for 24 to 72 h	[141]
		Human bone marrow mononuclear cells (hBMNCs)	(i) Reduced proliferation (ii Increased DDR (iii) Elevated SA-β-galactosidase activities	0.03, 0.3, 1 μM for 5 days	[29]
		Mouse dermal fibroblast	(i) Elevated SA-β-galactosidase activities (ii) Reduced population doubling (iii) p16 upregulation (iv) *LMNB1* reduction (v) SASP induction (vi) Increased DDR	50 nM for 24 h or 48 h	[30]
		Reporter p16-3MR mice	(i) Increased bioluminescence (ii) p16 upregulation in skin and lung	10 mg/kg for 3 consecutive days	
	Bleomycin	Lung cancer (A549)	(i) Elevated SA-β-galactosidase activities (ii) Reduced BrdU incorporation (iii) Increased cell size (iv) p53/p21 upregulation	50 μg/ml for 120 h 50 mU/ml for 48 h 0.05–50 μg/ml for 72 h	[33,142,143]
		Mouse lung tissue	(i) Elevated SA-β-galactosidase activities (ii) Upregulated p21 staining (iii) Reduced p-RB staining (iv) Increased DDR (v) Increased SASP	5 U/kg 50 mg/kg 2.5 mg/kg	[33–35]
		Rat alveolar epithelial cell/lung tissue	(i) Elevated SA-β-galactosidase activities (ii) Reduced BrdU incorporation	5 μg/ml for 24 h	[137]
		Human alveolar epithelial cell	(i) Elevated SA-β-galactosidase activities (ii) p21 upregulation (iii) p16 upregulation	0.1–10 μg/ml for 5 days	[33,143]
		Skin fibroblasts (HCA2)	(i) SASP induction	10 μg/ml for 24 h	[85]
		Prostate stromal cells (PSC27)	(i) Elevated SA-β-galactosidase activities (ii) Reduced BrdU incorporation (iii) SASP induction	50 μg/ml for 24 h	[136]
	Temozolomide	Glioma	(i) Elevated SA-β-galactosidase activities (ii) Reduced colony formation capacity (iii) p53/p21 upregulation	10 μg/ml for 24 h 100 μM for 24 h	[144–147]

(continued on next page)

Table 1. (continued)

Category	Drug	Cell type	Marker	Treatment	Refs
		Melanoma	(i) Reduced colony formation capacity (ii) p53/p21 upregulation	25 to 250 μM for 12 days	[148]
		Colorectal cancer	(i) Elevated SA-β-galactosidase activities	100–1000 μM for 48 h	[25]
		Reporter p16-3MR mice	(i) Increased bioluminescence (ii) p16 upregulation in skin and lung	10 mg/kg for 3 consecutive days	[30]
Radiotherapy		Fibrosarcoma (HT1080)	(i) Elevated SA-β-galactosidase activities (ii) G1 cell cycle arrest (iii) Increased micronuclei formation	1300 rad	[38]
		Breast cancer (MCF7)	(i) Elevated SA-β-galactosidase activities	Five times 2 Gy	[149]
		Breast cancer (MCF7 and MDA-231)	(i) Elevated SA-β-galactosidase activities (ii) p53 and p21 upregulation	10 Gy	[40]
		Glioblastoma (U87)	(i) Elevated SA-β-galactosidase activities	2 Gy	[41]
		Endothelial cells (HUVEC and BAEC)	(i) Elevated SA-β-galactosidase activities (ii) Reduced BrdU incorporation	8 Gy	[42]
		Keloid fibroblasts	(i) Enlarged cell size (ii) Elevated SA-β-galactosidase activities (iii) G1 cell cycle arrest (iv) p16, p21 upregulation	2, 4, 8 Gy	[43]
		Fibroblasts (HCA2)	(i) Elevated SA-β-galactosidase activities (ii) SASP induction (iii) Colony formation assay (irreversible)	10 Gy	[44]
		Fibroblasts (IMR90 and WI38)	(i) Elevated SA-β-galactosidase activities (ii) Reduced BrdU incorporation	2.5, 5, 7.5, and 10 Gy	[45]
		Mouse inguinal fat	(i) p16 upregulation in organs (ii) Elevated SA-β-galactosidase activities	10 Gy local irradiation on one leg	[46]
		Fibroblasts from naked mole rats	(i) Elevated SA-β-galactosidase activities (ii) p21 upregulation (iii) Reduced BrdU incorporation	10 Gy or 20 Gy	[47]
		Mouse tibialis anterior muscle	(i) Elevated SA-β-galactosidase activities	10 Gy local irradiation	[48]
		Reporter p16-3MR mice	(i) Increased bioluminescence (ii) p16 upregulation in organs (iii) SASP induction in organs	7 Gy total-body irradiation	[49]
		Mouse bone	(i) p16 upregulation in organs (ii) SASP induction in organs	20 Gy local irradiation	[114]
CDK4/6 inhibitors	Palbociclib	Breast cancer (MCF7)	(i) Elevated SA-β-galactosidase activities (ii) Reduced Proliferation	0.1, 0.3, 1 μM for 5 days	[29]
		Breast cancer (MCF7 and T47D)	(i) Elevated SA-β-galactosidase activities (ii) Reduced Proliferation (reversible)	1 or 5 μM for 6 days 5 μM shows off-target effects	[150]
		Melanoma (MEL10)	(i) Elevated SA-β-galactosidase activities (ii) Upregulated cyclin-D1	0.5 μM for 7 days	[151]
		Melanoma (1205Lu)	(i) G1 growth arrest (FACS) (ii) Reduced p-Rb (iii) Elevated SA-β-galactosidase activities (iv) SASP induction (v) Did not check reversibility	1 μM for 8 days	[69]
		Melanoma (SK-MEL-103)	(i) Elevated SA-β-galactosidase activities	1 μM for 14 days	[32]
		Melanoma (SK-MEL-103)	(i) Elevated SA-β-galactosidase activities (ii) Colony formation assay	1 μM for 7 days	[22]
		Liposarcoma (LS8817, LS141, LS0082)	(i) Elevated SA-β-galactosidase activities (ii) Reduced BrdU incorporation	1 μM for 2 days	[152]

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Table 1. (continued)

Category	Drug	Cell type	Marker	Treatment	Refs
		Liposarcoma (U2OS)	(i) Elevated SA-β-galactosidase activities	1 μM for 7 days	[153]
		Liposarcoma (LS8107)	(i) Elevated SA-β-galactosidase activities	1 μM for 7 days	[154]
		Gastric cancer (AGS and MKN-45)	(i) Elevated SA-β-galactosidase activities (ii) Reduced proliferation	0.5 μM for 2 or 4 days	[155]
		Hepatocellular carcinoma (Huh7 and skHep1)	(i) Elevated SA-β-galactosidase activities (ii) Reduced proliferation (drug on) (iii) Colony formation assay (drug off) (irreversible) (iv) Cyclin D1 upregulation	0.1, 0.5, 1 μM for 10 or 14 days	[156]
		hBMNCs	(i) Reduced proliferation (reversible) (ii) No DDR (iii) No SA-β-galactosidase activities	0.1, 0.3, 1 μM for 5 days	[29]
		Mouse embryonic fibroblast	(i) Elevated SA-β-galactosidase activities (ii) G1 cell cycle arrest (drug on) (iii) No DDR (iv) p16 upregulation (v) p21 upregulation (vi) SASP induction	4 μM for two times 4 days	[65]
		Human fibroblasts (IMR90)	(i) No SASP induction detected	10 μM for 7 days	[66]
	Ribociclib	Neuroblastoma	(i) Elevated SA-β-galactosidase activities	500 nM for 6 days	[55]
		Ewing sarcoma (SKNEP1)	(i) Elevated SA-β-galactosidase activities (ii) Cyclin D3 upregulation	1 μM for 5 days	[59]
	Abemaciclib	Breast cancer (MDA-MB-453)	(i) Elevated SA-β-galactosidase activities	25 nM for 48 h	[60]
		Breast cancer xenograft (MDA-MB-453)	(i) Elevated SA-β-galactosidase activities (ii) Reduced KI67^{+} cells	90 mg/kg daily for 5 days	[61]
		Breast cancer (MDA-MB-453 and BT474)	(i) Elevated SA-β-galactosidase activities (ii) Reduced proliferation (reversible)	250 or 500 nM for 7 days	[61]
		Breast cancer transgenic model (MMTV-rtTA/tetO-HER2)	(i) Elevated SA-β-galactosidase activities (ii) NO SASP induction detected	75–90 mg/kg daily for 12 days	[61]
		Breast cancer (MDA-MB-453)	(i) Elevated SA-β-galactosidase activities	500 nM for 72 h	[62]
		Breast cancer (MCF7)	(i) Elevated SA-β-galactosidase activities	500 nM for 4 doubling time	[63]
		Breast cancer (MCF7)	(i) G1 cell cycle arrest (ii) No SASP induction detected	500 nM for 6 h	[157]
Epigenetic modulators	5-Aza-2′ deoxycytidine	Osteosarcoma (U2OS)	(i) Elevated SA-β-galactosidase activities (ii) p16 upregulation	5 μM or 10 μM	[70]
		Lung mesothelioma (H28)	(i) Elevated SA-β-galactosidase activities (ii) p21/p27 upregulation (iii) Reduced proliferation (iv) DDR	1 μM or 10 μM for 2 days	[71]
		Liver cancer (HepG2, Hep3B, and Huh-7)	(i) Elevated SA-β-galactosidase activities (ii) SASP induction (iii) Reduced proliferation	20 μM for 24 h 12 μM for 72 h	[158,159]
	SAHA	Colon cancer (HCT116)	(i) Elevated SA-β-galactosidase activities (ii) Reduced proliferation (iii) Colony formation assay	2 μM for 24 h	[74]
		Leukemia (MOLM-7, HL-60, and JURL-MK1)	(i) Reduced proliferation (ii) Elevated SA-β-galactosidase activities	0.5–10 μM for 72 h	[75]
		Urothelial carcinoma	(i) Re-plated colony formation assay (ii) G1 cell cycle arrest	2.5 μM for 48 h	[76]

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Table 1. (continued)

Category	Drug	Cell type	Marker	Treatment	Refs
		Glioblastoma stem cell	(i) Elevated SA-β-galactosidase activities	2.5 μM for 7 days	[77]
		Adenoid cystic carcinoma primary cell	(i) Elevated SA-β-galactosidase activities (ii) p16 upregulation (iii) DDR	1 or 1.4 μM for 120 h	[73]
Immunotherapy	Rituximab (CD20-targeting antibody)	Human lymphoma (EHEB, RC-K8, SD1)	(i) Elevated SA-β-galactosidase activities (ii) DDR (iii) SASP induction (iv) p53 and p21 induction	20 μg/ml	[80]

Alkylating agents are reactive compounds that form DNA crosslinks by chemically reacting with atoms in DNA. Crosslinked DNA strands can break during DNA replication when the cell attempts division, resulting in DDR initiation [27]. Cisplatin and temozolomide are typical alkylating agents that can induce cellular senescence (Table 1).

Paclitaxel belongs to a family of microtubule inhibitors used to treat patients with ovarian, breast, and brain cancers and non-small cell lung cancer (NSCLC). The drug interferes with normal microtubule spindle dynamics, which compromises the metaphase–anaphase transition thereby arresting the cell at mitosis [28]. Treatment of MCF7 breast cancer cells [29] and various nonmalignant cell lines such as endothelial cells, bone marrow mononuclear cells, and human and mouse fibroblasts induces senescence [24,29,30]. Paclitaxel also increased bioluminescence in p16 reporter mice, thereby showing that the drug can induce senescence in noncancerous tissues *in vivo* [30] (Table 1).

Bleomycin is commonly used to treat Hodgkin's lymphoma and testicular germ cell tumors. The drug oxidizes DNA nucleotides to induce single- and double-strand breaks [31]. Bleomycin induces senescence in A549 lung cancer cells as shown by increased SA-β-galactosidase and upregulation of p21. Bleomycin is also commonly used to induce pulmonary fibrosis in cancer-free mice [32] and induces senescence in human alveolar cell lines and mouse lung tissue. Importantly, as well as demonstrating senescence by SA-β-galactosidase staining and p16 and p21 upregulation, some of these studies provided evidence of a SASP in lungs from treated mice [33–35] (Table 1). This is important considering that lung damage and pulmonary inflammation are major side effects in human patients [36].

Radiotherapy

Radiotherapy entails the use of high-energy electrically charged ions to inflict irreparable DNA damage in cancer cells with the intent of inducing cell death. Because of its lack of specificity, radiotherapy can be used to treat numerous cancers including lymphoma, soft tissue sarcomas, central nervous system tumors, and a wide range of carcinomas (lung, prostate, skin, breast, head and neck, bladder) [37]. As radiotherapy is administered locally to the site of the cancer, rather than systemically as with chemotherapy, it is less likely that noncancerous tissue areas are damaged. Numerous studies have demonstrated that ionizing radiation (IR) induces SA-β-galactosidase in breast, colon carcinoma, neuroblastoma, and fibrosarcoma cell lines (Table 1) [38–40]. Interestingly, the senescence response to irradiation appears to be dependent on p53 status. Breast cancer cells carrying a missense mutation in the p53 DNA-binding domain undergo apoptosis instead of senescence [40], while glioblastoma cells with a p53 mutation in the DNA-binding domain recover from irradiation and resume proliferation [41]. It is possible that the cell fate decision in response to radiotherapy in p53-mutant tumors depends on the cancer type or on additional mutations affecting survival pathways. Future work should further

characterize what variables influence the response to radiotherapy to select for treatment the patient population most likely to favorably respond to the treatment.

IR is commonly used to prematurely induce senescence in cells *in vitro* and *in vivo*. IR-treated cells show numerous markers of senescence including SA-β-galactosidase, p16, p21, and SASP induction [30,42–48]. Cancer-free mice irradiated with sublethal doses of γ-radiation also show several signs of senescence in various tissues, including p16 upregulation [49–51] (Table 1).

CDK4/6 Inhibitors

CDK4 and CDK6 are critical factors for the cell cycle transition from G1 to S phase [52]. Hyperactivation of the D-type cyclin–CDK4/6 axis in cancer cells has been observed in many preclinical studies, particularly in the context of breast cancer [53,54], making its targeted inhibition an attractive anticancer strategy. Recently, three pharmacological inhibitors (palbociclib, ribociclib, and abemaciclib) have been developed and approved by the FDA. Palbociclib was approved in combination with letrozole for estrogen receptor-positive (ER^+) advanced breast cancer patients in 2015. Two years later, ribociclib was approved in combination with an aromatase inhibitor to treat hormone receptor-positive (HR^+) human epidermal growth factor receptor 2-negative ($HER2^-$) metastatic breast cancer patients. In the same year, the last CDK4/6 inhibitor, abemaciclib, received approval in combination with fulvestrant for the treatment of HR^+HER2^- advanced breast cancer patients who failed to respond to endocrine therapy [55–57]. Because CDK4/6 inhibitors mimic the mechanism of action of p16 [58], induction of cellular senescence has been considered a likely outcome of the treatment. Palbociclib was shown to induce cellular senescence in breast and gastric cancer, melanoma, liposarcoma, and hepatocellular carcinoma, ribociclib in neuroblastoma cells [55] and Ewing sarcoma cells [59], and abemaciclib in various breast cancer cells [60–63] (Table 1). However, senescence induction was mainly evaluated based on SA-β-galactosidase positivity, and successful establishment of an irreversible proliferative arrest was rarely measured. While enhanced SA-β-galactosidase activity remains one of the best senescence-associated features, its use as a sole and unequivocal marker for senescent cells remains debated [1,2]. For example, conditions of increased lysosomal content or activity might also lead to SA-β-galactosidase activity. This has been demonstrated for CDK4/6 inhibitors in SK-Mel-103 melanoma cells and normal cells, where palbociclib and abemaciclib are trapped in the lysosomes and might induce lysosomal stress unrelated to growth arrest [64]. However, long-term treatments with palbociclib and abemaciclib are sufficient to induce senescence even when extra drug washouts to avoid lysosomal accumulation are applied [64]. Additional evidence for palbociclib being able to promote stable growth arrest derives from experiments using either mouse embryonic fibroblast [65] or IMR90 human fibroblasts [64,66]. These data are in line with the notion that prolonged overexpression of p16 (for 7 days) is needed to achieve permanent growth arrest, after which p16 itself becomes dispensable [67]. While the duration of the treatment is possibly an important determinant of cell fate, other variables might contribute to the sensitivity to CDK4/6 inhibitors. For example, palbociclib was also shown to induce reversible growth arrest in bone marrow mononuclear cells but irreversible growth arrest and senescence in MCF7 cells [29] (Table 1), suggesting a cell type-dependent mode of action. Studies on how senescence is induced by CDK4/6 inhibitors should focus on additional molecular and environmental conditions. Breast cancer patients carrying mutations in the coding region of the p53 DNA-binding domain are poor responders to abemaciclib [68]. However, it was shown that palbociclib-induced senescence is independent from the p53 pathway since prolonged treatment with palbociclib promoted G1 arrest in p53-deficient melanoma cells [69]. Nevertheless, the stability of the growth arrest after drug withdrawal, which would suggest successful induction of senescence, was not verified.

Epigenetic Modulators

5-Aza-2′-deoxycytidine (5-aza) is an inhibitor of DNA methyltransferase (DNMT), which can cause global demethylation of CpG-enriched promoters or enhancers [70]. Since the p16^{Ink4a} promoter contains CpG-rich regions, it was postulated that treatment with 5-aza could induce senescence by activating p16. Treatment with 5-aza was shown to induce p16 upregulation and growth arrest in osteosarcoma cells (U2OS) [70] and elevated SA-β-galactosidase activity and increased DDR in lung mesothelioma cells (H28) [71]. Notably, the p53–p21 pathway was also activated by 5-aza treatment in U2OS and MCF7 cells that entered a senescence state [72] (Table 1). Suberoylanilide hydroxamic acid (SAHA), also known as vorinostat, is a histone deacetylase (HDAC) inhibitor that was approved by the US FDA to treat cutaneous T cell lymphoma [73]. SAHA was shown to induce various senescence phenotypes in colon cancer cells (HCT116) [74], and senescence-like growth arrest in human leukemia cell lines (MOLM-7, HL-60, and JURL-MK1) [75] and urothelial carcinoma cells [76]. Interestingly, similar pro-senescence effects of SAHA were also observed for glioblastoma stem cells and adenoid cystic carcinoma primary cells [73,77] (Table 1). Other HDAC inhibitors, such as sodium butyrate and trichostatin-A, were initially tested in normal cells and shown to induce senescence in human normal fibroblasts [78,79].

Immunotherapy

As immunotherapeutic drugs mediate immune-mediated cancer cell apoptosis, it is possible that the SASP could be a modulator of this process. Riuximab is a CD20 targeting antibody used to treat leukemia and lymphoma. Interestingly this drug has been shown to induce senescence in B cell lymphoma lines, as judged by increased SA-β-galactosidase activity, p53/p21 signaling, a DDR response, and, indeed, a SASP [80] (Table 1). Owing to these results, it would be warranted to investigate whether other immunotherapies induce senescence and SASP in other cancer types.

Pro-tumorigenic Effects of Senescent Cells

Inflammatory SASP factors secreted by senescent cells can promote various aspects of tumorigenesis in a nonautonomous manner, including cancer cell proliferation, migration, invasiveness, angiogenesis, and epithelial–mesenchymal transition (EMT) as well as immune-mediated clearance.

The first evidence that the SASP can promote the proliferation and growth of cancer cells came from studying co-cultures of preneoplastic epithelial cell lines with senescent WI38 lung fibroblasts. When cultured together *in vitro*, the proliferation rate of preneoplastic epithelial cells was quicker than co-cultures with non-senescent fibroblasts [81]. Importantly, this pro-growth effect was independent from the senescence-inducing stimulus (H_2O_2, RAS^{V12}, or p14^{ARF} overexpression) but was not observed when senescent fibroblasts were co-cultured with normal epithelial cells [81]. In a xenograft murine model, faster-growing epithelial tumors were observed when co-injected with senescent fibroblasts [81]. IL-6 is a key SASP factor that can promote proliferation in a paracrine manner through binding to the IL-6 receptor and subsequent activation of STAT3 (Figure 1). STAT3 transcribes oncogenes and growth regulators including c-myc, c-Fos, cyclin D1, and mammalian target of rapamycin (mTOR) complex 1 (mTORC1) [82]. Co-injection of breast cancer cells with senescent fibroblasts (induced by γ-irradiation or bleomycin) in nude mice also results in faster-growing tumors [83].

The pro-tumorigenic SASP is also able to positively influence the invasive properties of cancer cells. Breast cancer cells displayed an increased ability to migrate across a porous membrane

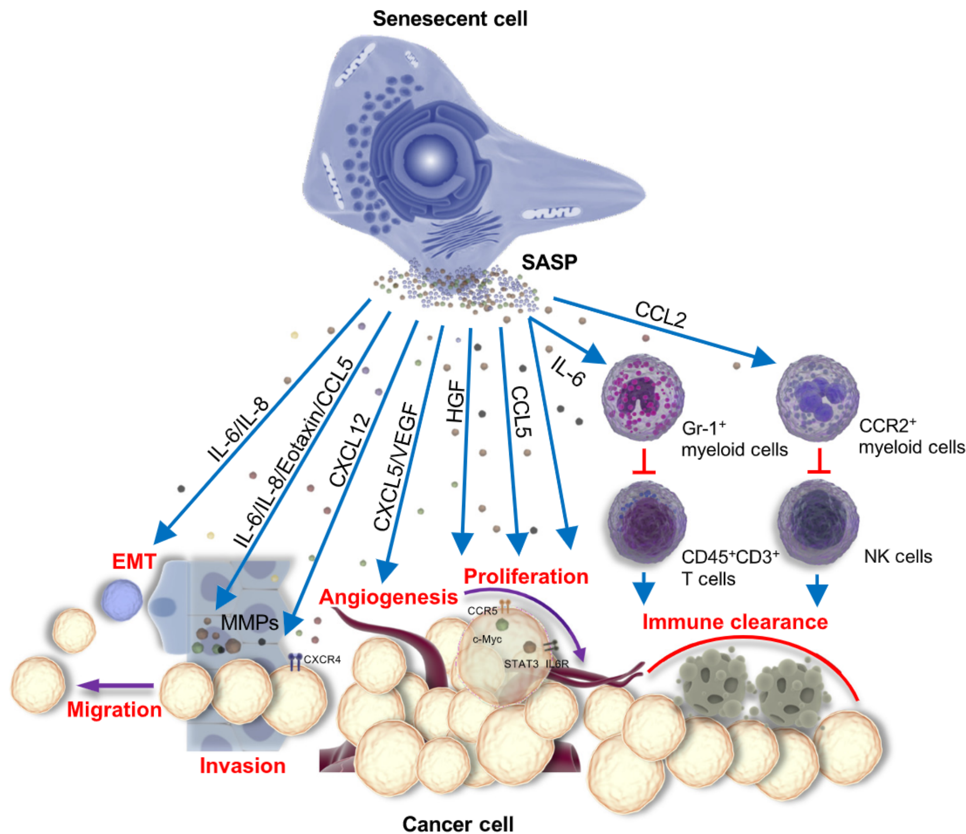

Figure 1. Pro-tumorigenic Effects of Senescent Cells. Senescence-associated secretory phenotype (SASP) factors are engaged in senescent cells through the activation of the NF-κB, C/EBPβ, and p38MAPK pathways. SASP factors contribute to various aspects of cancer progression: IL-6 and IL-8 promote EMT; IL-6–IL-6R–STAT3/CCL5–CCR5–c-Myc/HGF contribute to the proliferation of cancer cells; CXCL5 and VEGF promote angiogenesis; CXCL12–CXCR4/IL-6/IL-8/eotaxin/CCL5/MMPs promote the invasion and migration of cancer cells; and IL-6 suppresses the CD45$^+$CD3$^+$ T cell-mediated immune clearance of cancer cells by activating Gr-1$^+$ myeloid cells. CCL2 suppresses the natural killer (NK) cell-mediated immune clearance of cancer cells by activating CCR2$^+$ myeloid cells. The SASP is a collection of several factors that can act collectively. CCL, (C-C motif) chemokine ligand; CCR, (C-C motif) chemokine receptor; CXCL, (C-X-C motif) chemokine ligand; CXCR, (C-X-C motif) chemokine receptor; EMT, epithelial–mesenchymal transition; HGF, hepatocyte growth factor; IL, interleukin; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor.

when co-cultured with senescent cells [15]. These actions are reported to be modulated by various SASP factors. IL-6 and IL-8 seem to be major players, as invasiveness is rescued when blocking antibodies against these two interleukins are administered, while the addition of recombinant IL-6 or IL-8 promotes the invasiveness of preneoplastic epithelial cells co-cultured with non-senescent fibroblasts [15]. IL-6 and IL-8 are able to mediate the activation of STAT3, which consequently drives the transcription of various MMPs and promote cancer cell invasion [82,84]. For many decades MMPs have been known to positively influence cancer invasiveness through extracellular matrix degradation, allowing dissemination of cancer cells from the primary site [83]. It is also suggested that extracellular matrix degradation increases the permeability of adjacent capillaries, boosting access to mitogens and growth factors, such as hepatocyte growth factor (HGF) [85] (Figure 1).

The SASP also contributes to tumorigenesis by promoting EMT. Treating nonaggressive breast cancer cells with conditioned media from senescent fibroblasts induced various hallmarks of

EMT including reduced β-catenin and E-cadherin and increased vimentin expression, consistent with cells acquiring a mesenchymal phenotype [15]. Interestingly, senescent cancer cells can also promote EMT in non-senescent counterparts. Incubation of proliferating mesothelioma cells with conditioned media from mesothelioma cells induced to senescence by treatment with the chemotherapeutic pemetrexed results in the gain of various EMT markers, including vimentin upregulation [86]. Since STAT3 is a key transcriptional activator of EMT genes, IL-6 and IL-8 are therefore likely to be key SASP factors involved in this process. IL-6 and IL-8 have been shown to induce EMT in cancer cells *in vitro* [87–89] (Figure 1).

Vascular endothelial growth factor (VEGF) is a factor that promotes angiogenesis and provides access to growth factors, eventually favoring malignant growth [90]. VEGF is also part of the SASP [15] and co-injection of senescent fibroblasts with malignant epithelial cells was shown to promote the formation of blood vessels [91] (Figure 1).

Evidence of Senescent Cells in Cancer Patients

Considering the potential detrimental role of cellular senescence in promoting various aspects of tumorigenesis, measurement of senescence burst as a biomarker of toxicity has warranted investigation. Prostate cancer patients treated with the chemotherapeutic mitoxantrone showed increased expression of various senescence-associated genes (*p16*, *p21*) and numerous SASP markers including *IL-6* and *IL-8* [15]. SA-β-galactosidase and $p16^+$ and $p53^+$ cells have been detected in tumor tissues of breast cancer patients treated with cyclophosphamide, doxorubicin, and 5-fluorouracil [17]. An increase in $p16^+CD3^+$ T lymphocytes was also detected in breast cancer patients undergoing doxorubicin treatment [92]. Therefore, chemotherapy induces senescence in both malignant and nonmalignant cells in humans. *VEGF* and *CCL2* plasma concentrations in breast cancer patients were significantly upregulated after treatment commenced. Importantly these markers of senescence remained consistent even 12 months after treatment finished [92], suggesting that these senescent cells persist and are refractory to clearance.

SA-β-galactosidase and $p16^+$ cells are located at the invasive border of untreated human papillary thyroid carcinoma tissues, where they are found to promote cancer cell metastasis [93]. It is unclear where these senescent cells originate from, but since they are positive for $BRAF^{V600E}$ [93] they could be thyrocytes that have undergone oncogene-induced senescence (OIS). These cells may secrete proinflammatory factors, which promote invasion and metastasis of local cancer cells that have bypassed OIS from the gain of subsequent mutations in senescence maintenance genes.

Importantly, the presence of senescent cells in the tumor microenvironment is associated with worse disease-free survival. Human hepatocellular carcinoma (HCC) patients had worse overall survival and shorter recurrence-free survival when their tumors displayed higher expression of genes involved in senescence. This was presumably due to the presence of an immunosuppressive environment [94].

Roles of Senescent Cells in Side Effects of Cancer Therapies

Pro-tumorigenic Functions

While the studies described in previous sections highlight the potential pro-tumorigenic function of senescent cells and the evidence of the presence of senescent cells in treated cancer patients, the use of more sophisticated *in vivo* systems has recently allowed testing of the pathological and clinical relevance of therapy-induced senescence.

In the breast carcinoma mouse model MMTV-Wnt1, doxorubicin induces senescence, as measured by elevated SA-β-galactosidase activity, p21 expression, and expression of the SASP

factors eotaxin, CXCL5 and CCL5. Interestingly, these SASP factors mediate cancer relapse by stimulating the malignant transformation of surrounding non-senescent breast cancer cells [95] (Figure 1): eotaxin promotes cancer cell invasion through CCR3-ERK-mediated MMP3 activation [96]; CXCL5 simulates angiogenesis through activation of VEGF [97] and metastasis via activation of AKT/GSK3β/β-catenin signaling [98]; and CCL5 promotes proliferation through upregulation of cyclin D1 and c-myc and invasion through increased expression of MMP2 and MMP9 [99]. Genetic and pharmacological clearance of doxorubicin-induced senescent nonmalignant cells from an orthotopic model of breast carcinoma reduces cancer growth and limits cancer relapse [30]. Therefore, induction of senescence in nonmalignant and malignant cells may promote tumorigenesis and cancer relapse via the SASP.

Loss-of-function mutations and deletions affecting *PTEN* are a common feature of human prostate cancer cells, accounting for up to 40% of primary tumors [100]. Homozygous inactivation of *PTEN* in mouse prostate epithelial cells promotes the formation of adenocarcinomas *in vivo* [101,102]. Interestingly, docetaxel, a chemotherapeutic agent commonly used for prostate cancer treatment, is unable to efficiently induce senescence in these PTEN-null tumors [101]. A major mechanism explaining this protection is the infiltration of Gr-1^{+} myeloid cells, which secrete interleukin receptor antagonist (IL-1RA) to block IL-1α-mediated signaling and protect prostate cancer cells from the establishment of senescence. Importantly, blocking myeloid cell recruitment via CXCR2 inhibition sensitizes prostate cancer cells to docetaxel-induced senescence [102].

Forced expression of $NRAS^{G12V}$ in hepatocytes led to senescence and the secretion of CCL2, which in turn attracts CCR2^{+} myeloid cells and promotes senescent cell clearance. However, these immature myeloid cells promote the growth of nearby HCC cells by inhibiting NK cell-mediated cytotoxicity [94] (Figure 1). Myeloid infiltration was also found in a $KRAS^{G12V}$ lung adenoma model [102], suggesting that this immunosuppressive mechanism could occur independent of the type of cancer and/or oncogenic mutation.

p27 induction in mesenchymal cells promotes stromal senescence in skin and an immunosuppressive environment, due to recruitment of CD11b^{+} and Gr-1^{+} myeloid cells and a decrease in CD45^{+}CD3^{+} lymphocytes. Myeloid recruitment is partly mediated by IL-6 secretion from senescent stromal cells (Figure 1). Co-injection of isolated senescent fibroblasts with skin carcinoma cells in mice results in a suppressive myeloid cell-mediated increase in tumor growth, and this effect was absent in immunocompromised mice [103]. These data suggest that senescence-inducing interventions might skew the immune system in a tumor-permissive mode and warrant further investigation on the role of immune modulatory drugs in counteracting the tumor-promoting function of senescent cells.

IL-6 has also been reported to promote the metastasis of breast cancer cells to bone sites [104]. Using a conditional mouse model where osteoblasts can be specifically induced to senescence through p27 expression, implanted breast cancer cells metastasized at greater frequencies in bone when p27 was activated. This process was mediated by IL-6 secretion from the senescent osteoblasts, which can promote osteoclast formation and bone resorption in the local microenvironment. IL-6 inhibition prevented osteoclast formation and reduced the frequency of breast metastases. It is suggested that osteoclast-mediated bone resorption provides a greater surface area for breast cancer cells to disseminate [93]. Therapy-induced senescent cells located at sites distal to the tumor could therefore promote local niches for cancer cells to metastasize. Senescent thyroid cells have been proposed to induce the invasion and metastasis of surrounding non-senescent thyroid cancer cells *in vivo*. Orthotopic co-implantation of *BRAFV600E*-induced senescent thyrocytes and thyroid cancer cells resulted in increased lymphovascular

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invasion and metastases in lymph nodes. This process was mediated via a CXCL12/CXCR4 axis [105] (Figure 1). These results suggest that senescence-induced cancer cells could promote the aggressive features of cancer cells that did not respond to therapy.

Recent reports have also proposed that chemotherapy-induced senescent cancer cells acquire stemness-like properties. Doxorubicin treatment in Eμ-Myc transgenic mice results in B cell lymphoma senescence, but also in the upregulation of various stemness genes. Some of these senescent stem-like lymphoma cells were able to re-enter the cell cycle and showed increased aggressiveness, mainly dependent on canonical Wnt signaling [104].

Contribution to Therapy Side Effects

Treatment-related toxicities of cancer patients represent a major clinical issue. In the short term, these toxicities might lead to treatment discontinuation and decrease the efficacy of the intervention. In the long term, adverse reactions and excessive damage by the therapy can cause cancer relapse and additional morbidities, including secondary tumors. This is becoming particularly relevant because of the increased number of cancer survivors [106], who decades after successful anticancer treatment are suffering a multitude of pathologies that develop at an accelerated pace [107]. Additionally, survivors of childhood cancers are much more likely to develop secondary cancers, spinal disorders, and pulmonary diseases in adulthood [108].

Chronic inflammation is a predominant factor that not only influences the outcome of cancer treatment and contributes to cancer progression and cancer relapse, but also promotes potential side effects. Factors such as IL-1A, IL-6, IL-8, CCL2, and CXCL12 are overexpressed after anticancer therapy [109] and have been associated with fatigue, cardiovascular morbidity, physical function decline, and appetite loss [110–112]. Strikingly, these factors are also the major components of the SASP [15], and *in vivo* studies have shown that senescent cells are a major contributor to aging and age-related disease and dysfunctions, many of which are observed in cancer patients [17,92]. Considering that senescence and the SASP are promoted by many anticancer interventions (Table 1), it is logical to speculate that senescent cells might mediate part of the short- and long-term adverse reactions to cancer interventions.

A recent study from our laboratory demonstrated a correlation between detrimental senescent cells/SASP and the healthspan of chemotherapy-treated mice. Removal of chemotherapy-induced systemic senescent cells reduced the burden of inflammatory SASP factors and myelosuppression, thus preventing physical decline, frailty, and cardiac dysfunctions [30]. Additionally, another group showed that inhibition of the p38MAPK pathway in paclitaxel-treated mice improved trabecular bone volume and bone density [113]. This beneficial effect is probably also mediated by inhibition of the SASP, as paclitaxel treatment could induce systemic senescence [30] and the p38MAPK pathway promotes SASP development [13]. This hypothesis was confirmed by another study showing that therapy-induced senescence contributed to bone loss and that clearance of senescent cells rescued bone homeostasis [114] (Figure 2). Total-body γ-irradiation of mice also induces senescence and an upregulated SASP in various tissues [49,51]. Irradiated mice also developed early onset of symptoms associated with frailty including decreased cognition, weight loss, and reduced grip strength [50].

Senotherapy in Cancer

Targeted removal of senescent cells (senolysis) is an emerging strategy in cancer treatments, particularly in combination with more traditional anticancer interventions [115]. ABT-263 (navitoclax) is a pan-BCL2 inhibitor that interferes with senescence-associated antiapoptosis and leads to the elimination of senescent cells [45]. Moreover, ABT-263 was able to kill senescence-like ovarian

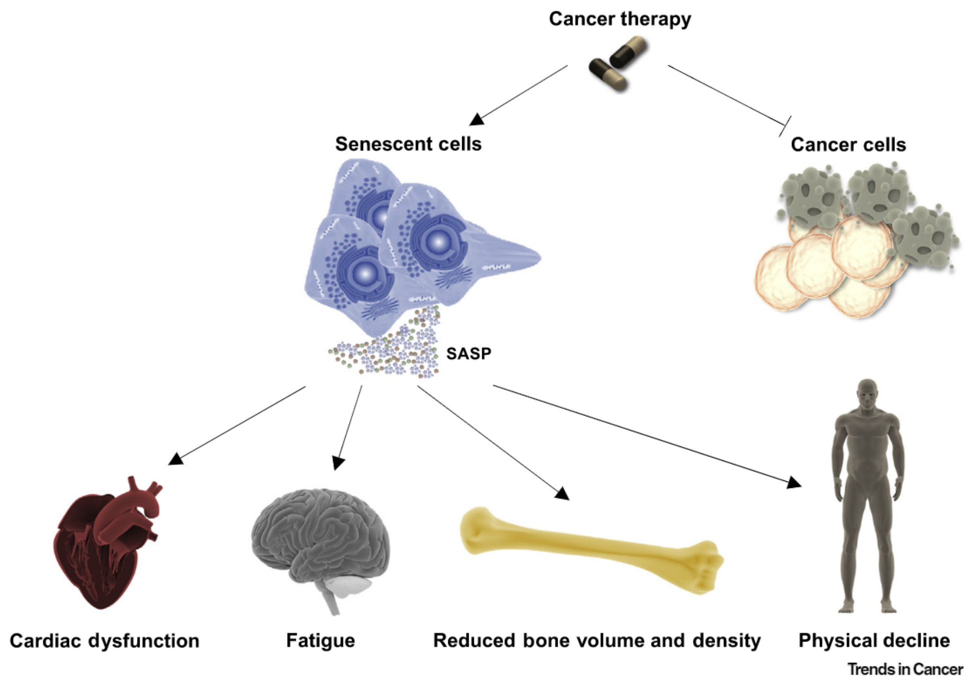

Figure 2. Side Effects Caused by Senescent Cells and the Senescence-Associated Secretory Phenotype (SASP) Induced by Cancer Therapy. Cancer therapy is effective in inhibiting cancer progression but can induce premature cellular senescence in normal or cancer cells. The SASP of senescent cells contributes to many side effects, including cardiac dysfunction, fatigue, bone loss, and physical decline in treated patients.

(OV4453 and OV1946) and breast (MDA-MB-231) cancer cells, in culture and in xenografts, pretreated with the PARP inhibitor olaparib [116]. The bromodomain and extraterminal (BET) protein BRD4 is overexpressed in triple-negative breast cancer (TNBC), which makes the BET inhibitor a potential therapeutic agent [117]. Another pan-BCL2 family inhibitor, obatoclax, showed synergetic effects with the BET inhibitor to kill senescence-like TNBC cells [117].

mTOR is an essential regulator of the SASP [44,118] and mTOR inhibitors have shown senolytic effects in senescent cancer cells. Both concurrent and sequential treatments with chemotherapy (docetaxel) and the mTOR inhibitor temsirolimus (CCI-779) showed advanced antitumor effects in prostate (PC3) and breast (MDA-MB-468) cancer cells and xenografts [119]. A recent report showed that an inhibitor of the DNA-replication kinase CDC7, XL413, selectively induced senescence in liver cancer cells with p53 mutations (Huh7 and MHCC97H), and further administration of the mTOR inhibitor AZD8055 boosted the suppressive effects on tumor growth [120]. Since interfering with the SASP not always achieves senolysis, the lethal effect of mTOR inhibitors seem more dependent on the senescent cell type.

The HDAC inhibitor panobinostat was also shown to promote senolysis of chemotherapy-treated NSCLC and head and neck squamous cell carcinoma (HNSCC) cell lines (A549, H460; UMSCC47, FaDu) [121]. More recently, cardiac glycosides, in particular digoxin, were also characterized as universal senolytics able to kill various types of therapy-induced (bleomycin, gemcitabine, doxorubicin, etoposide, and palbociclib) senescent cancer cells both *in vitro* and *in vivo* [122]. Inhibition of the SASP (senostasis) might also suffice to improve the response to cancer therapies. Rapamycin dramatically reduces the growth of prostate cancer cell xenografts in mice treated with the chemotherapeutic mitoxantrone [44]. Similar results were seen in breast

cancer xenografts when mice were treated with both doxorubicin and the SASP inhibitor metformin [123].

Together with potentiating cancer cell elimination, senotherapies have the potential to improve the healthspan of cancer patients. In a preclinical mouse model, ABT-263 cleared doxorubicin-induced senescent cells in mice bearing MMTV-PyMT breast carcinomas and significantly delayed cancer metastasis and recurrence [30]. Rapamycin and metformin also increased healthspan in middle-aged wild-type mice [124,125]. Administration of SASP inhibitors may therefore alleviate non-tumorigenic side effects associated with senescence-inducing chemotherapy.

Can a Foe Turn into a Friend?

Besides the aforementioned detrimental effects, some of the proinflammatory SASP factors also hold the potential to contribute to the immune clearance of cancer cells. Restoration of p53 functions in lymphomas, sarcomas, and liver carcinomas leads to the features of cellular senescence and tumor regression [126,127], which is mainly mediated by the chronic inflammation and innate immune system triggered by senescent stromal cells [127]. Immune surveillance by $CD4^+$ T cells of premalignant senescent hepatocytes also prevents the development of hepatocellular carcinomas [128], indicating that senescence and its secretory phenotypes provide an extrinsic barrier to malignancy in the early stages of cancer progression. Inactivation of Stat3 in a *Pten*-null prostate cancer model reduces levels of immunosuppressive SASP factors without affecting the expression of SASP chemoattractants, thus favoring immune-mediated reduction of tumor size. Moreover, treatment with a JAK2 inhibitor targeting the Stat3 pathway in combination with docetaxel modulated the chemotherapy-induced SASP and triggered a stronger immune-mediated antitumor response [129]. Recently, it has been shown in a mouse model of pancreatic ductal adenocarcinoma that a combination of MEK and CDK4/6 inhibitors induces cellular senescence, with the SASP favoring vascularization, drug delivery to the tumor site, and sensitivity to immunotherapy [130].

Concluding Remarks and Future Directions

Cancer therapies inflicting high levels of DNA damage are still the primary treatment option for many malignancies, but they can inevitably lead to senescence induction and the secretion of proinflammatory SASP molecules in both malignant and nonmalignant cells. Using various *in vivo* models that genetically mimic human tumors, these SASP factors have been shown to promote various hallmarks of tumorigenesis including proliferation, invasion, immunosuppression, and metastasis. However, these studies are limited to a few cancer types, and whether the findings are representative of most tumors needs to be addressed. This is particularly important because of the heterogeneous phenotypes of the different senescence programs; for example, the dependence of SASP composition on the cell type [131,132]. For instance, in some cases the SASP might have antitumor functions; thus, it remains to critically assess the mechanisms by which senescent cells drive tumorigenesis and determine whether we can identify subsets of senescent cells that might instigate more detrimental functions (see Outstanding Questions).

Inhibiting the production of specific SASP factors via the use of senolytics or senomorphics has been shown to suppress tumorigenesis and to suppress the immunosuppressive environment promoted by senescent cancer cells. Moreover, senolysis might reduce the risk of some (pseudo)senescent cancer cells escaping growth arrest and resuming uncontrolled proliferation. Thus, a ‘one–two punch’ strategy based on sequential pro- and antisenescence interventions might be an effective strategy to treat cancer [120]. However, this approach requires more characterization of the response of senescent cancer cells to the senotherapy. For example,

Outstanding Questions

What are the mechanisms by which senescent cells promote cancer therapy-induced adverse reactions?

Do all senescent cells share the same properties? Are there more dangerous subsets of senescent cells?

Can we pharmacologically interfere with detrimental senescence?

What would be the potential differences between treating young and old patients?

the BCL-2 inhibitors ABT-263 and ABT-737 initiate a response only in senescent cells that upregulate Bcl-xl or Bcl-w [7,8]. If a specific type of senescent cancer cell does not rely on these proteins for survival, they may not be effectively cleared via BCL-2 inhibitors.

Strategies towards senescence-inducing therapies in old and young human cancer patients should be considered carefully (Figure 3). Young adults who have a low baseline of senescence prior to cancer treatment might benefit from adjuvant senotherapy that could reduce the likelihood of tumor relapse and premature aging phenotypes (Figure 3).

By contrast, older patients, who normally have a higher level of senescence pre-cancer treatment [133,134], might benefit from additional neoadjuvant senotherapy. Reducing the burst of senescence could potentially improve healthspan and prepare the patients to better cope with the highly damaging stress deriving from the anticancer interventions. Senolytics or senomorphics would then be given again after the end of the anticancer therapy, to prevent further tumorigenesis from proinflammatory SASP factors (Figure 3). The feasibility of this approach should be tested further using appropriate cancer models with aged mice and

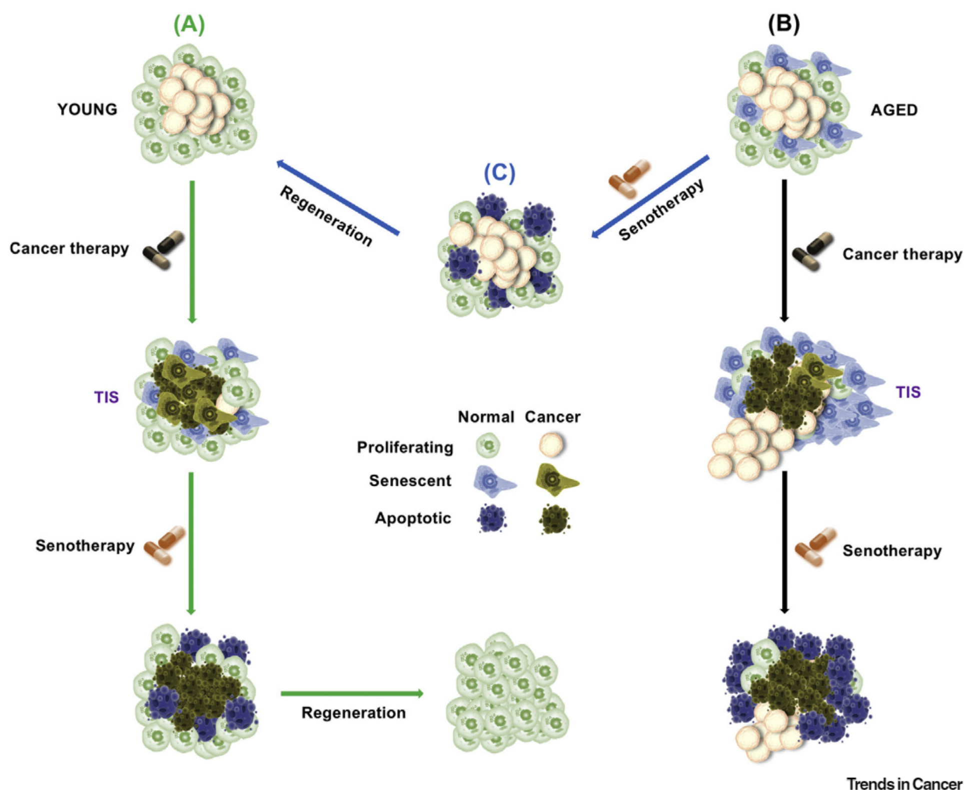

Figure 3. Potential Strategies for Senotherapy-Mediated Cancer Treatment. (A) Cancer therapy in a young tumor microenvironment leads to cancer cell death and therapy-induced senescence (TIS) in cancer cells and with normal stromal cells. Senotherapy selectively kills senescent cancer and normal cells induced by therapy and a better treatment outcome is achieved after regeneration (green-arrow route). (B) Senescent normal stromal cells occur at the highest rates in old tumor microenvironments and contribute to cancer progression during therapy and cancer relapse post-therapy. Senotherapy might not achieve the same level of beneficial effects that is observed in young microenvironments (black-arrow route). (C) Applying senotherapy before cancer therapy might remove detrimental age-associated senescent normal stromal cells. This step might rejuvenate an aged microenvironment and predispose to a better treatment outcome (blue-arrow route–green-arrow route).

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comparing the pro-cancer functions of senescence in young versus old environments (see Outstanding Questions). This is a particularly urgent question, as antisenescence compounds are entering clinical trials and will be tested mainly in older individuals with other pathologies.

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