Potential Use of Senolytics for Pharmacological Targeting of Precancerous Lesions

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ABSTRACT

Senescence is a cell state that contributes to several homeostatic and pathologic processes. In addition to being induced in somatic cells in response to replicative exhaustion (replicative senescence) as part of organismal aging, senescence can also be triggered prematurely by oncogene hyperactivation or tumor suppressor dysfunction [oncogene-induced senescence (OIS)]. Consequently, senescent cells comprise a major component of precancerous lesions of skin, oral mucosa, nasopharynx, prostate, gut, and lung. Unfortunately, invasive (or minimally invasive) interventions are currently the only available approach employed to eradicate premalignant lesions that carry the potential for cancer progression. Senolytics are a newly emerging drug class capable of selectively eliminating senescent cells. Although senolytics have been successfully demonstrated to mitigate a myriad of aging-related pathologies and to cull senescent cancer cells, there is a paucity of evidence for the potential use of senolytics as a novel approach to eliminate oncogene-induced senescent cells. This Emerging Concepts commentary will 1) summarize evidence in established models of OIS including B-Raf–induced nevi, transgenic lung cancer, and pancreatic adenocarcinoma models, as well as evidence from clinical precancerous lesions; 2) suggest that OIS is targetable; and 3) propose the utilization of senolytic agents as a revolutionary means to interfere with the ability of senescent premalignant cells to progress to cancer *in vitro* and *in vivo*. If proven to be effective, senolytics will represent an emerging tool to pharmacologically treat precancerous lesions.

SIGNIFICANCE STATEMENT

The treatment of premalignant lesions is largely based on the utilization of invasive (or minimally invasive) measures. Oncogene-induced senescence (OIS) is one form of senescence that occurs in response to oncogene overexpression in somatic cells and is present in precancerous lesions. Although the contribution of OIS to disease progression is undetermined, recent evidence suggests that senescent cells are permissive for malignant transformation. Accordingly, the pharmacological targeting of oncogene-induced senescent cells could potentially provide a novel, less invasive, means for the treatment of premalignant disease.

Precancerous Lesions: On the Road of Malignant Transformation

Precancerous (premalignant) lesions describe histopathological changes that have increased risk of malignant transformation. Although not all cancer develops from premalignant lesions and may instead form *de novo*, premalignant lesions are frequently detected in tissues amenable to the development of cancer and are often considered a precursor to subsequent malignant tumor formation. The rate of diagnosis of precancerous lesions has increased dramatically due to the availability of reliable cancer screening modalities. For example, ductal carcinoma *in situ* is a precursor of invasive breast carcinoma that is readily identified through routine breast mammography as part of breast cancer screening (Pinder, 2010), whereas prostatic intraepithelial neoplasia (PIN), which is the main precursor lesion to invasive prostate carcinomas,

is identified in 5% of diagnostic fine-needle aspirations of the prostate (Zhou, 2018). Importantly, the risk of precancerous conditions to progress to cancer is variable. For example, whereas uterine leiomyomas or thyroid adenomas have a very limited potential of malignant transformation, lesions such as precancerous skin conditions or colonic adenomas have a relatively higher risk to transform to their respective cancer types (Tanaka, 2009; Lee et al., 2010). Accordingly, these lesions warrant clinical intervention designed to reduce the risk of life-threatening cancer.

The clinical management of precancerous lesions is variable and largely dependent on the type of the lesion and its risk for progression to malignancy. For example, low-grade cervical intraepithelial neoplasia, which has a considerable risk of progressing into cervical carcinoma, is often treated by minimally invasive ablative cryotherapy, thermal ablation, cold knife conization, or large loop electrosurgical excision where hysterectomy is spared for a selected minority of patients (Basu et al., 2018). The treatment of ductal carcinoma *in situ* can be more invasive, involving surgical intervention in the form of breast-conserving therapy (lumpectomy) or mastectomy

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ABBREVIATIONS: AKT, protein kinase B; Bcl-2, B-cell lymphoma-2; MAPK, mitogen-activated protein kinase; OIS, oncogene-induced senescence; SASP, senescence-associated secretory phenotype; SA-β-gal, senescence-associated β-galactosidase.

followed by adjuvant radiotherapy (Fisher et al., 2001). Actinic keratosis, which can progress to squamous carcinoma of the skin, is often treated with photodynamic therapy, cryosurgery and/or chemotherapy (5-fluorouracil) (Eisen et al., 2021); however, dysplastic nevi, which rarely progress to cutaneous melanoma, can be removed by excisional biopsies (Strazzula et al., 2014). Idiopathic leukoplakia, which represents a fundamental premalignant lesion of the oral cavity, is frequently managed with surgical excision, cryotherapy ablation, and CO_2 laser ablation (Jeong et al., 2012). This variability in treatment options can be explained, in part, by the uncertainty of the risk for malignant transformation, since the estimated risk is currently based on mostly epidemiologic evidence with a limited understanding of the molecular processes that facilitate progression to cancer. Subsequently, understanding these cellular and molecular events that drive the transition to malignancy is essential for utilizing state-of-the-art, novel therapeutic options to treat premalignant lesions, reduce their risk of malignant transformation, and possibly replace invasive or minimally invasive interventional therapy with pharmacological treatment.

Senescence in Cancer: Guilty Until Proven Innocent

The Senescent Phenotype. Senescence is a cell stress response that drives proliferating cells into a terminal state (Gorgoulis et al., 2019). The primary feature of cellular senescence is the cessation of replicative activity whereby cells become stably growth-arrested (Sharpless and Sherr, 2015). However, senescence is also characterized by several signature hallmarks that collectively constitute the senescent phenotype (Hernandez-Segura et al., 2018). In addition to growth arrest, senescent cells become enlarged and flattened, exhibit a neuron-like morphology (Cho et al., 2004), and possess a reduced nucleocytoplasmic ratio (Son et al., 2019). Despite maintaining a metabolically active state, senescent cells develop dysregulated energetics and mitochondrial dysfunction and accumulate reactive oxygen species (Kaplon et al., 2013). This is usually accompanied by macromolecular damage to DNA (Von Zglinicki et al., 2005; Rodier et al., 2011), proteins (Ahmed et al., 2010), and lipids (Ogrodnik et al., 2017). Furthermore, senescent cells have enhanced lysosomal biogenesis, which is commonly reflected by the upregulation of the senescence-associated β-galactosidase (SA-β-gal) enzyme (Kurz et al., 2000). Senescent cells undergo broad alterations in the expression of several gene sets, including those involved in cell cycle and cytoskeletal regulation, interferon-related, insulin growth factor–related, mitogen-activated protein kinase (MAPK), and oxidative stress pathways (Fridman and Tainsky, 2008; Casella et al., 2019). Changes in gene expression result in the activation of the highly active secretory function of senescence, whereupon senescent cells secrete a spectrum of soluble and insoluble factors into the microenvironment, collectively representing the functional arm of senescence, termed the senescence-associated secretory phenotype (SASP) (Coppe et al., 2008). Lastly, senescent cells undergo epigenetic changes in the form of heterochromatic condensations, referred to as senescence-associated heterochromatic foci (SAHF) (Kosar et al., 2011).

Oncogene-Induced Senescence Is a Component of Premalignant Lesions. Cells can enter into senescence in response to a variety of exogenous and endogenous stimuli or stresses. Classically, senescence develops as an end-stage cell fate in dividing cells that have reached their maximum number of divisions due to telomere dysfunction (Karlseder et al., 2002). This form of senescence is termed replicative senescence and is an insidious process that occurs as part of organismal aging (Liu et al., 2019). However, senescence can also be induced prematurely in response to stressful stimuli (Fridlyanskaya et al., 2015). For example, somatic cells undergo senescence under oxidative stress, DNA damage (d'Adda di Fagagna et al., 2003), or oncogenic hyperstimulation [oncogene-induced senescence (OIS)] (Galluzzi and Vitale, 2018). Tumor cells, which are fundamentally immortal, can also be forced into senescence by 1) stress elicited by exposure to cancer chemotherapeutics, termed therapy-induced senescence (TIS) (Saleh et al., 2020a), or 2) by interference with oncogene addiction (Wu et al., 2007).

Senescence has a distinct tumor suppressive function, reflected in the arrested replication of somatic cells harboring oncogenic mutations, thereby serving as a defensive barrier against malignant transformation. Consequently, transforming cells undergoing senescence are committed into a stable growth arrest that halts further proliferative progression, contributing to effective tumor suppression (Mooi and Peeper, 2006). This tumor-suppressive function of senescence is further supported by observations indicating that reversal (or evasion) of the senescent growth arrest can accelerate tumorigenesis (Rane et al., 2002; Beausejour et al., 2003; Sarkisian et al., 2007; Carrière et al., 2011), which explains, in part, the accumulation of OIS cells in premalignant lesions of both spontaneous and ectopic (vector-driven oncogene expression) experimental models (Collado et al., 2005). In this context, early evidence of senescent cell accumulation in spontaneous premalignant phenotypes was marked by evident $p16^{INK4a}$ expression (a major cell cycle regulator of the senescent growth arrest) in dysplastic skin and oral mucosal lesions (Natarajan et al., 2003). Subsequently, evidence for senescent cell accumulation has been reported in an array of precancerous processes including human preneoplastic gastrointestinal lesions (Bartkova et al., 2006; Tateishi et al., 2006; Miyasaka et al., 2011), pre-melanoma nevi (Gray-Schopfer et al., 2006), prostatic intraepithelial neoplasia (Majumder et al., 2008), oral leukoplakia (Bascones-Martínez et al., 2012), and premalignant nasopharyngeal epithelium (Tsang et al., 2012). The evidence on the existence of senescence in precancerous lesions is ample, and some examples are summarized in Table 1. In experimental models where oncogene overexpression is induced exogenously, senescent cells are also identified in abundance. For example, senescent cells are found more frequently in a Ras-driven mouse lung adenoma model than when the lung adenocarcinoma lesions have been established spontaneously (Collado et al., 2005; Baek et al., 2013). Similar observations to the accumulation of senescent cells in precancerous lesions were reported in Ras-driven pre-lymphoma murine phenotypes (Braig et al., 2005), murine mammary epithelial hyperplasia (Sarkisian et al., 2007), and murine prelymphomagenic thymocytes (Xu et al., 2008).

The induction of OIS can be demonstrated experimentally by the overexpression of an oncogene in a somatic cell. For

TABLE 1
Evidence on the existence of senescence as a component of precancerous lesions

Precancerous condition	Identified senescence-associated markers	Reference
Actinic keratosis	γH2AX	Hida et al., 2009
Bowen's disease	p27[Kip1]	Oh and Penneys, 2004
Ductal carcinoma in situ	p53, DEC1, and DCR2	Pare et al., 2019
Lichen planus	p21[Cip1]	Bascones-Ilundain et al., 2007
Oral leukoplakia	Rb	Bascones-Martínez et al., 2012
Colonic adenomas	p16[INK4a]	Kriegl et al., 2011
Cervical intraepithelial neoplasia	p15[INK4b], p16[INK4a], and p21[Cip1]	Zhang et al., 2014

This table summarizes examples of experimental evidence demonstrating the expression of senescence-associated biomarkers in human tissue specimens derived from premalignant lesions. Increased expression of SA-β-gal is the canonical marker of the senescent phenotype. Rb, p15[INK4b], p16[INK4a], p21[Kip1], and p21[Cip1] are cell cycle regulators that are often involved in the initiation or maintenance of the senescent growth arrest. p53 is an established player in mediating DNA damage–induced senescence, whereas the phosphorylated form of H2A histone family member X (γH2AX) is a histone modification that reflects the development of DNA double-stranded breaks that often accompany senescence induction. Deleted in esophageal cancer 1 (DEC1) is a basic helix-loop-helix transcription factor that is involved in p53-mediated senescence, and decoy receptor 2 (DCR2) is a novel marker of senescence especially of the renal epithelium. None of these markers is specific to senescence, but all are frequently utilized in the experimental identification of senescence.

example, the vector-mediated ectopic expression of oncogenic Ras (H-Ras V12) results in the promotion of accelerated senescence in human and murine cells *in vitro* (Serrano et al., 1997; Malumbres et al., 2000; Mason et al., 2004; Takaoka et al., 2004). The enduring expression of mutant BRAF (*BRAF*-V600E) in melanocytes induces senescence marked by growth arrest, p16[INK4a] expression, and SA-β-gal upregulation (Michaloglou et al., 2005; Kaplon et al., 2013). Constitutively active protein kinase B (AKT) can also drive primary human endothelial cells into senescence (Miyauchi et al., 2004), as might be predicted given AKT's role in signal transduction downstream of RAS and BRAF. Overexpression of cyclins, such as cyclin E, also promotes OIS (Bartkova et al., 2006). The induction of senescence in response to oncogene overexpression is a consequence of DNA damage resulting from a hyper-replicative state, which is consistent with other forms of senescence in which the activation of the DNA damage repair response is a central event in the development of the phenotype (Di Micco et al., 2006). Interestingly, similar to oncogene hyperactivation, the inactivation of certain tumor suppressor genes can also commit somatic cells into senescence. For example, the loss of phosphatase and tensin homolog function (PTEN) can precipitate p53-dependent OIS that halts further cellular proliferation and interferes with initial malignant transformation *in vivo* (Chen et al., 2005).

Cell-Autonomous and Nonautonomous Pro-Malignant Potential of Senescence. Although the cell-autonomous tumor suppressive potential of senescence is established, the overall contribution of senescence to tumor development is double-edged. Despite the pronounced and durable growth arrest, senescent cells engage in an active secretory function through the SASP, which enables senescent cells to interact with both senescent and nonsenescent neighboring cells. Importantly, the cells impacted by the SASP can be nonmalignant (such as fibroblasts and immune cells), premalignant, or malignant. Components of the SASP can elicit differing effects on the surrounding environment, and the role of the SASP in its entirety as either a pro- or antitumorigenic signal strongly depends upon its composition (Faget et al., 2019). For example, in a model of KRAS[G12D]-induced OIS in pancreatic cells, the secretion of Activin A as a component of the OIS-associated SASP interfered with the growth of preneoplastic lesions (Zhao et al., 2020); in dramatic contrast, inhibition of Activin A binding or knockout of the Activin A receptor promoted growth of preneoplastic lesions and disease progression (Zhao et al., 2020). This enhanced growth after

Activin A receptor knockout correlated with a loss of the senescent phenotype, suggesting that Activin A secreted by senescent cells contributes to delayed disease progression, at least in part, by reinforcing the senescent growth arrest throughout the lesion. In contrast, replicative- or stress-induced senescent fibroblasts have been shown to *promote* growth of co-cultured transformed human keratinocytes via secretion of osteopontin (Pazolli et al., 2009). Furthermore, the SASP can promote epithelial-to-mesenchymal transition, invasion, plasticity, and proliferation of nonsenescent tumor cells (Ortiz-Montero et al., 2017; Ritschka et al., 2017; Alimirah et al., 2020). These less-desirable effects of the SASP on the non-senescent cells within both premalignant and malignant tumor cell populations support the hypothesis that senescence induction favors malignant transformation of neighboring cells, rather than malignant transformation and outgrowth of senescent cells themselves. In this respect, increased senescent tumor burden and the resulting SASP were shown to elevate p38 MAPK and MAPK/extracellular signal-regulated kinase signaling , contributing to the formation of squamous cell skin carcinomas (Alimirah et al., 2020). The authors from this work concluded that senescent cells are therefore "tumor promoters" that drive progression via paracrine interactions with nonsenescent neighbors, and not tumor "initiators." Although we agree that the SASP itself likely drives tumor promotion from neighboring, nonsenescent cells, we maintain that there exists a distinct potential for OIS cells to serve as "initiators" as well, should they be capable of escaping the senescence-associated growth arrest.

This assertion is based on evidence showing that, although the cell-autonomous tumor suppressive function of senescence is largely a function of the stability of the senescent growth arrest, the senescent growth arrest is not always inescapable (Chakradeo et al., 2016). Escape from senescent growth arrest has perhaps been best demonstrated in models of TIS, where a subpopulation of senescent tumor cells can recover proliferative capacity (Sabisz and Skladanowski, 2009; Wang et al., 2013; Saleh et al., 2019) and, more strikingly, allow for the evolution of more aggressive tumor phenotypes (Yang et al., 2017; Milanovic et al., 2018). However, the evasion of the stable growth arrest is not limited to senescent tumor cells and has been reported in other models of senescence. For example, short hairpin RNA–mediated suppression of *TP53* expression is sufficient to reverse the senescent growth arrest of murine fibroblasts after telomere dysfunction (Dirac and Bernards, 2003). Further disabling of

p53 or p16^INK4a, which play important roles in mediating the stability of the senescent cell cycle arrest, allows senescent human fibroblasts, induced by replicative exhaustion, to resume proliferation (Beausejour et al., 2003). In the case of OIS, proliferative recovery is likely permissive for progressive malignant transformation, as somatic cells harboring oncogenic mutations that were forced into senescence can resume growth with increased risk of acquiring additional carcinogenic mutations (de Carné Trécesson et al., 2011). This premise is supported by the fact that the loss of p16^INK4a function is an early step during tumorigenesis of many malignancies (Liggett and Sidransky, 1998), whereas inactivation of p53 in premalignant cells accelerates the development of cancerous lesions through evasion of senescence (Harajly et al., 2015). Moreover, the loss of Rb function in Ras-induced senescent pancreatic cells results in an increased rate of cystic neoplasms formation and accelerated progression towards pancreatic adenocarcinoma (Carrière et al., 2011). Loss of Rb function has also been shown to facilitate the transformation of oncogene-induced senescent astrocytes into malignant gliomas (Seoane et al., 2008). Another relevant example involves the induction of p27^Kip1-dependent OIS in an AKT1-induced prostatic intraepithelial neoplasia model, where the genetic suppression of p27^Kip1 function facilitates the escape from senescence and progression into prostatic carcinoma (Majumder et al., 2008). Although the expectation is that the majority of oncogene-induced senescent cells will remain in a terminal growth arrested state, the natural acquisition of additional mutations that produce critical dysfunction of senescence-associated effector proteins might lead to the slippage of a premalignant cell into a replicative phase. Accordingly, OIS cells might represent dormant foci that harbor the potential to erupt into cancer progenitor cells.

Altogether, the evidence that senescence is a component of precancerous lesions, that senescent cells contribute to a pro-tumorigenic microenvironment through the SASP, and that the tumor-suppressive senescent growth arrest can be reversed, support the premise that the elimination of senescent cells could serve as a strategy for preventing premalignant lesions from developing into actual cancers (Galluzzi and Vitale, 2018). In fact, this exact proposition was put forward after the first report by Serrano et al. (1997) that identified OIS. However, it remained largely hypothetical due to the lack of effective and selective pharmacological agents that can target senescent cells.

Senolytic Removal of Oncogene-Induced Senescent Cells: New Approach to Prevent Cancer?

Recently, a number of compounds have been identified to exert a "senolytic" effect, i.e., the ability to selectively eliminate senescent cells (Zhu et al., 2015). The primary distinguishing feature of senolytics is their potential to induce cell death, primarily apoptosis, in senescent cells while sparing proliferating counterparts (Chang et al., 2016). Senolysis can be accomplished through the targeting of multiple, different pro-survival pathways in senescent cells (Zhu et al., 2015). The ability of senolytic agents to eliminate senescent cells *in vitro* has been robustly demonstrated in several models of

senescence including somatic cells undergoing replicative exhaustion (Chang et al., 2016) and tumor cells exposed to anticancer therapy (Saleh et al., 2020b). Functionally, the senolytic removal of senescent cells resulted in the amelioration of several aging-related processes in mouse models of tau-dependent neurodegenerative disease (Bussian et al., 2018), Alzheimer's disease (Musi et al., 2018; Zhang et al., 2019), insulin resistance (Aguayo-Mazzucato et al., 2019), osteoarthritis (Sessions et al., 2019; Yang et al., 2020), and aging-associated hepatic steatosis (Ogrodnik et al., 2017). Moreover, senolytics have improved the outcome of other disease models where the accumulation of senescent cells appears to contribute to their pathogenesis such as myocardial infarction and ischemia-reperfusion injury (Walaszczyk et al., 2019; Dookun et al., 2020), chronic kidney disease (Nath et al., 2018), pulmonary fibrosis (Pan et al., 2017), and bone degeneration (Kim et al., 2017; Yao et al., 2020). Furthermore, senolytics are being investigated in several ongoing clinical trials for their ability to produce symptomatic relief or slow down the progression of chronic kidney disease, pulmonary fibrosis, and Alzheimer's disease (NCT02848131, NCT02874989, NCT04210986).

Although OIS models are commonly used to investigate senescence-related biology, the majority of studies evaluating senolytics have thus far been focused on alternative senescence-inducing stimuli, such as radiation or chemotherapy (Shahbandi et al., 2020; Carpenter et al., 2021). However, there is still strong, albeit limited, evidence supporting the ability of certain senolytics to selectively kill oncogene-induced senescent cells (Table 2). For example, human WI-38 fibroblasts induced into senescence by ectopic expression of oncogenic H-Ras were selectively eliminated in culture by the BH3 mimetic navitoclax (ABT-263) (Chang et al., 2016). Similarly, navitoclax successfully exerted senolytic effect in the targeting of the transgenic, KIAA1549:*BRAF* fusion–driven, pilocytic astrocytoma DKFZ-BT66 cell model (Selt et al., 2017; Buhl et al., 2019). This evidence supports the utility of ABT-263 in exerting a universal senolytic activity against senescent cells induced by different stimuli (Carpenter et al., 2021). Other agents that have demonstrated senolytic activity against OIS cells include the natural product piperlongumine (Wang et al., 2016) and the cardiac glycoside ouabain (Guerrero et al., 2019), but not the dasatinib plus quercetin (D+Q) combination (Buhl et al., 2019).

Ouabain is capable of eliminating senescent hepatocytes induced by a transposon-mediated transfer of oncogenic N-Ras (Guerrero et al., 2019). In C.B17 SCID/beige mice engineered to overexpress N-Ras, ouabain treatment reduced the number of N-Ras–positive, SA-β-gal–positive senescent hepatocytes *in vivo*. Guerrero et al. (2019) have extended the utilization of ouabain to a mouse model of adamantinomatous craniopharyngioma, a rare but relevant pediatric pituitary tumor (Gonzalez-Meljem et al., 2017). In this model, expression of oncogenic β-catenin in Sox2+ pituitary stem cells and Hesx1+ embryonic precursor cells results in senescence induction coupled with a robust paracrine activity that drives the development of adamantinomatous craniopharyngioma supporting the pro-tumorigenic contribution of the SASP (Gonzalez-Meljem et al., 2017). β-catenin–positive Hesx1+ embryonic precursor cell clusters were dissected, extracted, and cultured *ex vivo*, and β-catenin–driven senescence in Hesx1+ embryonic precursor cells was confirmed by their

TABLE 2
Senolytic agents capable of eliminating OIS

OIS model	Senolytic	Reference
In vitro: H-Ras–induced WI-38 fibroblasts	Navitoclax (ABT-263)	Chang et al., 2016
In vitro: KIAA1549:*BRAF* fusion–driven pilocytic astrocytoma DKFZ-BT66 cells	Navitoclax (ABT-263), ABT-737	Buhl et al., 2019
In vitro: Ras-induced WI-38 fibroblasts	Piperlongumine	Wang et al., 2016
In vitro: N-Ras^{G12V}–induced murine hepatocytes	Ouabain	Guerrero et al., 2019
In vivo: C.B17 SCID/beige mice	Ouabain	Guerrero et al., 2019
Ex vivo: β-catenin–positive Hesx1+ embryonic precursor cells	Ouabain, ABT-737	Guerrero et al., 2019
In vivo: Ras-induced IMR90 lung fibroblasts	Navitoclax (ABT-263)	Guerrero et al., 2019
In vivo: K-Ras–induced pancreatic premalignant senescence in transgenic mice	ABT-737	Kolodkin-Gal et al., 2021
In vitro: BRAF-V600E–induced BJ human fibroblasts	Ouabain	L'Hôte et al., 2021

The table lists examples of senolytics that have been successfully tested in models of OIS both *in vitro* and *in vivo*. Despite the limited number of studies, senolytics such as Bcl-2 inhibitors or cardiac glycosides have a promising potential for eliminating oncogene-induced senescent cells accumulating in premalignant lesions. Both ABT-263 and ABT-737 are pan-Bcl-2 inhibitors that interfere with Bcl-2, Bcl-X$_L$, and Bcl-w. Ouabain is a cardiac glycoside that inhibits the Na$^+$/K$^+$-ATPase ion pump. Piperlongumine is a phytochemical that exerts its senolytic activity through inducing oxidative stress in senescent cells.

p21^{Cip1} expression, SA-β-gal expression, and lack of Ki67 expression (Guerrero et al., 2019). Interestingly, ouabain and ABT-737 [another pan–B-cell lymphoma-2 (Bcl-2) inhibitor], were able to senolytically eliminate β-catenin–positive senescent cells sparing other nonsenescent pituitary cell types (Guerrero et al., 2019). Observations from the reviewed literature confirm that senolytics are effective in eliminating oncogene-induced senescent cells both *in vitro* and *in vivo*; however, direct evidence on the outcome of the senolytic removal of oncogene-induced senescent cells on malignant progression is lacking. Despite the ability of ouabain to eliminate oncogene-induced senescent cells, it is noteworthy that, in the previous literature, ouabain's concentrations used to demonstrate its senolytic potential are significantly higher than the clinically relevant plasma concentrations, which are typically within the picomolar range (Selden and Smith, 1972). However, a recent report by L'Hôte et al. (2021) provided new evidence confirming the ability of ouabain to eradicate oncogene-induced senescent cells using a nanomolar concentration range of the drug (albeit still higher than the physiologic ranges). In this work, OIS was induced in BJ human fibroblasts using exogenous overexpression of the oncogene *BRAF*-V600E (L'Hôte et al., 2021). Ouabain was successful in inducing cell death in senescent cells via its established ability to inhibit the Na$^+$-K$^+$-ATPase but also through interfering with autophagic flux where autophagy appears to play a critical role in the survival of senescent cells in this model (L'Hôte et al., 2021).

Perhaps the most direct effort that investigated the potential use of senolytics against OIS to prevent malignant progression was provided by Kolodkin-Gal et al. (2021) in a model of pancreatic adenocarcinoma. In this work, the authors used a triple-transgenic mouse model that allows for the activation of K-Ras specifically in pancreatic acinar cells upon exposure to tamoxifen, which consequently led to 1) the induction of OIS *in vivo*, 2) the development of precancerous pancreatic lesions, i.e., pancreatic intraepithelial neoplasia, and more importantly, 3) the confirmation that the developing precancerous lesions have an abundance of senescent cells (Kolodkin-Gal et al., 2021). When oncogene overexpression was coupled with treatment with caerulein, an inflammation inducing agent, the premalignant pancreatic lesions progressed rapidly to the adenocarcinoma phase. However, the treatment of mice with ABT-737 reduced the burden of senescent cells in the pancreatic premalignant lesions, reduced the expression of the SASP, and consequently, reduced premalignant lesion formation. Interestingly, only 25% of the ABT-737–treated mice (as opposed to 71% of the control mice) developed full pancreatic adenocarcinoma after dual Ras overexpression and caerulein treatment, strongly suggesting that the removal of oncogene-induced senescent cells directly interferes with disease progression (Kolodkin-Gal et al., 2021). Although this work provided evidence on the role of paracrine senescence in malignant transformation, it did not investigate whether escape from OIS was a participating factor in the transition from premalignancy to malignancy. Moreover, this work did not explain why the removal of the senescent cells from the pancreatic premalignant lesions would increase the division rate of the surviving, nonsenescent premalignant cells that were not amenable to senolysis (Kolodkin-Gal et al., 2021). This study indicates that the elimination of senescent cells likely has more complex ramifications that reiterate the dual role of the SASP in tumor suppression. Consequently, further studies to investigate the characteristics of the protumorigenic senescent cell subpopulations in premalignant lesions and methods to improve the selective removal of only "harmful" senescent cells would be of significant preclinical importance (Carpenter et al., 2021). Collectively, the removal of senescent cells could provide a novel approach to delay the progression of premalignant lesions pharmacologically, especially in lesions that are inaccessible for surgical resection (Fig. 1).

This proposed strategy is still subject to several concerns. First, senolytic targeting is not restricted to the "harmful" oncogene-induced senescent cells and can potentially interfere with other physiologic functions of senescence and the beneficial elements of the SASP (Zhu et al., 2020). Second, and as can be interpreted from the work by Kolodkin-Gal et al. (2021), the elimination of senescent cells existing in precancerous lesions could be associated with increased proliferation of nonsenescent cells after senolysis, which poses the risk for the selection for rapidly growing cells in a precancerous lesion. Furthermore, certain senolytics, particularly the most established agents such as BH3 mimetics, have been associated with adverse effects (such as thrombocytopenia), which might limit their clinical use. Moreover, further understanding of the mechanisms underlying OIS might pave the way for the development of senolytics that target processes specific to OIS (Kaplon et al., 2013). Third, the lack of reliable *in vivo* markers for OIS, or for *in vivo* senescence in general (Saleh et al., 2021), can lead to failure of detecting senescent
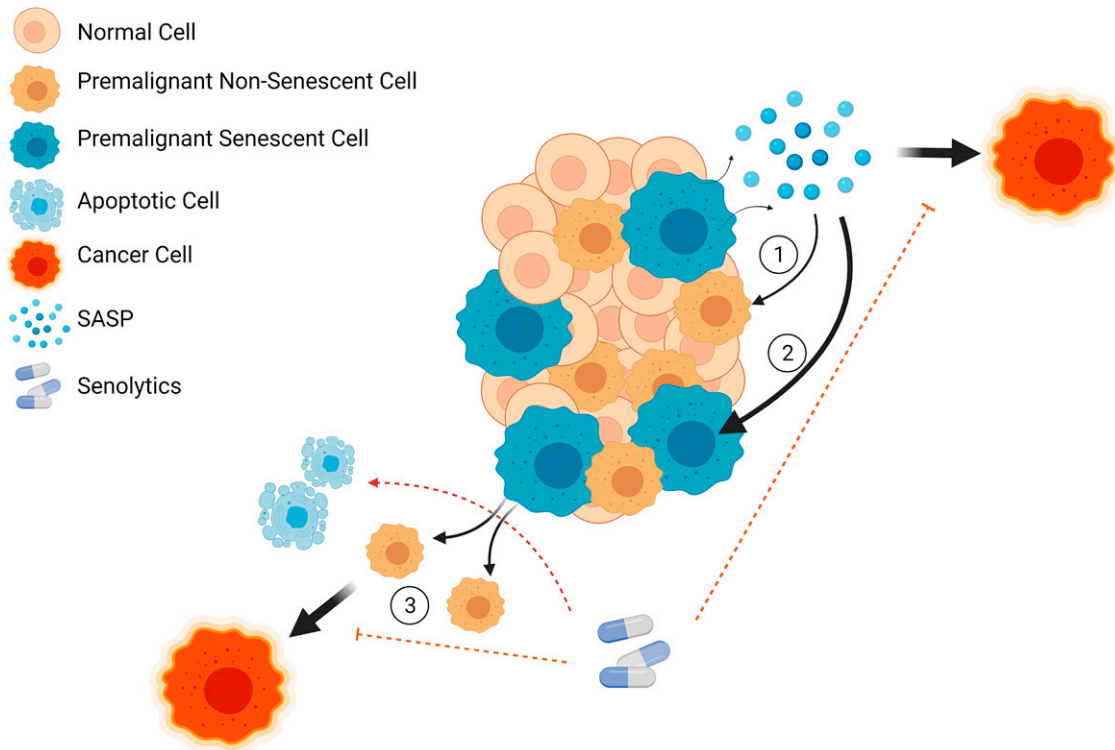
Fig. 1. Senolytic removal of premalignant senescent cells. Senescent cells accumulate in precancerous lesions in response to oncogene hyperactivation. Oncogene-induced senescent cells interact with neighboring nonsenescent premalignant cells and normal cells through secreting several mediators collectively called the SASP. (1) However, the SASP has dual effects in that it can either promote or hinder the progression of nonsenescent premalignant cells depending on its composition. (2) Moreover, and through the SASP, senescent cells can reinforce the growth arrest in cells in the precancerous lesion that are already senescent. (3) In addition to their cell-nonautonomous effects, senescent cells can give rise to proliferating cells, where evasion of escape the durable growth arrest is a potential step during malignant transformation. Overall, the contribution of senescent cell accumulation in premalignant lesions is yet to be fully defined, although early evidence suggests that the removal of oncogene-induced senescent cells may delay progression into full malignancy. This figure illustrates the proposed strategy of removing senescent cells that accumulate in precancerous lesions using senolytic agents as a novel approach to interfere with malignant progression. Senolytic agents are hypothetically expected to cull oncogene-induced senescent cells thereby reducing the risk for evading the durable growth arrest and decreasing the pro-tumorigenic, inflammatory drive mediated by the SASP. This figure was generated through biorender.com.

cells among premalignant cells (Tran et al., 2012; Baek and Ryeom, 2017). The development of routine methods to identify senescent cells in premalignant samples/biopsies can pave the way for a more individualized usage of senolytics. Finally, since several currently investigated senolytics are associated with undesirable adverse effects, particularly the Bcl-2 inhibitors, the identification or development of safer senolytics would accelerate their consideration for the use to mitigate aging-related pathologies including cancer. Nevertheless, we propose that further investigation into senolytic agents and their effectiveness against OIS models may produce a noninvasive method to treat precancerous lesions that carry high risk of malignant transformation.

Authorship Contributions

Participated in research design: Saleh, Carpenter.

Wrote or contributed to the writing of the manuscript: Saleh, Carpenter.

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