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Senescence and apoptosis: dueling or complementary cell fates?

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Abstract

In response to a variety of stresses, mammalian cells undergo a persistent proliferative arrest known as cellular senescence. Many senescence-inducing stressors are potentially oncogenic, strengthening the notion that senescence evolved alongside apoptosis to suppress tumorigenesis. In contrast to apoptosis, senescent cells are stably viable and have the potential to influence neighboring cells through secreted soluble factors, which are collectively known as the senescence-associated secretory phenotype (SASP). However, the SASP has been associated with structural and functional tissue and organ deterioration and may even have tumor-promoting effects, raising the interesting evolutionary question of why apoptosis failed to outcompete senescence as a superior cell fate option. Here, we discuss the advantages that the senescence program may have over apoptosis as a tumor protective mechanism, as well as non-neoplastic functions that may have contributed to its evolution. We also review emerging evidence for the idea that senescent cells are present transiently early in life and are largely beneficial for development, regeneration and homeostasis, and only in advanced age do senescent cells accumulate to an organism's detriment.

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See the Glossary for abbreviations used in this article.

Introduction

Over half a century ago, Hayflick and Moorhead demonstrated that primary human cells in culture have a limited capacity for replication [1]. After undergoing a finite number of divisions, these cells entered into a permanent cell cycle arrest, subsequently termed replicative or cellular senescence. They hypothesized that cellular senescence was a model-in-miniature of processes leading to organismal aging. They also noted that cancer cells divided indefinitely in culture, suggesting a role for replicative senescence in preventing cancer.

The intracellular signals that drive senescence remained obscure until the discovery of telomere erosion and telomerase. Telomeres are repetitive DNA sequences that comprise the ends of many linear chromosomes and protect them from degradation and recombination. Telomeres erode with each cell division due to the biochemical nature of DNA replication: the use of RNA-based priming of the lagging strand and unidirectionality of DNA polymerases. Thus, telomeres have been proposed to be the "molecular clock" that determines the number of divisions a cell can undergo before reaching replicative senescence [2]. Telomerase—an enzyme expressed in many human stem and cancer cells [3], as well as broadly in the mouse [4]—adds telomeric DNA repeats to the telomere and is capable of conferring an indefinite division potential to several types of primary cells in culture, including fibroblasts [5]. Without telomerase, telomeres become critically short and lose their protective function [6], which elicits a DNA damage response (DDR) that upregulates inhibitors of cell cycle progression to effect and enforce the senescence growth arrest [7].

The concept of replicative senescence established a framework for understanding the signaling pathways that drive senescence. Damage sensor proteins, such as ATM in the replicative senescence of human cells, recognize a stress—for example short telomeres—and activate a master regulator (generally p53 through ATM-dependent phosphorylation), which in turn upregulates effectors of cell cycle arrest [8]. p53 can also be stabilized through the action of $p19^{Arf}$ ($p14^{Arf}$ in human cells), an inhibitor of the ubiquitin ligase MDM2 that targets p53 for degradation. However, ATM can suppress ARF in some cancer cells, so the final effect on cell cycle progression depends on the balance between upstream signals [9].

p21, a cyclin-dependent kinase inhibitor (CDKi) responsible for the initial cell cycle arrest, is one of the most important targets of p53 transcriptional activity in senescent cells. The p21-mediated cell cycle arrest can act as a temporary respite for cells with low to moderate amounts of damage, preventing S-phase entry under unfavorable conditions for DNA replication [10]. If the damage is successfully repaired, cells may resume the transiently interrupted cell cycle. However, prolonged arrest leads to upregulation of the CDKi $p16^{Ink4a}$

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Glossary

AKT	Protein kinase B
ARF	Alternate reading protein from CDKN2a locus; p19 in mice and p14 in humans
ATM	Ataxia telangiectasia mutated
BAD	Bcl-2-associated death promoter protein
BAX	Bcl-2-associated X protein
BCL-2	B-cell lymphoma 2, an important anti-apoptotic protein
BCL-XL	B-cell lymphoma extra large
BRAF	Rapidly accelerated fibrosarcoma; a serine–threonine protein kinase in the RAS-RAF-MEK-ERK signaling cascade
CCN1	CCN family member 1; a matrix-associated extracellular signaling protein
CD158	Cluster of differentiation 158; a plasma membrane receptor
CDK	Cyclin-dependent kinase
CDKi	Cyclin-dependent kinase inhibitor
CREB	Cyclic-AMP response element binding protein
DDR	DNA damage response
DNMT3a	DNA methyltransferase 3a
eNOS	Endothelial nitric oxide synthetase
ERK	Extracellular signal-regulated kinase
ETS1	Erythroblastosis homolog 1; a transcription factor
FKBP	FK506 binding protein
FOXO	Forkhead Box O-containing protein a transcription factor
HDF	Human diploid fibroblast; a descriptor of cell karyotype and origin
HLA	Human leukocyte antigen; human equivalent of the major histocompatibility complex genes
HUVEC	Human umbilical vein endothelial cell
IL	Interleukin
INK-ATTAC	Senescent cell killing transgene: apoptosis through targeted activation of caspase (ATTAC) in p16^{Ink4a} (INK)-positive cells
MCF7	Michigan Cancer Foundation-7 human breast cancer cell line
MDM2	Mouse double minute 2
MOMP	Mitochondrial outer membrane permeabilization
MRC5	Human diploid fetal lung fibroblast cell line
mTORC1	Mechanistic target of rapamycin complex 1
NK	Natural killer
NO	Nitric oxide
NOXA	Phorbol-12-myristate-13-acetate-induced protein 1
OIS	Oncogene-induced senescence
p38	MAPK Mitogen-activated protein kinase
PI3K	Phosphoinositide-3 kinase
PIP	Phosphatidylinositol phosphate
PKC	Protein kinase C
PTEN	Phosphatase and tensin deleted in chromosome 10
PUMA	P53 upregulated modulator of apoptosis
Rb	Retinoblastoma
SKN-SH	Bone marrow-derived cell line from a human patient with neuroblastoma
SMAD	Transcription factor downstream of TGF-β signaling
TGF-β	Transforming growth factor-β
UVB	Ultraviolet B light
WI-38	Human diploid fetal lung fibroblast cell line

[11], which activates the transcriptional regulator Rb. Prolonged p16^{Ink4a} expression results in a permanent cell cycle arrest, often with a 2N G1 DNA content. Using the FUCCI reporter system to monitor cycle stages in live human cells, Nakanishi and colleagues recently found that senescence stimuli leading to a DDR can also result in "skipped" mitosis and a G1 arrest with 4N DNA content [12].

Senescent cells accumulate with organismal aging, but telomere erosion is not the sole cause [13,14]. Other stresses that engage the DDR, such as exposure to oxidants, γ-irradiation, UVB light, and DNA damaging chemotherapies, can all induce senescence. Overexpression of oncogenic Ras drives cultured cells into senescence due to the DNA damage induced during the initial period of hyperproliferation, supporting the idea that senescence is a barrier to the proliferation of pre-cancerous or potentially neoplastic cells [15]. Other oncogenes, such as BRAF^{V600E} [16], and the loss of tumor suppressors, such as PTEN [17], also promote senescence but through proliferation-independent mechanisms that are quite different from Ras. BRAF^{V600E}-induced senescence involves multiple mechanisms, including suppression of the metabolic enzyme pyruvate dehydrogenase [18], direct activation of p16^{Ink4a} [19,20], and upregulation of IL-6 and IL-8 [21], whereas loss of PTEN leads to senescence through mTORC1 [17]. These results show that different oncogenic stimuli can induce an irreversible senescent state, termed oncogene-induced senescence (OIS), despite acting through different signaling pathways. Further work is needed to establish whether these "stimulus-specific pathways" are distinct or shared by multiple types of senescence (Sidebar A). For example, is pyruvate dehydrogenase suppressed in replicative senescence or PTEN-loss-induced senescence [18]? Regardless of differences in signaling pathways, Ras overexpression, BRAF mutation, and Pten deletion ultimately activate common effectors of senescence such as p19^{Arf} and p16^{Ink4a} [22]. These cell culture results suggest that senescence is a barrier to transformation and are supported by studies showing that mice lacking p19^{Arf} [23] or p16^{Ink4a} [24] are predisposed to cancer.

Many stimuli leading to a DDR can also induce apoptosis, which is a form of programmed cell death. Apoptosis removes damaged or pre-neoplastic cells, suggesting it should be more capable of restricting tumorigenesis than senescence (Sidebar A). This expectation is especially true in light of the fact that senescent cells actively secrete a suite of cytokines, chemokines, and matrix-remodeling enzymes known as the senescence-associated secretory phenotype (SASP) [25,26] or senescence-messaging secretome (SMS) [27]. Thought to be responsible for stimulating the clearance of senescent cells by the innate immune system or to elicit autocrine signaling to maintain the senescent state, many SASP factors also have pro-tumorigenic properties [25]. Indeed, senescent cells encourage the growth and invasion of breast cancer [28,29] and mesothelioma cells [30] through their SASP.

Given that senescence seems to be an imperfect tumor-suppressive mechanism, what advantage could it have over apoptosis? How is the choice between senescence and apoptosis determined? Guiding our first inquiries into these questions are two studies demonstrating that senescence is a non-essential but integral part of embryogenesis, a stage in the life of every metazoan that also depends on apoptosis (Sidebar A).

Developmental versus stress-induced senescence and apoptosis

Apoptosis *in vivo* was originally associated with pathology and identified as a form of non-necrotic cell death during liver injury [31].

Sulston and colleagues were the first to identify apoptosis in a non-pathologic process during the embryonic development of the nematode *Caenorhabditis elegans*. This organism undergoes a fixed, genetically determined period of embryogenesis in which each developing hermaphrodite loses exactly 131 cells, mostly neurons, through apoptosis [32]. Many of the molecular effectors of apoptosis, including caspases, were discovered through mutagenic screens that disrupted this process [33]. Although differing in details—for example, macrophages engulf apoptotic debris in mammals, whereas non-specialized neighboring cells have this role in nematodes—most factors identified in *C. elegans* screens have human and mouse homologs [34].

Apoptosis is also functionally conserved during development. Many cells produced in abundance in the embryo are subsequently eliminated by apoptosis. Such cells include mammary tissue in males [35] and the interdigital webbing [36]. Likewise, peripheral afferent neurons extend from the spinal ganglia in numbers far exceeding their targets, so only those that successfully contact muscle or skin avoid apoptotic death [37]. Thus, apoptosis regulates patterning in the embryo by altering cellularity in the most direct way possible: cell death (Sidebar A).

Three groups have recently identified cellular senescence during development. Rajagopalan and Long found that HLA-G secreted by trophoblast cells in the extra-embryonic placenta induces senescence of nearby NK cells by binding the receptor CD158d [38]. The SASP from these senescent cells promotes vascular tube formation in culture and is hypothesized to drive vascularization of the placenta *in vivo*. In addition, two groups independently reported the existence of senescence in embryos. Serrano and colleagues identified senescence in the endolymphatic sac and mesonephros of mouse and human embryos and determined it has a morphogenetic role analogous to that of apoptosis [39]. They proposed that cellular senescence, followed by macrophage-mediated clearance of senescent cells or by the overgrowth of nearby cells, alters cellularity, resulting in tissue patterning. Keyes and colleagues found evidence of senescence in the apical ectodermal ridge and neural floorplate and proposed that the SASP of these cells induces tissue remodeling [40]. All three groups found that p21, and not $p16^{Ink4a}$, is the key enforcer of the cell cycle arrest, in combination with the $p16^{Ink4a}$-related protein $p15^{Ink4b}$. $p15^{Ink4b}$-positive cells were identified in the mesonephros and endolymphatic sac. Macrophages clear senescent cells in the embryo [39,40], whereas the cell type responsible for eliminating senescent NK cells in the placenta is unknown. However, immune clearance is not necessary in the endolymphatic sac, where senescent cells are overgrown by their dividing neighbors [39].

Together, these studies suggest that cellular senescence during embryogenesis is a programmed, transient phenomenon that contributes to tissue remodeling through the SASP or to altered cellularity through clearance (Sidebar A).

In the young adult, transient senescent cells also exist. This is not to suggest that the senescence cell cycle arrest is reversible, but that these cells serve a similar transient purpose to those in the embryo: the SASP directs tissue repair and regeneration [41,42]. Like senescence in development, immunosurveillance clears these cells after their programmed function is performed. Transient senescent cells in the embryo and the adult can be termed "acute senescent cells", although the molecular pathways involved are slightly different (Fig 1). In advanced age, senescent cells may accumulate due to several factors: declining immune function [43], decreased ability to stabilize p53 to levels required to cause apoptotic death [44], or slow accumulation of macromolecular damage that does not reach the threshold for cell death [14]. These "chronic senescent cells" may act to the detriment of the organism by promoting tumorigenesis and tissue dysfunction through the SASP (Sidebar A).

Apoptosis versus senescence in the adult

Whether an individual cell in an embryo is faced with apoptosis and senescence as alternative fates is unknown, as is how it might decide between them, although recent work demonstrates that in p21-knockout animals, embryonic senescence is partially replaced by compensatory apoptosis [39,40]. This observation raises the possibility that senescence and apoptosis pathways are simultaneously engaged in certain processes or stress responses and that it is the particular wiring of each cell type that decides which outcome —senescence or apoptosis—will occur first (Sidebar A). Here, we focus on the cell-autonomous features of the choice between senescence and apoptosis in the adult animal, as well as in cultured somatic cells. The emerging molecular factors in this fate choice are the activity of the p53-p21 axis, the role of signaling through PTEN-PI3K-AKT-mTOR, and the degree of macromolecular damage (Fig 2).

Stress level

In some circumstances, apoptosis is a response to overwhelming stress, whereas senescence is a consequence of less severe damage [45]. For example, doxorubicin leads to senescence at low doses and apoptosis at high doses in MCF7 breast cancer cells [46]. A similar dose-dependent response to doxorubicin is seen in neonatal rat cardiomyocytes [47,48]. Other stresses that lead to DNA damage induce senescence at low doses and apoptosis at higher ones, such as fibroblast exposure to etoposide [49] or UVB [50], and keratinocyte exposure to UVB [51]. Oxidative damage also induces a dose–response effect: high-dose H_2O_2 causes apoptosis, whereas lower doses of H_2O_2 induce senescence in F65 and IMR90 human diploid fibroblasts [52,53]. However, some DNA damaging agents that produce bulky adducts, such as busulfan [49], cause senescence but not apoptosis regardless of the dose. This suggests that the nature of the DNA damage, in addition to its severity, can determine the cellular response. Finally, the cell type determines the response to a given stress. Human stromal fibroblasts senesce in response to up to 50 Gy ionizing radiation (IR), whereas T lymphocytes undergo apoptosis in response to only 2 Gy IR (J. Campisi, unpublished data). It is unclear whether these different outcomes are the result of differences in DNA repair efficiency or downstream "preferences" for apoptosis or senescence. Thus, the crucial determinants of whether a cell responds to damage by undergoing senescence or apoptosis are the cell type and the nature and intensity of the damage.

The p53-p21 axis

In addition to the nature and degree of stress, the balance between pro-senescent and pro-apoptotic pathways also decides cell fate. One such pathway is controlled by the tumor suppressor protein p53. p53 was first shown to trigger apoptosis in response to cellular

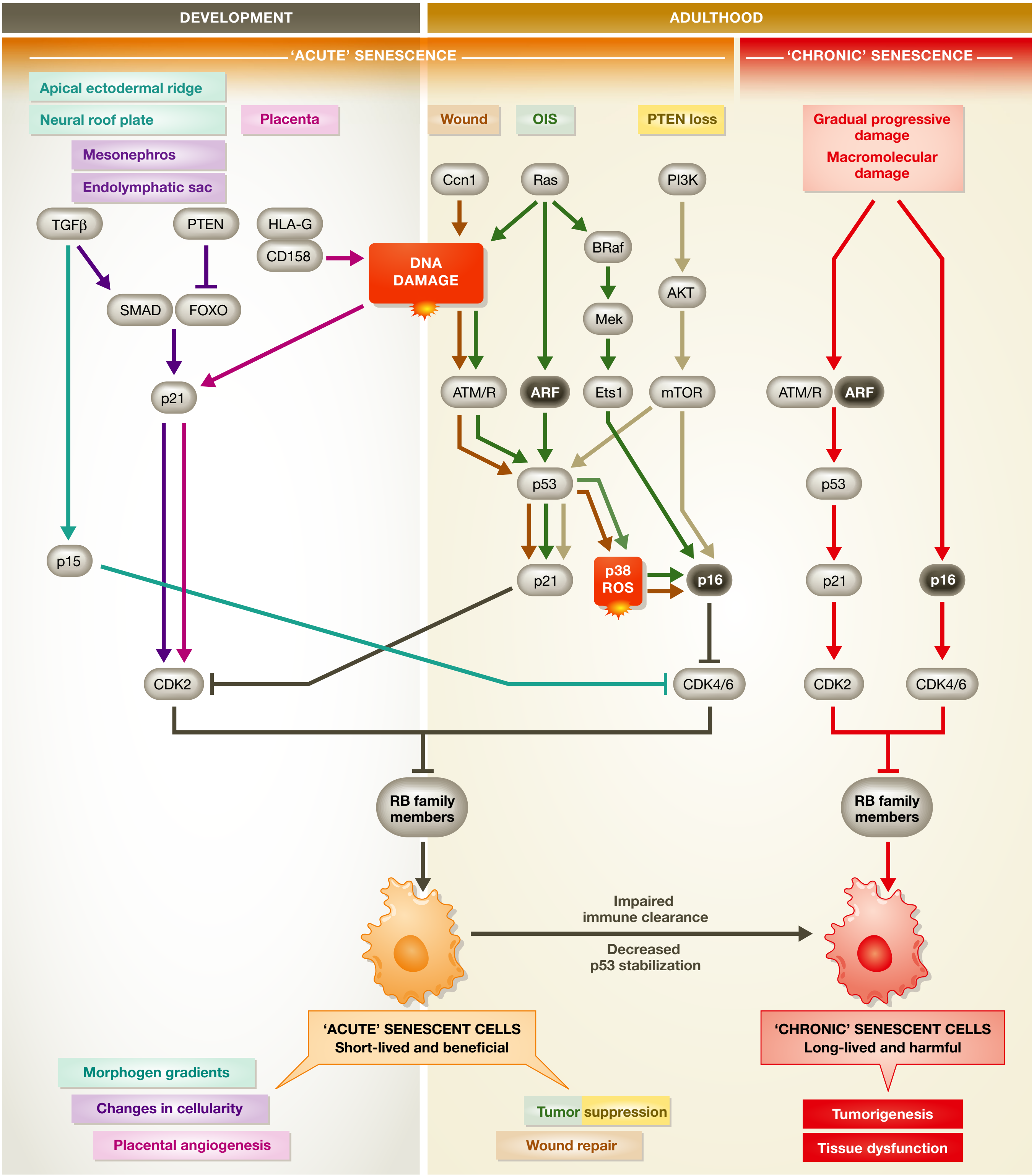

Figure 1. Senescence in development and in the adult.

During development and in the healthy adult, cells can undergo acute senescence, a permanent cell cycle arrest that is physiologically normal. In the embryo and placenta, these cells secrete signaling molecules as part of their SASP to promote morphogenesis. Cell death through immune clearance also complements cell death through apoptosis to change cellularity in developing tissues. In the adult, acute senescent cells function to suppress tumorigenesis and promote wound repair, using different molecular mechanisms than in the embryo. Upon immune dysfunction, acute senescent cells that would normally be cleared by immune surveillance may be chronically present. As they also have a decreased ability to stabilize p53 to the levels required for apoptosis, senescent cells not killed by the immune system may contribute to tumorigenesis and tissue dysfunction. See Glossary for definitions and the text for details.

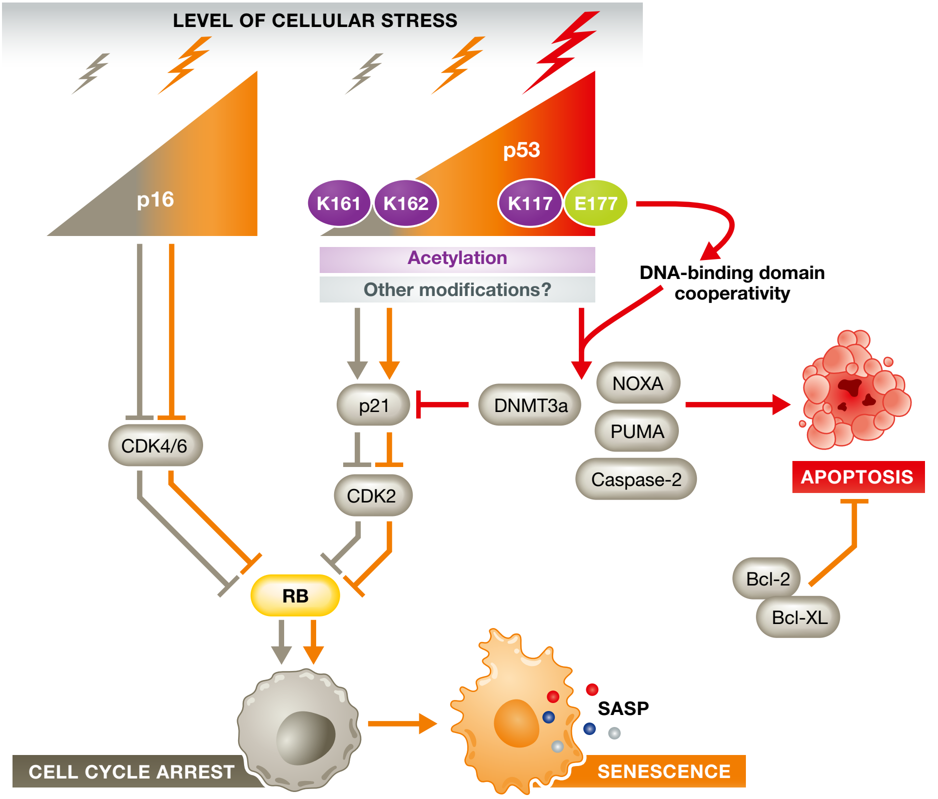

Figure 2. Signaling pathways that enforce the choice between cell cycle arrest, senescence, and apoptosis.

Pathways are colored as follows: leading to arrest in gray, leading to senescence in orange, and leading to apoptosis in red. The weight of the arrow reflects the level of stress. The p16-RB and p53-p21 pathways are known to be important for cellular response to stress. The decision to activate p16, p53, or both is determined by the stress level and cell type. Low levels of p16 promote a transient arrest, whereas high levels lead to senescence. Low levels of p53, with transient kinetics and K161/K162 acetylation, promote cell cycle arrest and senescence. High levels of p53, K117 acetylation, and cooperativity of DNA binding domains within the p53 tetramer lead to the transcription of apoptotic genes and ensuing apoptosis, both directly and by blocking pro-senescence signals. See Glossary for definitions and the text for details.

stress, but is now known to, depending on the stress and cell type, modulate genes involved in homeostasis, transient cell cycle arrest, and senescence [54]. p53 levels, kinetics, and transcriptional activity are all key determinants of how cells respond to various stressors (Fig 2). For example, MEFs expressing the hypomorphic R172P p53 mutation senesce rather than apoptose in response to UVB, fail to upregulate the pro-apoptotic factors PUMA and NOXA and express high levels of the pro-survival gene BCL-2 [55]. Conversely, human diploid fibroblasts treated with a dose of H_2O_2 sufficient to induce a mixture of apoptosis and senescence lead to p53 induction in both cases, but twice as much p53 is expressed in cells destined for apoptosis [53]. Intriguingly, PKC family members are upregulated in MRC5 human lung fibroblasts undergoing IR-induced senescence. Knockdown of PKCζ or PKCλ reduces the levels of BCL-2, phospho-BAD and phospho-CREB, leading to a marked p53 induction and apoptosis [56]. p53-dependent apoptosis relies on a combination of p53 expression level and post-translational modifications, which regulate its activity and localization. The observation that p53 expression is necessary, but not sufficient, for oncogene- and DNA damage-induced senescence suggests that, likewise, changes in p53 post-translational modifications are important for senescence [57–59].

The kinetics of expression is an additional way through which p53 is regulated to control cell fate. Low levels of γ-irradiation—such as 2.5–5 Gy—usually induce a transient rise in p53 levels [60], leading to a transient cell cycle arrest followed by recovery [61]. However, when p53 degradation is prevented with the MDM-2 inhibitor Nutlin-3a, the ensuing higher, stable p53 levels lead to cellular senescence [61]. Furthermore, p53 stabilization by Nutlin-3a treatment promotes senescence, with no evidence of apoptosis, in cultured MEFs upon oxidative stress [62]. Finally, stabilization of p53 is impaired with age in splenocytes [44]. If this is a general phenomenon, attenuated p53 signaling may prevent severely damaged cells from undergoing apoptosis and contribute to senescent and neoplastic cell accumulation with age.

Preferential p53-mediated transactivation of apoptosis- or senescence-specific target genes can also direct cell fate (Fig 2). p53 transactivation activity is fine-tuned by both post-translational modifications [63] and cooperativity between the DNA binding domains of the p53 tetramer. For instance, separation-of-function mutants show that individual acetylation sites on p53 can control the choice to senesce or apoptose [64]. Thus, cells from $p53^{K117R/K117R}$ mutant mice—in which the acetyl acceptor lysine is replaced by arginine—cannot upregulate PUMA and NOXA to induce apoptosis, but can still undergo cell cycle arrest and senescence through p21 upregulation. Ablation of K161 and K162, in addition to K117, also eliminates the arrest and senescence responses. Despite the fact that p53 has been shown to be phosphorylated at different positions after replicative senescence or DNA damage [65], it is unclear whether mutation of these residues would lead to "preference" for an apoptotic fate.

The quaternary structure of the p53 complex also appears to be important for the cellular response to stress. Disrupting cooperation between p53 DNA binding domains with the point mutation E177R interferes with activation of pro-apoptotic genes, but not other p53 targets involved in senescence and metabolism [66,67].

One important p53 target gene is p21, which enforces the initial cell cycle arrest in cells undergoing senescence and apoptosis [68]. p21 expression has been proposed to negatively regulate p53-dependent apoptosis [69]. Low concentrations of doxorubicin promote senescence in SKN-SH neuroblastoma [70] and colorectal carcinoma cells [71], associated with high p21 expression, whereas high doses of doxorubicin result in low p21 expression and apoptosis. These findings show the inverse relationship between p21 and apoptosis sensitivity expected for an anti-apoptotic protein and suggest the possibility that p21 is actively suppressed in apoptosis. Zhang and colleagues demonstrated that, in apoptotic colorectal carcinoma cells, the p53-target DNMT3a is responsible for suppressing p21. When this p21 antagonism is relieved by DNMT3a knockdown, the high-dose doxorubicin that would normally cause apoptosis leads to senescence instead. Similarly, as mentioned above, developmental senescence does not occur in p21-knockout animals and is partially compensated by apoptosis [39,40]. In addition, p21 disruption tips the balance from senescence to apoptosis in colon cancer cells treated with the topoisomerase inhibitors irinotecan or camptothecin [72]. Whether this is because p21 knockdown allows cell cycle reentry, with Topo1 inhibition leading to aberrant replication and a pro-apoptotic DDR, is unclear.

PTEN-AKT signaling

PTEN converts the lipid second messenger PIP3 to PIP2, thereby suppressing the activity PI3K and AKT, which are kinases that control pathways important for cell cycle progression [73], size [74], and metabolism [75]. The PTEN/PI3K/AKT axis is also important for the choice between apoptosis and senescence. Complete loss of PTEN induces senescence in certain mouse and human cells independently of hyperproliferation, subsequent DNA damage, and ATM kinase activation [17]. This is in striking contrast to the senescence caused by overexpression of Ras, which requires hyperproliferation and a DDR [15]. However, some cell types proliferate in the absence of PTEN, although the response to stress is altered by PTEN status. For instance, in response to irradiation, human glioma cells with wild-type PTEN undergo apoptosis, whereas PTEN-null glioma cells undergo ROS/p53/p21-dependent senescence [76]. Conversely, AKT deficiency confers resistance to replicative- and Ras-induced senescence and promotes apoptosis under conditions of oxidative stress [77]. The nuclear functions of PTEN, including DNA damage repair, have been recently shown to depend on SUMOylation at K254 [78]. Whether selectively altering the nuclear functions of PTEN in the DDR instead of disrupting its cytosolic phosphatase activity would also lead to a pro-senescent, anti-apoptotic phenotype is unknown, and a promising area of research.

Cellular senescence arising from PTEN deficiency or AKT activation occurs through activation of mTORC1, requires p53 activity, and engages p21 [79]. Consistent with these findings, rapamycin—which inhibits mTORC1—delays replicative senescence and the senescence induced by progerin, the truncated form of Lamin A found in patients with Hutchinson-Gilford progeria syndrome [80,81]. If mTOR is the signaling center of the PTEN-PI3K/AKT pathway relevant to a senescence or apoptosis decision, rapamycin should promote apoptosis under conditions of stress. Indeed, rapamycin enhances apoptosis in human and mouse cells treated with cisplatin, although under basal conditions—such as in untreated cultured keratinocytes—rapamycin does not promote apoptosis [82,83].

Effectors and inhibitors of apoptosis

Blocking the ability of damaged cells to execute the apoptotic program can also switch cell fate toward senescence (Sidebar A). Although the extrinsic apoptotic pathway has an arm that is completely caspase independent [84], the intrinsic pathway relies on caspases at multiple stages [85]. For example, DNA damage stabilizes p53 to activate the intermediary caspase-2, which triggers mitochondrial outer membrane permeabilization (MOMP) [86]. MOMP allows the release of mitochondrial cytochrome c activating the caspase-9-containing apoptosome [87]. The apoptosome then triggers the executioner caspases 3, 6, and 9, which degrade protein targets to effect the morphological changes of apoptosis and cause cell death. Inhibition of caspases therefore blocks intrinsic apoptosis at many steps of the pathway, leaving senescence as an alternative cell fate. For instance, treating SKN-SH neuroblastoma cells with doxorubicin and a pan-caspase inhibitor prevents apoptosis and promotes senescence [70]. Similarly, FANC-deficient hematopoietic progenitor cells usually undergo apoptosis when damaged by oxidative stress, but blocking apoptosis by caspase inhibition allows cells to survive and become senescent [88].

Manipulating upstream mediators of apoptosis, such as the anti-apoptotic Bcl-2 family proteins, can also influence the choice between senescence and apoptosis. Overexpression of Bcl-2, which prevents cytochrome c release during MOMP [89], forces senescence in fibroblasts treated with an otherwise lethal dose of doxorubicin [90]. This also occurs in cancer cells, in which chemotherapy-induced senescence is an important alternative cell fate to apoptosis (Sidebar A). One of the first studies to report an apoptosis-independent function to p53 in cancer showed that murine lymphomas overexpressing Bcl-2 undergo p53/p16-dependent senescence rather than apoptosis in response to cyclophosphamide [91]. Conversely, knockdown of the anti-apoptotic BCL-XL protein in colon cancer cells subject to DNA damage induced by irinotecan switches senescence to apoptosis [72].

Apoptosis resistance or sensitivity in senescence

If senescence and apoptosis are truly alternative cell fates, one hypothesis would be that cellular changes that are pro-senescent are actively anti-apoptotic and that senescent cells are resistant to apoptosis (Fig 3). Seluanov and colleagues provided strong evidence for the existence of apoptosis resistance in replicatively senescent HDFs, which they showed occurs through p53 signaling. They found that early-passage WI-38 cells undergo p53-dependent apoptosis in response to actinomycin D, low-dose cisplatin, or UVB irradiation, whereas p53-independent apoptosis occurred with high-dose cisplatin and etoposide. When senescent cells were challenged with p53-dependent apoptotic stimuli, they underwent necrosis instead [92]. Exogenous expression of p53 in senescent cells restored their

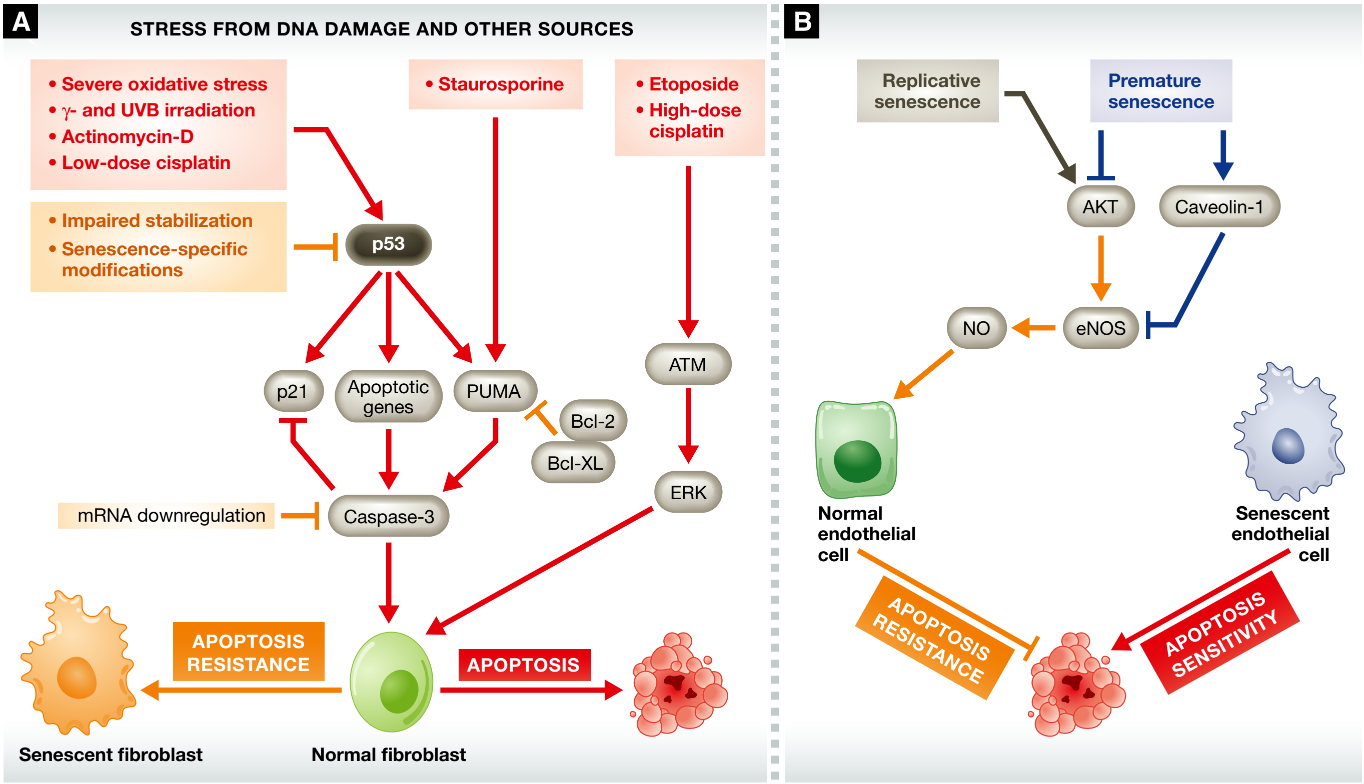

Figure 3. The interplay between senescence and apoptosis is cell type specific.

The pathways leading to apoptosis are depicted in red, those leading to apoptosis resistance in orange, and those that sensitize endothelial cells to apoptosis in blue. (A) Senescent fibroblasts are resistant to p53-mediated apoptotic stimuli, such as actinomycin D and low-dose cisplatin, as well as to stimuli—such as staurosporine—that rely on p53 target genes. This resistance can be explained by low p53 levels due to decreased stabilization in senescent cells, as well as the existence of senescence-specific p53 post-translational modifications. Senescent and non-senescent cells have a similar sensitivity to p53-independent apoptotic stimuli. (B) Senescent endothelial cells have increased sensitivity to apoptosis. Senescent endothelial cells lose eNOS expression and express reduced levels of pro-survival NO. This eNOS loss may be due to the loss of the positive regulator AKT, or to the upregulation of negative regulators, such as caveolin-1. However, AKT levels increase during replicative senescence, so the issue of PTEN/PI3K/AKT signaling in the senescent endothelium is unresolved. See Glossary for definitions and the text for details.

ability to undergo p53-dependent apoptosis. The response of senescent cells to the p53-independent agents was similar to that of early-passage cells, suggesting that apoptosis resistance in senescent cells may be mediated by changes in p53 signaling. Supporting this idea, exposure to Fas ligand induces p53-independent apoptosis to the same extent in senescent and non-senescent WI-38 cells [93].

The outcome of apoptosis resistance can also be cell survival, rather than necrotic cell death. Senescence induced by mild H_2O_2 promotes survival rather than apoptosis in response to apoptotic stimuli such as UVB [94] or high-dose H_2O_2 [53], in part by upregulating the anti-apoptotic BCL-2 despite global repressive epigenetic changes [95]. Like UVB, high-dose H_2O_2 upregulates p53 [53], leading to p53-dependent apoptosis [96]. Similarly, replicative senescent human fibroblasts resist apoptosis in response to serum withdrawal by maintaining high levels of BCL-2 [97]. This upregulation of anti-apoptotic proteins in senescent cells may explain staurosporine resistance [93,98,99]. Staurosporine triggers apoptosis in a p53-independent manner, indicating that alterations in p53 during senescence cannot be directly responsible for this resistance. However, staurosporine requires PUMA to execute apoptosis in MEFs [100] and human colon cancer cells [99]. Overexpression of BCL-2 counteracts the pro-apoptotic genes PUMA and NOXA in cancer cells [101], and a similar process occurs during senescence.

Not only fibroblasts can escape apoptotic death. Replicatively senescent primary keratinocytes upregulate NF-κB to resist UVB-induced apoptosis, whereas immortalized HaCat keratinocytes remain sensitive to apoptosis [102]. Furthermore, when keratinocytes enter G0 after reaching confluence, they become UVB resistant. Exit from the cell cycle has also been shown to lead to apoptosis resistance in colon cancer cell lines, but this phenomenon has not been widely studied [103]. Similar to confluence, cell cycle extension can promote resistance to apoptosis by allowing additional time to repair damage. Consistent with this scenario, late passage, non-senescent human fibroblasts—which have an extended cell cycle time—upregulate BCL-XL and resist UVB-induced apoptosis [104]. Modulation of the cell cycle, either by its extension or exit to G0, is therefore a confounding element in studies about the apoptosis resistance of senescent cells that precludes definitive conclusions about the effects of senescence *per se*.

In contrast to fibroblasts and keratinocytes, senescent endothelial cells are more susceptible to apoptosis than their non-senescent counterparts. For example, porcine pulmonary artery endothelial cells passaged to replicative senescence undergo more spontaneous apoptosis than at early passage, with reduced BCL-2 and increased BAX expression [105]. Senescent human umbilical vein endothelial cells (HUVECs) similarly undergo increased apoptotic death in response to exogenous ceramide C2 [106]. Conversely, senescent human foreskin fibroblasts treated in the same manner are resistant to this compound compared to their early-passage counterparts [106]. Apoptosis by exogenous ceramide C2 has been reported to be p53 dependent, although in radiation-induced apoptosis, ceramide C2 production is p53 independent [107].

Fibroblasts show reduced sensitivity to apoptosis as passage number increases, whereas HUVECs are increasingly susceptible to apoptosis with passage [108], showing reduced activity of the anti-apoptotic endothelial nitric oxide synthase (eNOS) [109–112]. Hoffman and colleagues attributed the loss of NO production to reduced AKT [108], which phosphorylates and activates eNOS [113]. Other mechanisms may also be involved in endothelial cell senescence, such as increased levels of the eNOS negative regulator caveolin-1 [112]. PTEN/PI3K/AKT pathway activity is reduced during senescence of irradiated endothelial cells [114] and HUVECs exposed to high glucose [115]. However, AKT activity rises during replicative senescence of endothelial cells, and its inhibition extends replicative lifespan *in vitro* [116]. Thus, the variations in sensitivity to apoptosis between senescent endothelial cells and fibroblasts may reflect differences in the way these cells modulate pro-survival factors, such as eNOS, or how the PTEN/PI3K/AKT pathway is altered with senescence.

Mice expressing low amounts of the mitotic checkpoint protein BubR1 due to hypomorphic alleles (BubR1$^{H/H}$) accumulate senescent cells in several tissues early in life [117] and rapidly develop progeroid phenotypes [118]. BubR1$^{H/H}$ mice that are prevented from developing senescent cells through genetic ablation of p16^{Ink4a} have a delayed time to onset of these phenotypes; however, they still die early from p16^{Ink4a}-independent effects of BubR1 depletion [117]. p16^{Ink4a}-positive cells can be effectively eliminated from BubR1 hypomorphic mice using an INK-ATTAC transgene, in which a fragment of the p16^{Ink4a} promoter is used to drive the expression of a drug-inducible caspase-8/FKBP fusion protein in senescent cells. Administration of the synthetic drug AP20187 causes dimerization of the FKBP domains, forcing caspase-8 activation and apoptotic death.

The clearance of p16^{Ink4a}-positive cells in this manner also delays the development of progeroid phenotypes [119]. The cell types undergoing senescence and expressing the INK-ATTAC transgene in BubR1$^{H/H}$ fat and muscle have been defined as adipocyte progenitors/stem cells and fibroadipogenic progenitors, respectively [120]. While it is tempting to conclude that these cells are not resistant to apoptotic death because the apoptotic program can be initiated through caspase-8 dimerization, the role of caspase-8 is downstream of any apoptosis-resistance changes described in senescent cells. Nevertheless, these results in BubR1$^{H/H}$ INK-ATTAC mice provide hope for the development of a senescent cell killing therapy, by demonstrating that senescent cells can undergo apoptosis *in vivo* with the proper stimulus.

Senescence signaling within tissues

Apoptosis leads to a rapid elimination of dysfunctional cells by phagocytes in a manner that does not stimulate inflammation [121]. On the other hand, the pro-inflammatory secretion of growth factors and cytokines from senescent cells has the potential to generate prolonged paracrine signaling. In this way, apoptosis can be viewed almost solely as a cell-intrinsic mechanism, as compared to the dual cell autonomous and non-autonomous nature of senescent cells. Emerging data suggest that the presence of senescent cells has an advantage over apoptosis due to this ability to communicate with other cells, raising the possibility that signaling from senescent cells within tissues can be both beneficial and detrimental (Sidebar A).

The senescence program is activated in a variety of benign and pre-malignant lesions *in vivo* to limit tumor progression in a cell-autonomous manner [16,122–124]. Various components of the SASP, however, promote pre-malignant cell growth or invasion through their ability to induce angiogenesis, epithelial–mesenchymal transitions and differentiation within the local microenvironment [25,29,125–127]. These effects are clearly pro-neoplastic and thus are detrimental side effects of the SASP.

However, several studies have suggested that the SASP is not always pro-tumorigenic [128]. First, the SASP can reinforce and maintain the senescent state in cell culture models of senescence [21,129–131]. Second, the SASP attracts the immune system to clear both premalignant and established tumor cells by phagocytosis or cytotoxic-mediated killing, through a "senescence surveillance" process that entails both innate and adaptive immune responses [132–134]. Oncogene-induced, pre-malignant hepatocytes present many features of senescent cells, including high levels of p16^{Ink4a}, p21 and senescence-associated (SA)-β–galactosidase activity. It is thought that these cells generate a SASP that initiates a CD4^{+}-T-cell-mediated adaptive immune response to subsequently remove these pre-malignant lesions. Furthermore, reactivation of p53 in a Ras-induced liver-carcinoma mouse model resulted in rapid regression of the existing tumor. Surprisingly, the tumors were not eliminated through apoptosis but through cellular senescence and a SASP, consistent with observations from a sarcoma mouse model [135]. The SASP that is generated within the liver tumors triggers the innate immune system to respond to the senescent cells and remove them through the action of macrophages, neutrophils, and NK cells.

With these observations in mind, one could argue that senescence in pre-malignant and established tumor cells has some advantages over apoptosis (Fig 4), although it should be emphasized that apoptosis provides a preferred and effective anti-tumor mechanism in various contexts, including malignancies with Myc mutations [136,137]. First, when a cell within an emerging tumor undergoes senescence, it has the potential to negatively impact its neighboring non-senescent tumor cells through the SASP. For instance, it has been shown that senescence and SASP production can trigger senescence in neighboring cells via paracrine signaling, a phenomenon that has been referred to as bystander senescence [138]. Second, the mobilization of immune responses to these areas of senescence in pre-malignant and established tumors could have a greater impact on reducing tumor cell burden than apoptosis of single cells. This is in contrast to what is observed with apoptosis of large proportions of neoplastic cells, such as is seen with cytotoxic agents and various mouse models, illustrating that apoptosis is a potent anti-cancer mechanism when it occurs in a coordinated manner in a large percentage of cells. When a limited number of cells must decide between senescence and apoptosis, perhaps senescence has a greater consequence, as there is potential to impact other cells in the microenvironment. However, it is still unclear why certain pre-neoplastic lesions—such as benign nevi that are often caused by

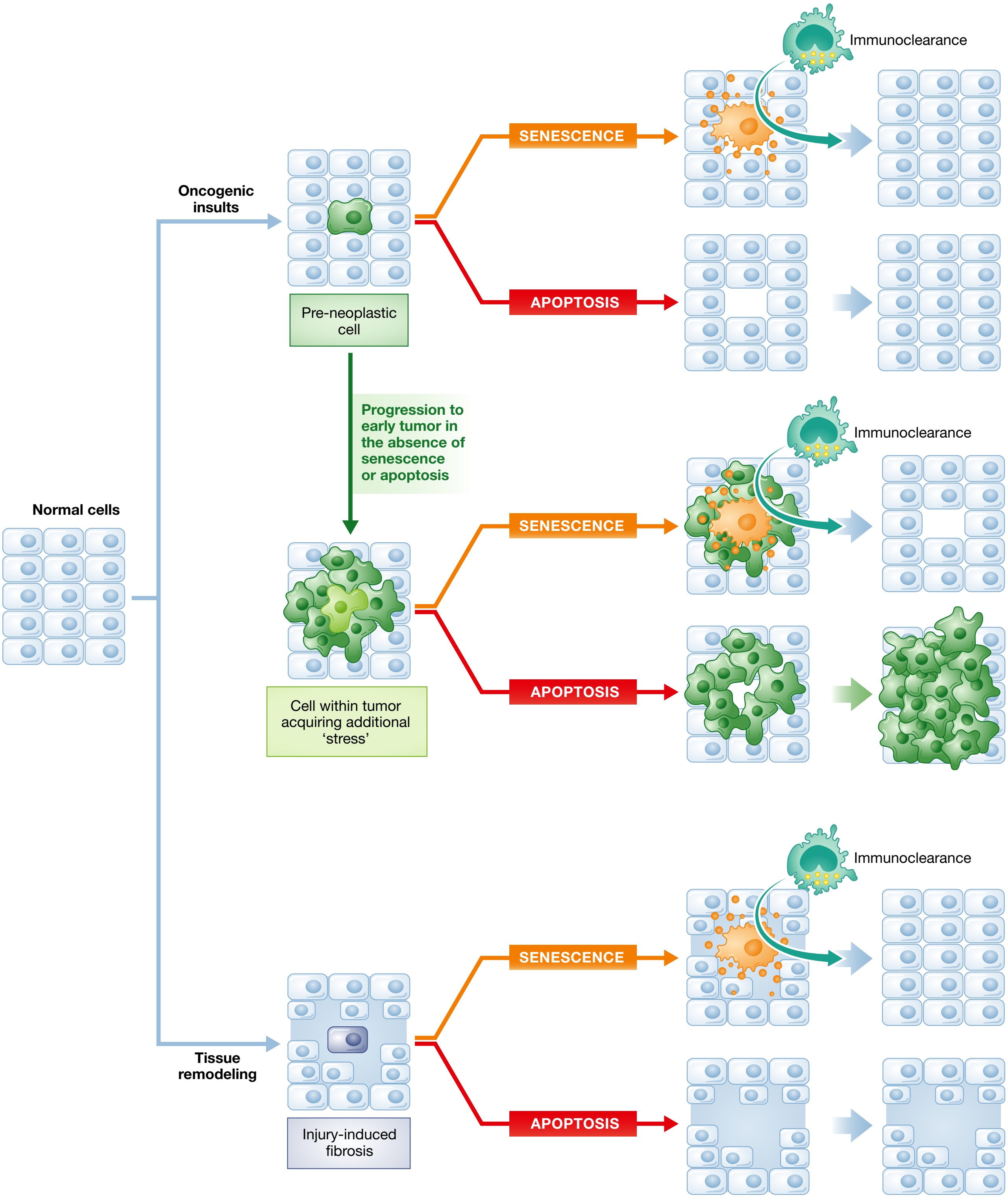

Figure 4. Consequences of senescence and apoptosis in stressed tissues.
Normal cells are subject to a variety of stressful stimuli, including oncogenic insults (top) and tissue damage (bottom). Cells that have acquired a pre-neoplastic lesion may undergo senescence or apoptosis. The outcome of this decision is largely the same if the senescence surveillance machinery—which ensures that the lesion is efficiently removed—is intact. If pre-neoplastic lesions do not induce senescence or apoptosis, they continue to grow and progress (middle). In this scenario, if senescence is engaged in a fraction of the now established tumor, the SASP and recruitment of the surveillance machinery may be much more effective at removing tumor cells than a single cell that undergoes apoptosis and does not initiate an immune response. Although not illustrated, if a large percentage of tumor cells are coerced into apoptosis, this would also lead to reduction in tumor volume. In response to tissue damage (bottom), senescence would also theoretically be advantageous compared to apoptosis, as the production of the SASP would limit tissue fibrosis and promote tissue remodeling, as long as the SASP-producing cell is ultimately removed by the immune system.

oncogenic BRAF mutations—remain for extended periods of time, avoiding immune-mediated senescence surveillance. Whether analogous avoidance processes occur outside the context of tumorigenesis, for example, during senescence in aged tissues, is unknown (Sidebar A).

One potential reason why senescent cells become more abundant with age is that the immune system deteriorates over time and becomes less efficient in clearing senescent cells. On the other hand, perhaps mechanisms that maintain tissue/organ size act to suppress the clearance of senescent cells by the immune system to prevent potential tissue dysfunction through cell loss. Future studies to investigate these phenomena and assess whether immune cells target both acute and chronic senescent cells for destruction are clearly needed and warranted. In addition, aging was not selected for during the evolution of most organisms. Thus, the programs that mediate changes with advancing age may be more malleable than previously thought.

Other tissues seem to benefit by initiating senescence upon damage. Hepatic stellate cells of the liver, for example, which promote fibrosis upon activation by injury, undergo senescence upon chronic damage to restrict fibrosis [134]. Similarly to what is observed in established liver tumors, NK cells subsequently clear these senescent stellate cells. During the wound-healing response in skin, myofibroblasts proliferate and produce extracellular matrix [139]. These cells then senesce and destroy the matrix through the SASP, thereby limiting fibrosis. A number of additional tissues undergo senescence with age and disease (reviewed in [14,128, 140, 141]); however, additional studies are needed to understand the contribution of these senescent cells to disease initiation, progression, and maintenance.

Future directions

Important molecular mechanistic insights about the relationship between senescence and apoptosis have emerged over recent years, although a number of questions about the elimination of senescent cells *in vivo* remain unresolved. The first concerns the interaction between the immune system and cellular senescence. If, as hypothesized, a decline in immune surveillance underlies the accumulation of senescent cells with age, the rates with which senescent cells are generated could be relatively constant with age and, thus, short-lived senescent cells would exist transiently even earlier in life. One test of this idea would be to compare senescent cell numbers in young normal mice and mice that are deficient in various arms of the immune surveillance network, including mice lacking $CD4^+$ T cells, macrophages or NK cells. An increase in senescent cells in young immunodeficient animals would suggest that immunosurveillance normally limits their presence in healthy young animals.

The question of rates of senescence in youth and old age can be partially addressed by inducible immunodeficiency models. If the rate of senescent cell production increases with age, abrogation of immune surveillance in aged animals should cause greater accumulation of senescent cells than disrupting the immune system in youth. A potential confounder in this experimental design is that immune dysfunction could *per se* drive *de novo* formation of senescent cells, rather than sparing existing cells. A complimentary approach would be to label senescent cells during a period of time in young animals and compare them with the number of cells accumulated during an identical interval in old age. An estrogen receptor–Cre fusion protein driven by the p16 promoter, in combination with a floxed cassette inhibiting the expression of GFP, for example, might suffice for this purpose.

Another open question is whether and how apoptosis resistance is altered with the establishment and progression of senescence (Sidebar A). Some features of late senescence, such as the recently described cytoplasmic chromatin processing [142], are reminiscent of blebbing during apoptosis. Whether chromatin blebbing is a hallmark of senescent cells or whether it represents a transition from senescence to apoptosis remains to be determined. Activation of latent transposons occurs late in senescence [143,144], which may cause non-self antigen presentation on the cell surface, leading to cytotoxic T-cell-mediated apoptosis. Thus, additional apoptotic pressures may overwhelm the anti-apoptotic machinery engaged in established senescent cells. However, these hypotheses assume that senescence occurs because it is preferable to apoptosis in certain contexts. One alternative to this model is that pre-neoplastic cells already have critical mutations that inhibit apoptosis, leaving senescence as a fail-safe mechanism. The ability of "single hits"—such as Ras overexpression or $Braf^{V600E}$ mutation—to induce senescence might argue against this possibility, but perhaps these mutants compromise apoptosis and thereby lead to senescence, rather than vice versa.

Whether senescent cells in aged organisms are more detrimental than beneficial to healthy life remains unclear. The lack of any overt downside to senescent cell clearance in the $BubR1^{H/H}$; INK-ATTAC model suggests that apoptosis of senescent cells might be safe [119]. It may therefore be reasonable to develop therapies that kill senescent cells to prolong healthy organismal life. The question then would be to identify the molecular target(s) that constitute an Achilles' heel of senescent cells, which express a balance of pro-survival and pro-apoptotic signals. Although enhancing p53 levels might tip the balance toward apoptosis, drugs that do so risk having significant off-target effects. Understanding when and how to tip this balance will require more basic research and the ability to translate this research to pre-clinical and eventually clinical settings.

Conclusions

Regardless of the theoretical mechanisms discussed above, the function of senescent cells *in vivo*, when unrelated to pathologies such as cancer and wounding, remains obscure. Crucial conclusions about senescence are based on cell culture work using non-physiological stresses. Many of the most interesting features of senescent cells observed *in vitro*, such as G1 arrest with 4N DNA content [12] or cytoplasmic heterochromatin processing [142], have yet to be verified *in vivo* or in culture by other groups. Given the variability in apoptosis sensitivity observed between senescent endothelial cells and fibroblasts, it is imperative that *in vivo* senescent cells be identified, isolated, and characterized (Sidebar A).

An even larger challenge to the field is to put what has been learned about senescence and apoptosis, not only in the context of the human or mouse, but into the bigger picture of multicellular life. What other species have cellular programs analogous to senescence? Does *C. elegans*, the poster child for developmental apoptosis, also

Sidebar A: In need of answers

- (i) Is senescence advantageous over apoptosis in some contexts or a "backup" pathway for apoptosis? How do damaged cells decide whether to continue living in a dysfunctional state or die?
- (ii) Most hallmarks of senescence have been established in cell culture. How similar are the features of senescent cells *in vivo*? Furthermore, do these traits vary by tissue and cell type?
- (iii) Apoptosis is conserved across metazoans. Are developmental and adult senescence conserved across taxa and, if so, can comparative phylogeny determine the origin of senescence?
- (iv) What is the lifespan of senescent cells *in vivo*, and do acute and chronic senescent cells have different life spans? Do senescent cells accumulate with age due to faulty immune clearance, changes in stresses that the organism experiences, or a reduced ability to undergo apoptosis? Do senescent cells contribute to normal aging and age-related diseases?
- (v) Can cancer be conceptualized as a cellular process that evades apoptosis and senescence? Can senescent cells escape from senescence and undergo transformation?

use cellular senescence during embryogenesis? If enough data are gathered about the use of these processes in development and aging in vertebrates and invertebrates, it would maybe be possible to ascertain which mechanism is ancestral. The field can also learn whether senescence originated as a developmental force or a tumor suppressor. This question has not been resolved from the apoptosis viewpoint either. While perhaps inappropriate to ask the teleological "Why?" about the choice between apoptosis and senescence, we can ask "How?"—how did they evolve, how do cells choose between them, and, ultimately, how can we make use of this choice to promote a healthy life (Sidebar A).

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Conflict of interest

The authors declare that they have no conflict of interest.

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