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Review

Fundamental Cell-Intrinsic Mechanism Underlying Age-Dependent Accumulation of Senescent Cells

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ABSTRACT: The accumulation of senescent cells during aging contributes to the progression of various age-related pathologies. Ineffective immune clearance, increased half-life of senescent cells, and bystander senescence are considered the primary drivers of this age-associated accumulation. Most of these causes stem from the aging of the immune system, which results in a prolonged persistence of damaged/nonfunctional cells within tissues and allows the internal senescence program to progress to a more severe phenotype. Here, we propose the existence of an additional immune-independent mechanism underlying the accumulation of senescent cells during aging. By reanalyzing existing experimental evidence, we show that cells of diverse identities and tissue origins become increasingly susceptible to senescence with age. The latter implies that epigenetic and molecular changes that cells acquire during aging create a permissive background for the activation of the senescence program. In light of our findings, senotherapeutic interventions alone may be insufficient to substantially alter the trajectory of organismal aging. Effective strategies may need to target upstream drivers of cellular dysfunction, including age-associated epigenetic alterations. Epigenetic rejuvenation could, in principle, enhance cellular stress resilience and thereby reduce the rate at which senescent cells emerge and accumulate.

Keywords: epigenetic aging, senescence, stress resistance, accumulation of senescent cells

1. Introduction

Aging is a complex phenomenon that still lacks a universally accepted definition. Several "schools of thought" offer different perspectives, framing aging as the accumulation of damage, a decline in the strength of natural selection, a continuation of development, a loss of function, increased mortality, or a collection of age-related changes [1, 2]. In contrast, there is broader consensus regarding senescence, which is typically defined as the stable cell cycle arrest of damaged cells that remain viable but exhibit widespread functional impairments and acquire a proinflammatory secretory phenotype [3, 4]. Although cellular senescence is commonly associated with aging, it remains debated whether it acts as a driver or a consequence of the aging process [2, 4, 5]. The "senescence-centric" perspective of aging is supported by several experimental observations. First, senescent cells accumulate in multiple organs and tissues over time [4, 6]. Second, even a small proportion of senescent cells can compromise tissue renewal, impair homeostasis, and reduce organismal fitness, including in young, healthy animals [4, 6]. Finally, clearance of senescent cells—either through genetic (using genetically-modified mice) or chemical (using senolytics) approaches—has been shown to delay the onset of age-associated disorders in both mice and humans [4, 6]. Collectively, these findings suggest that the accumulation of senescent cells contributes to age-related pathologies and that their removal may restore tissue and organ function. Nevertheless, evidence supporting a primary, driving role for senescence in aging remains limited.

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In contrast to the "senescence-centric" view, recent study revealed that alterations in the epigenetic landscape are key drivers accelerating aging of mammalian organs and tissues [7]. Epigenetic changes caused by acts of DNA repair lead to a gradual loss of transcriptional networks, cellular identity, and functionality, and ultimately to cellular senescence [7]. From this perspective, the senescence burden is rather a consequence than a cause of aging. In line with this, it was recently shown that epigenetic aging, as measured by the epigenetic clock, is distinct from cellular senescence [8]. The latter provides evidence that this cellular phenomenon is unrelated to aging on its own. Senescence is more likely to represent a fundamental nonspecific stress response mechanism against external or internal stimuli when cellular damage exceeds the repair capacity [2, 5].

Although senescence is not considered a primary driver of aging from the perspective of epigenetic theory, its contribution to the progression of organismal aging is indisputable. Aging appears to create a permissive environment that allows dysfunctional senescent cells to accumulate within tissues. Several mechanisms underlying this age-related accumulation have been identified. The most well-established is impaired immune clearance due to age-associated declines in immune function [9, 10]. Another factor is the induction of senescence in the surrounding microenvironment through paracrine and bystander effects [11]. A more recently described mechanism states that senescent cells slow their own removal rate during aging [12]. The latter may be due to altered senescence-associated secretory phenotype (SASP) composition, disrupted tissue architecture, or an abundance of senescent cells that exceeds immune capacity. Here, we propose an additional mechanism driving the age-related accumulation of senescent cells. We hypothesize that cells become more prone to senescence during aging. In other words, as cells age, their stress resilience declines, shifting the balance from repair toward senescence. In the present study, we summarized the relevant experimental *in vivo* and *in vitro* data to investigate whether reduced stress resistance and/or repair capacity of aging cells contributes to the elevated levels of senescence observed during aging (Table 1). If confirmed, this would suggest that cellular senescence represents a maladaptive response of aging cells that exacerbates the aging phenotype. Understanding the mechanisms that underlie age-dependent accumulation of senescent cells may adjust the existing anti-aging strategies.

Table 1. Experimental evidence for predisposition of aged cells towards induced senescence.

Experimental model	Age groups	Stress / tissue of interest	Estimated senescence markers	Time of the analysis	Potential confounding by immune-mediated clearance	Ref
Stress -> tissue or cell isolation -> analysis	Young (~8–10 weeks) and aged (~46–49 weeks) C57BL/6 mice	Bilateral renal ischemia-reperfusion / kidney	Expression of p53 and p21, SA-β-gal activity	6 weeks post stress	high	[13]
	Young (12 weeks) and aged (88 weeks) C57BL/6 mice	Intra-tracheal installation of bleomycin / lung	SA-β-Gal activity	14 days post stress	high	[12]
	Young (12 weeks of age) and old (72 weeks of age) C57BL/6 mice	Moderate-level controlled cortical impact or sham surgery / microglia and cortical tissue	p16 gene and protein expression	72 h post stress	minimal	[14]
	Young (10-11 weeks) and aged (72 weeks) C57BL/6JRJ mice	Intranasal administration of lipopolysaccharide / lung	Expression of p53 and p21, γH2AX level	24 h post stress	minimal	[15]
	Young (16-28 weeks) and aged (80 weeks) C57BL/6J mice	Injecting the tibialis anterior muscles with notexin / skeletal muscles	Fraction of muscle stem cell and progenitors that co-express Cdkn2a and Cdkn1a	1, 2, 3.5, 5, 7 days post stress	minimal/high (at later time points)	[16]
	Young (8 weeks) and aged (88 weeks) C57BL/6J mice	Cryoinjury of the quadriceps femoris / muscle	"apoptosis", "aging", and "cellular senescence" gene sets expression	2, 4, 7 days post stress	minimal/high (at later time points)	[17]

	Young (12 weeks) and aged (72 weeks) C57BL/6 mice	Unilateral intraocular pressure elevation / retina	Expression of genes within "Inflammatory", "apoptosis" and "senescence" pathways	2, 3 days post stress	minimal	[18]
Cell isolation -> stress -> analysis	Fibroblasts from young (25.5 years) and aged (90.2 years) human donors	Rotenone- and glucose-induced senescence	SA-β-gal activity	7 days post stress	absent	[19]
	Fibroblasts from young (16 years) and aged (75 years) human donors	Ionizing radiation	γH2AX foci, SA-β-gal activity	16 h post stress for DNA damage and 10 days post stress for SA-β-Gal	absent	[20]
	Muscle stem cells from young (8 weeks) and aged (96 weeks) C57BL/6 mice	Isolation procedure with no additional stress	γH2AX	-	absent	[21]
	Muscle stem cells from young (8-16 weeks) and aged (80-96 weeks) C57BL/6 mice	Ionizing radiation	γH2AX	24 h post stress	absent	[22]
	Mouse embryonic fibroblasts with tamoxifen-inducible I-PpoI endonuclease prior (young) and 96-h post-tamoxifen supplementation (aged)	etoposide, camptothecin, and hydrogen peroxide	γH2AX and 53BP1	The values are missing in the original study	absent	[7]
	Retinal pigment epithelium cells from young (9-20 years) and aged (62-76 years) human donors	oxidative stress	PI staining, LDH activity	The values are missing in the original study	absent	[23]

NB! Because no single marker is fully specific to identify senescent cells, "bona fide" senescence should be confirmed using a combination of markers, typically:
1. Stable cell-cycle arrest (associated with activation of p16, p21, and p53 pathways)
2. SA-β-gal activity
3. Persistent DNA damage response (γH2AX and 53BP1)

2. Tissues from aged mice exhibit higher level of induced senescence in various *in vivo* models

The first evidence of increased levels of induced senescence in aged mice dates back to 2013 [13]. In this study, acute kidney injury was modeled in young (~8–10 weeks) and aged (~46–49 weeks) C57BL/6 mice via 30-minute bilateral renal ischemia-reperfusion. Senescence in kidney tissues was assessed six weeks post-reperfusion by measuring p53 and p21 expression, as well as senescence-associated β-galactosidase (SA-β-gal) staining (Fig. 5 in the original study). Notably, p53 and p21 expression levels were similarly low in young and aged control kidneys, indicating minimal age-dependent accumulation of senescent cells under baseline conditions. However, six weeks after the injury all measured senescence markers were elevated in both young and aged kidneys. Importantly, the increase in p53 and p21 expression throughout the kidney, as well as the proportion of SA-β-gal–positive tubules, was significantly greater in aged animals. These findings demonstrate that aged tissues are more prone to accumulate senescent cells following stress, although the experimental design does not distinguish whether this reflects enhanced senescence induction or impaired immune clearance.

Another line of evidence comes from a study published in 2019 [12]. In this work, senescence was induced in the lung epithelium of young (12 weeks) and aged (88 weeks) C57BL/6 mice via intra-tracheal administration of bleomycin. Senescent lung epithelial cells were identified as double-positive for SA-β-gal and the epithelial marker pan-cytokeratin and were quantified using imaging flow cytometry at multiple time points post-treatment. Compared to the above study [13], here the experiment was performed using much older animals, and the authors witnessed a minimal abundance of senescent cells in the aged untreated tissues, though not exceeding a few percent. Remarkably, by the earliest analyzed time point after bleomycin administration (day 14), the proportion of senescent cells in aged lungs was approximately three times higher than in young lungs, reaching nearly 40% (Fig. 5 in the original article). These findings further support an age-dependent increase in the severity of stress-induced senescence, although the underlying mechanisms remain to be elucidated.

Additional support for our hypothesis comes from analyses of age-related differences in the progression of

acute traumatic brain injury [14]. The authors compared various parameters between young (12 weeks) and aged (72 weeks) C57BL/6 mice after moderate-level controlled cortical impact or sham surgery. Microglia from aged animals exhibited a higher basal level of senescence, as evidenced by increased cell size, lipofuscin accumulation, and γH2AX staining (Fig. 4 of the original study). The effects of traumatic brain injury were assessed after 72 h. Notably, the authors reported markedly elevated levels of p16 transcripts and protein in the cortical tissue and microglia of aged mice following injury, significantly exceeding those observed in young animals (Fig. 5 of the original article). These findings suggest that brain aging is associated with enhanced propensity for stress-induced cellular senescence.

Further evidence relevant to our study is provided in a subsequent report [15], in which the authors investigated age-dependent responses to acute lung injury. In this model, lipopolysaccharide (LPS) was administered intranasally to young (10–11 weeks) and aged (72 weeks) C57BL/6JRj mice. The following analysis was performed on alveolar epithelial type II cells isolated from young and aged control or LPS-treated mice 24 h after the start of the experiment. Importantly, the authors did not reveal any signs of age-related accumulation of senescent cells prior to stress. Namely, epithelial cells from aged animals were characterized by the same proliferation rate as those from young animals, with no evidence of relative telomere shortening, DNA damage response markers, or cell cycle arrest. Despite the absence of overt baseline senescence, LPS stimulation elicited a markedly stronger response in aged epithelial cells. Within 24 hours of exposure, aged epithelial cells exhibited significantly greater upregulation of p21 and p53 transcripts, along with increased γH2AX staining intensity, compared to cells derived from young animals (Fig. 4 and 6 of the original study). These findings underscore the heightened sensitivity of aged cells to stress-induced senescence.

Another support for our hypothesis is provided by a recent study examining the role of cellular senescence in skeletal muscle regeneration throughout the murine lifespan [16]. In this work, muscle injury was induced in young (4–7 months), old (20 months), and geriatric (26 months) C57BL/6J mice by intramuscular injection of notexin into the tibialis anterior. Injured and uninjured muscles were harvested at six time points (days 0, 1, 2, 3.5, 5, and 7) and subjected to single-cell transcriptomic analysis. The authors considered the double-positive *Cdkn2a*+ and *Cdkn1a*+ cells as senescent muscle stem cells and progenitors. At baseline (day 0), no significant age-related differences were observed in the proportion of $Cdkn2a^{+}/Cdkn1a^{+}$ double-positive cells. However, by day 3.5 post-injury, the fraction of senescent muscle stem cells and progenitors was significantly increased in old and geriatric mice compared to young animals (Fig. 3E in the original study). These findings indicate that aging does not necessarily increase the steady-state burden of senescent cells in this compartment but markedly amplifies the senescence response following injury. Consistent observations were reported in an independent study employing a cryoinjury model of skeletal muscle regeneration in young (2 months) and aged (22 months) C57BL/6J mice [17]. Single-cell RNA sequencing performed before injury and at 2, 4, and 7 days post-injury revealed that, upon damage, muscle stem cells derived from aged tissue exhibited significantly higher expression of gene sets associated with apoptosis, aging, and cellular senescence (Fig. 5C–H in the original study). Collectively, these results are in line with our speculation on increased sensitivity of aged cells towards senescence.

Finally, we identified a study whose findings closely align with our conceptual framework [18]. In this work, the authors examined age-dependent transcriptional responses in the retina following unilateral elevation of intraocular pressure. To do so, intraocular pressure was unilaterally elevated to 30 mmHg for 1 hour in young (3 months) and aged (18 months) C57BL/6 mice and then RNA-sequencing analysis on bulk retinas collected 2 days after the procedure. As shown in Figure 2H and Supplementary Figures S2K, S2L, the core genes within significantly enriched pathways—inflammatory, apoptosis and senescence—were upregulated more in aged retinas than in young retinas upon elevated intraocular pressure stress (Fig. 2H, Supplementary Figures 2K, 2L in the original study). Moreover, ATAC-sequencing revealed pronounced age-related differences in chromatin dynamics following stress. Notably, nearly fourfold more genomic regions exhibited increased chromatin accessibility in aged retinas than in young retinas after stress elevation (Fig. 4A and Supplementary Figure 4A in the original study). Based on the data obtained the authors suggested that during aging retinal cells accumulate the competence on the epigenetic level to respond to stress faster and stronger, eliciting more damage to the tissue in the form of the inflammation and senescence.

Taken together, the findings discussed in this section and summarized in Table 1 underscore several important conclusions. First, aging per se is not invariably associated with an elevated basal burden of cellular senescence. In the majority of studies reviewed here, uninjured tissues from young and aged animals exhibited comparable baseline levels of senescence-associated markers. Second, in response to stress, tissues from aged mice display a more rapid and pronounced activation of senescence-related pathways. As early as 24 hours following exposure to damaging stimuli, aged tissues demonstrate higher levels of DNA damage and increased

expression of cell cycle arrest markers relative to their young counterparts. Elevated levels of senescence-associated markers persist in aged tissues for extended periods after injury, including at 3, 14, and even 35 days post-stress. Of note, comparisons of stress-induced senescence between aged and young animals were performed within each individual study. Across diverse tissues and experimental paradigms, a consistent trend emerges—aged organisms exhibit a greater magnitude of senescence induction in response to injury or stress. Collectively, these observations provide convergent, albeit indirect, evidence supporting the notion that aging enhances the susceptibility of cells and tissues to stress-induced senescence. A key limitation of *in vivo* approaches for directly testing our hypothesis is the difficulty of distinguishing the contribution of increased stress sensitivity in aged cells from other well-established mechanisms driving senescent cell accumulation. Specifically, the age-associated decline in immune clearance likely prolongs the persistence of senescent cells in tissues, which, through SASP-mediated paracrine signaling, may promote senescence in neighboring cells and thereby exacerbate age-related accumulation. Furthermore, a prolonged residence of senescent cells within tissues may alter the composition of their secreted factors, which, according to recent observations, could further slow their clearance [12]. Together, these factors may contribute to the progressive increase in senescent cell burden observed with age.

3. Cells derived from aged donors exhibit increased susceptibility to DNA damage and stress-induced senescence

A direct evaluation of our hypothesis requires a controlled comparison of stress resilience and senescence induction *in vitro* using cells derived from young and aged donors. Unlike *in vivo* models, *in vitro* systems eliminate the confounding influence of age-associated decline in immune-mediated clearance of senescent cells, thereby enabling assessment of cell-intrinsic susceptibility. We identified six experimental studies that address this question (Table 1). Although each has methodological limitations, their findings collectively support our hypothesis.

The first study compared stress-induced responses in primary human dermal fibroblasts isolated from young (mean age 25.5 years) and aged (mean age 90.2 years) donors [19]. Two distinct senescence-inducing stimuli were employed: rotenone and high glucose. Senescence was quantified by measuring SA-β-gal activity using flow cytometry. At baseline, fibroblasts from aged donors exhibited higher SA-β-gal activity than those from young individuals (Fig. 1B in the original study). To account for this initial difference, the authors normalized stress-induced changes to baseline levels and subsequently compared the magnitude of induction between the age groups (Fig. 1C and D). Both rotenone exposure and glucose overload elicited a significantly greater increase in SA-β-gal activity in fibroblast strains derived from aged donors. Although senescence was assessed using a single marker in this study, the results nevertheless support the concept that cells from aged individuals are intrinsically more prone to undergo senescence in response to stressful stimuli.

Additional evidence is provided by a study published in 2016 [20], which likewise employed primary fibroblast cell lines derived from young (mean age 16 years) and aged (mean age 75 years) donors. The aim of the study was to reveal age-related alterations in the efficiency of DNA double strand breaks repair. To this end, fibroblasts from both age groups were exposed to ionizing radiation, and the formation and resolution of γH2AX foci were quantified at multiple time points. Interestingly, no difference in the number of foci between young and aged cells was observed prior to irradiation or at 2 h post irradiation (Fig. 3A and B in the original study). However, a significantly increased number of γH2AX foci was detected in aged cell lines compared to young ones at 16 h post DNA damaging stress (Fig. 3A and B in the original study). Concordantly, fibroblasts from aged donors displayed a more pronounced elevation in SA-β-gal activity at 10 days post irradiation than cells from young donors, as shown in Figure 7A and B in the original study (of note x-axis labels in Figure 7B are mixed up). Based on the data obtained, the authors conclude that young cells are more resistant to ionizing radiation than aged cells. The results of this study confirm our suggestion and reveal an age-related decline in DNA double strand breaks repair as a possible mechanism that may underlie the higher sensitivity of aged cells to stress-induced senescence.

Further experimental evidence relevant to this review is provided by two independent studies from the same laboratory [21, 22], both examining age-related alterations of muscle stem cells In the study published in 2018, the authors isolated muscle stem cells from young (8 weeks) and aged (96 weeks) C57BL/6 mice and then performed γH2AX staining to quantify the proportion of cells that exhibited signs of DNA damage [21]. Strikingly, approximately 80% of MuSCs derived from aged animals exhibited γH2AX foci, compared with fewer than 20% of cells from young mice (Fig. S2C and S2D in the original study). In the following study, the number of γH2AX foci in muscle stem cells isolated from young (8-16 weeks) and aged (80-96 weeks) mice was assessed 24 h after irradiation (Fig. 2K in the original study) [22]. As expected, the authors revealed a significantly greater number of X-irradiation-induced foci in aged cells than in

the young ones, demonstrating the higher susceptibility of aged cells to genotoxic stress. Moreover, aged muscle stem cells exhibited a higher rate of cell death in response to the selected irradiation dose (Fig. 2M, N), further underscoring their diminished stress resilience. Given that both senescence and apoptosis represent alternative cellular responses to stress, the ultimate cell fate decision is likely influenced by the magnitude of the insult and by intrinsic properties of the cell type. Notably, additional experiments in the latter study identified loss of global heterochromatin and increased chromatin accessibility in aged muscle stem cells as mechanistic contributors to their heightened sensitivity to genotoxic stress [22]. These findings provide a plausible epigenetic basis for the increased susceptibility of aged stem cells to DNA damage and its downstream consequences.

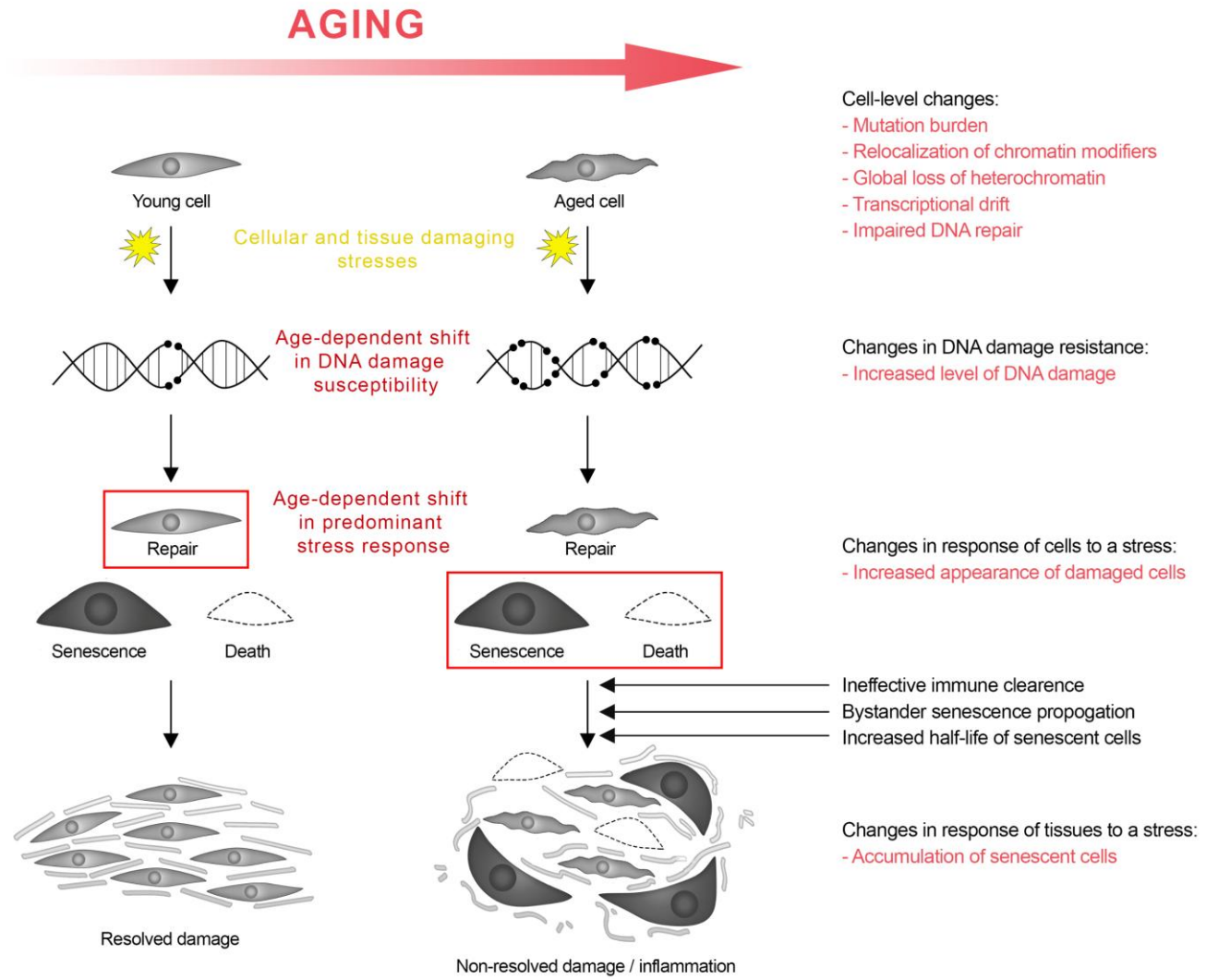


Figure 1. Schematic illustration of a novel immune-independent mechanism underlying age-dependent accumulation of senescent cells. Alterations that cells acquire during aging lead to reduced stress-resistance of cells and increased level of induced senescence.

The next part of this section draws on experimental evidence from a recent study proposing the loss of epigenetic information as a driver of aging [7]. The authors used an elegant approach to mimic the effects of organismal aging by expressing endonuclease I-Ppol. This enzyme generates a limited number of double-strand breaks (DSBs), predominantly within non-coding regions, thereby perturbing chromatin organization without causing widespread genomic disruption. Induction of I-Ppol expression in mouse embryonic fibroblasts derived from transgenic embryos resulted in a measurable increase in epigenetic age within 96 hours. Importantly, despite this acceleration of epigenetic aging, no detectable differences in cell cycle progression, apoptosis, or senescence were observed either during or 96 hours after I-Ppol induction when compared with cells from control mice, which were epigenetically younger. These findings indicate that epigenetic aging, in this context, does not

intrinsically elevate the basal level of senescence. Finally, the authors compared the sensitivity of control (young) and I-Ppol-expressing (aged) cells to various DNA damaging agents, including etoposide, camptothecin, and hydrogen peroxide. All of the tested compounds induced a higher level of damage in I-Ppol-expressing cells, confirming an age-related decline in resistance of cells to DNA damage. Although the precise timing of DNA damage assessment was not specified, these observations are in agreement with the findings discussed above [20], reinforcing the concept that aged or epigenetically aged cells display heightened vulnerability to DNA damage.

Additional evidence supporting the altered stress resistance of aged cells comes from a study published in 2010 [23]. The authors investigated the oxidative stress sensitivity of human retinal pigment epithelium (RPE) cells derived from young (9–20 years) and aged (62–76 years) donors. Using multiple complementary approaches, they demonstrated a pronounced increase in cell death in RPE cells from aged donors (Fig. 3A and B in the original study). Furthermore, aged RPE cells exhibited clear signs of mitochondrial deterioration, bioenergetic deficits, and compromised antioxidant defenses. The authors concluded that age-associated mitochondrial dysfunction in RPE cells may sensitize the retina to oxidative and age-related injury.

Together, the evidence summarized in this chapter clearly demonstrates an age-related decline in the stress-resistance of cells. Several possible molecular reasons may underlie this phenomenon. Firstly, the efficiency of DNA damage repair is reduced with age. Secondly, DNA organization into higher order chromatin structures is gradually lost as cells age, resulting in higher accessibility of DNA to damaging agents. Thirdly, mitochondrial dysfunction leading to increased reactive oxygen species (ROS) generation and heightened oxidative stress may compromise cellular defense mechanisms. These age-related alterations elevate the level of stress-induced damage in aged cells, leading either to death or to senescence. Therefore, the accumulation of senescent cells during aging indeed may be mediated by the reduced stress-resistance of aging cells (Fig. 1).

4. Discussion

Here we tested the assumption that aged cells become more prone to stress-induced senescence, resulting in the immune-independent accumulation of senescent cells during aging. The fundamental basis for this suggestion arose from several key points.

The first point concerns the nature of senescence and its relation to organismal aging. Historically, the idea that senescence contributes to aging stemmed from the discovery of replicative senescence. This form of senescence was considered an intrinsic mechanism that limits the proliferative capacity of normal cells when the predetermined limit for division is reached [5, 24]. To put it simply, repeated rounds of proliferation, each accompanied by telomere shortening, should result in replicative senescence of cells within tissues, thus leading to aging. Such a hypothesis mostly comes from *vitro* studies, whereas there are some arguments that cast doubt on a role of replicative senescence in aging *in vivo*. First, the prevalence of senescent cells in aged tissues appears minimal [6, 13, 15, 25, 26]. For instance, when the fraction of cells expressing *Cdkn2a* (p16) was compared between young (12 weeks) and aged (72 weeks) mice, only ~1–2% of cells were p16-positive in the older animals [25]. Moreover, this level was almost equal between mice 72, 84, 96, and 120 weeks of age, indicating the absence of age-related increase in the number of senescent cells. Second, there is no clear relation between the number of population doublings cells can endure and the age of the donor [27, 28]. Namely, if health status and biopsy conditions are strictly controlled, the replicative lifespan of fibroblasts in culture does not correlate with donor age [27]. Third, earlier studies on centenarians and at least one described here have questioned whether telomere shortening reliably occurs *in vivo*, as telomere length often shows no correlation with chronological age [15, 29]. Therefore, it still remains unclear whether telomere shortening reflects a mitotic clock-like process or it is rather a biomarker of stress [30-33]. In addition, numerous studies have demonstrated that normal cells can undergo senescence in response to various physiologic stresses [34]. These arguments along with another data on the topic are summarized in the outstanding review [5] favors that telomere shortening revealed *in vitro* is rather a result of non-physiologic cultural conditions than an intrinsic clock-like mechanism that operates independently of extrinsic physiological stresses. As such, replicative senescence is unlikely to be a faithful model of aging occurring *in vivo*, instead it should be considered just a subtype of stress-induced senescence. From this perspective, senescence—whether replicative, stress-induced, or oncogene-induced—represents a core cellular program, akin to apoptosis or repair, that can be activated by the cell at any age in response to physiological stress. Further support for this notion comes from studies employing epigenetic clocks [8, 35, 36]. Using multiple models of senescence (X-ray-induced, Ras-induced, and replicative) and multitissue clock, it was demonstrated that epigenetic aging is distinct from both cellular senescence and telomere attrition [8, 35].

The second key point is based on a set of results from studies focused on genetic or chemical approaches to remove senescent cells from the organism (reviewed in [4]). The main conclusion that outcomes from these

studies is that accumulating senescent cells may promote certain aspects of the aging phenotype and thus contribute to the age-related pathologies. Indeed, mounting evidence shows that the accumulation of senescent cells increases the risk of many age-related diseases, including osteoarthritis, neurodegeneration, and atherosclerosis (summarized in [12]). Moreover, transplantation of relatively low numbers of senescent cells into young healthy mice reproduces some pathologies, e.g. osteoarthritis, that typically accompany organismal aging [11, 37-39]. Together with the points mentioned above, these arguments demonstrate that the deleterious effects often attributed to senescence, in fact are not caused by senescence itself, but results from the accumulation of damaged non-functional cells within tissues. This observation raises a critical question: which age-related alterations predispose organisms to the retention and accumulation of these damaged senescent cells?

Currently, numerous studies are addressing this question. Among the factors identified, age-related changes in the immune system—encompassing both adaptive and innate immune compartments—have emerged as primary contributors, given their central role in the clearance of senescent cells [9, 10]. Aging affects most of the organs and compartments in which immune cells differentiate, mature, or circulate, including bone marrow, thymus, spleen, lymph nodes, and blood, leading to perturbed immune cell quantity and quality [37, 40]. Another mechanism facilitating the age-related accumulation of senescent cells is the bystander effect. The impact of bystander senescence *in vivo* was verified in an elegant set of experiments when senescent cells were xenotransplanted into immunocompromised NSG mice [11]. A more recently revealed factor promoting the accumulation of senescent cells during aging is that senescent cells slow down their own removal rate with increasing age [12]. By using experimental and theoretical approaches, the authors designed the saturating removal model to estimate senescent cell turnover during aging. Interestingly, the authors considered slowdown of removal of senescent cells during aging as the core mechanism that leads to age-related rise in production of senescent cells.

The third key point integrates the evidence discussed above and underscores several important inconsistencies. Although aging is inevitably associated with immune system decline and reduced turnover of senescent cells, the basal levels of senescent cells in most tissues remain relatively low under non-stressful conditions [6, 13, 15, 25, 26]. Notably, much of the data on the adverse consequences of accumulated levels of senescent cells on proper organ and tissue functioning are performed when senescence is triggered by certain stress [41-48]. Along with that, separate observations demonstrate that cells from aged individuals are more susceptible to stress [19, 20]. Since senescence can be considered as stress reaction, we hypothesized that intracellular alterations during aging reduce stress resistance of cells, thus less stress will be needed to push aged cells to senescence. This is where other mechanisms that contribute to senescent cells accumulation come into play: the more pronounced senescence initially induced in aged tissues by stress is further enhanced by less effective immune clearance, reduced removal rate and bystander effects.

Analysis of existing literature provides several lines of experimental evidence supporting our hypothesis. First, cells and tissues from aged animals displayed increased level of induced senescence compared to the young ones as was demonstrated using animal models of acute kidney injury, acute traumatic brain injury, bleomycin- or LPS-induced lung injury, notexin-induced injury or cryoinjury of skeletal muscle, and elevated unilateral intraocular pressure elevation in the eye [13-18]. A key limitation of some of these studies is that senescence markers were assessed long after the initial stress, making it difficult to distinguish between a higher rate of stress-induced senescence and subsequent accumulation due to other factors. More direct evidence comes from studies in which cells were stressed *in vitro* after isolation procedures. This approach minimizes the influence of immune-mediated clearance on senescent cell accumulation. A limited number of experimental results highlights higher sensitivity of cells from aged donors towards stressful stimuli, which led to higher level of senescence in some cases or of cell death in the other [7, 19-22, 23]. The most apparent mechanistic contributor to this age-related decline in stress resilience is impaired DNA repair, which may involve reduced expression or delayed recruitment of repair factors, as well as decreased repair fidelity [20, 49]. Another contributing factor is the age-associated loss of global heterochromatin and increased chromatin accessibility [18, 22]. Consistent with this, earlier work has shown that proper histone-DNA interactions and higher-order chromatin organizations protect against induced DNA strand breaks [50]. Importantly, restoring heterochromatin content in aged cells to youthful levels mitigates age-associated phenotypes, reduces irradiation-induced DNA damage, and decreases cell death [22]. Furthermore, the reduced stress resilience of aged cells may be linked to mitochondrial dysfunction. Aging is associated with a decline in mitochondrial ATP production, which promotes increased generation of reactive oxygen species (ROS) and exacerbates oxidative stress [23, 51]. Elevated mitochondrial ROS can directly induce DNA damage, while diminished ATP availability compromises the activity of DNA repair enzymes [52, 53]. This combination of heightened oxidative stress and impaired repair capacity creates a cellular environment

that limits the ability of aged cells to cope with additional stressors.

Summarizing all of the above data, we can conclude that cellular alterations during aging may result in reduced resistance of aged cells to stresses, what may lead to higher levels of senescent cells that is further intensified by altered immune clearance (Fig. 1). In this context, our review supports a complementary mechanism underlying age-dependent accumulation of senescent cells, operating alongside established mechanisms such as altered immune surveillance, bystander effects, and changes in senescent cellular turnover. The present findings open important directions for future investigation. In particular, it will be interesting to determine whether the observed relationship is maintained in the context of oncogene-induced senescence (OIS). To date, direct experimental comparisons evaluating the susceptibility of primary human cells derived from young versus aged donors to OIS are, to our knowledge, lacking. Addressing this gap would provide critical insight into the mechanistic interplay between aging and cancer progression and could refine our understanding of how age-associated cellular states influence oncogenic responses. Moreover, in light of our findings, senotherapeutic interventions alone may not be sufficient to fully counteract the complex, multifactorial processes underlying organismal aging. While the selective elimination of senescent cells has shown promise in alleviating specific age-associated pathologies, it remains unclear whether such approaches can substantially modify the broader trajectory of aging. It is conceivable that more comprehensive strategies may be needed to target upstream drivers of cellular dysfunction, including progressive epigenetic alterations. Interventions aimed at epigenetic rejuvenation may, in principle, enhance cellular stress resilience and potentially reduce the rate at which senescent cells emerge and accumulate; however, the extent to which such effects translate into durable systemic benefits remains to be determined.

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Authors' contributions

A.V.B. designed the review framework, and led the planning, writing of the original draft, figure preparation, and manuscript editing. P.I.D. designed the review framework, provided expert review, manuscript and figure editing. Both authors have read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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