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Impact of Senescent Cell Subtypes on Tissue Dysfunction and Repair

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Abstract

Cellular senescence, first observed and defined through cell culture studies, is a cell fate associated with essentially permanent cell cycle arrest and that can be triggered by a variety of inducers. Emerging evidence suggests senescence is a dynamic process with diverse functional characteristics. Depending on the tissue, type of inducer, and time since induction, senescent cells can promote tissue repair and re-modeling, prevent tumor development, or contribute to age-related disorders and chronic diseases, including cancers. Senescent cell characteristics appear to depend on multiple factors and be influenced by the *milieu* and other senescent cells locally and at a distance. We review diverse phenotypes of senescent cells originating from different cell types, senescence inducers over time since induction of senescence, and across conditions and diseases. This background is essential to inform further understanding about senescent cell subtypes and will point towards rational senescence-modulating strategies for achieving therapeutic benefit.

Keywords

Senolytics; Cellular senescence; Deleterious senescent cell subtype; Helper senescent cell subtype

1. Introduction

Senescence is a cell fate that entails an essentially permanent cell cycle arrest in response to multiple factors, including repeated replication, potentially oncogenic mutations and activated oncogenes, DNA damage, metabolic insults, damage/danger signals, neutrophils, radiation, and cytotoxic and other drugs [1–4]. Senescent cells often undergo profound morphological and functional changes, including overall cellular, nuclear, and mitochondrial enlargement, chromosomal deconvolution and chromatin remodeling, gene expression changes, heightened metabolic activity, and sustained survival with resistance to apoptosis [5–9]. Some, but not all senescent cells acquire a senescence-associated secretary phenotype (SASP), with release of interleukins and other cytokines, chemokines that attract, activate, and anchor immune cells, pro-apoptotic factors, matrix metalloproteinases, growth factors,

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reactive metabolites, bioactive lipids including saturated fatty acids and ceramides, bradykines, and prostanoids, micro-RNAs, mitochondrial DNA, and other non-coding nucleotides, and extracellular vesicles, among other factors [10–12].

Senescence appears to be a programmed process that can contribute to tissue homeostasis and such physiological functions as embryonic and fetal development [13], parturition [14], tumor suppression [15], wound healing [16], and tissue repair [17]. Normally, senescent cells are cleared by the immune system soon after their appearance, but with chronic stress or immune dysfunction, senescent cells can persist and develop an increasingly pro-inflammatory, pro-apoptotic SASP [18–20]. Accumulation of senescent cells has emerged as a root cause contributor to stem cell, progenitor, tissue, and systemic dysfunction, predisposing to multiple disorders that account for much age- and chronic disease-related morbidity and mortality, including cancers, diabetes and its complications, neurodegenerative, cardiovascular, hepatic, and kidney diseases, osteoporosis, arthritis, and many more [10].

The molecular architecture of senescent cells determines their pathophysiological functions and secretory phenotype. Senescence is a dynamic multistep process that is impacted by intra- and extra-cellular signals leading to heterogeneity in functional outcomes [21]. The molecular characteristics of senescent cells depend on the stage of senescence progression, the nature of the inducer, and cell type of origin. The anti-apoptotic mechanisms of senescent cells and SASP profiles are shaped continuously by the microenvironment, immune cells, pathogens, and other neighboring senescent cells [20, 22].

2. Differences among senescent cells

2.1. Cell type of origin

Senescent cells in tissues and organs contribute to not only aging phenotypes but also to the genesis of multiple chronic diseases. Senescent cells can have SASP profiles that vary among cell types of origin. *In vitro* comparisons of senescent preadipocytes, endothelial cells, myoblasts, fibroblasts, and epithelial cells reveal differences in the extent of upregulation of SASP factors [23]. Senescent endothelial cells and preadipocytes tend to have greater SASP factor expression than other cell types, such as epithelial cells or myoblasts [23]. Higher SASP expression by such cells implies that some senescent cells may contribute more to chronic inflammation and organismal age-related dysfunction than others. Senescent cells in different tissues exert their effects through distinct mechanisms. For example, senescent preadipocytes release activin A, which impairs adipogenesis, leading to metabolic dysfunction [24, 25]. Senescent melanocytes induce telomere dysfunction in surrounding cells through the chemokine, CXCR3, contributing to impaired skin regenerative potential with aging [26]. Currently, it is unclear if all cell types can become senescent. Traditionally, only replicative or mitotically competent cells have been thought to be susceptible to senescence. However, it appears that terminally differentiated cells can also acquire features of senescence [27]. For example, cortical and hippocampal neurons from old mice can develop DNA damage, SASP production, heterochromatinization, and senescence-associated β-galactosidase (SA β-gal) activity, among other features of cellular senescence, many of which may contribute to neural aging phenotypes [28]. Similarly,

several features of senescence have been identified in terminally differentiated, non-dividing hepatocytes [29] and cardiomyocytes [4] of aged mice, with increased expression of the DNA damage markers, $p21^{Cip1}$ and $p16^{Ink4a}$, along with pro-fibrotic SASP induction. Emerging evidence suggests that specific immune cell populations, such as macrophages [30] and T cells [31, 32], may display senescent-like features, which raises the question of whether these cells actually are or can become senescent. Additionally, the SASP comprises many molecules indicative of inflammation, adding to the complex nature of discerning whether immune cells are truly senescent or are reacting as non-senescent normal immune cells to age- or disease-related signals.

2.2. Type of inducers

Many of the intra- and extra-cellular signals that contribute to cells' entering the senescent cell fate include those related to tissue or cellular damage or cancer development. These include damaged DNA, telomeric uncapping or dysfunction, exposure to extra-cellular DNA, oncogene activation, replicative stress or inducers of proliferation (such as growth hormone/IGF-1), protein aggregates, misfolded proteins, failed protein removal through decreased autophagy, saturated lipids and other bioactive lipids (bradykines, ceramides, certain prostaglandins, *etc.*), reactive metabolites (*e.g.*, reactive oxygen species [ROS], hypoxia, or hyperoxia), mechanical stress (*e.g.*, bone-on-bone stress in osteoarthritis), inflammatory cytokines (*e.g.*, TNFα)[33], damage-associated molecular patterns (DAMPs, *e.g.*, released intracellular contents signaling breakage of neighboring cells), and pathogen-associated molecular patterns (PAMPs; *e.g.*, bacterial endotoxins or components of viruses) [18, 22]. These inducers can activate one or more senescence-promoting transcription factor cascades, in some cases involving $p16^{INK4A}$ and retinoblastoma protein (Rb), in others, p53 and $p21^{CIP1}$, both of these pathways, or other pathways [34]. These transcription factor cascades enforce replicative arrest and influence epigenetic changes, causing altered expression of hundreds of genes. The SASP may vary depending on the type of inducers. Variations in the SASP can occur both in the specific factors released and in the magnitude of their expression. For example, senescent fibroblasts can release factors such as platelet-derived growth factor AA (PDGF-AA) [16] and matricellular protein CCN1 [35], accelerating wound closure upon acute skin injury. Additionally, senescent fibroblasts can drive angiogenesis by secreting vascular endothelial growth factor (VEGF), promoting endothelial cell invasion and increasing vascularization [36]. Prolonged aberrant persistence of senescent cells can have detrimental effects in promoting cancer. For example, chemotherapy-induced senescent fibroblasts can promote tumor recurrence and metastasis by releasing SASP factors such as interleukins (IL)-6 and −8 [37].

2.3. Time since induction of senescence

Cellular senescence takes longer to become established than other cell fates: replication, differentiation, apoptosis, or necrosis. From initiation to the attainment of a complete state of cellular senescence takes from 10 days to 6 weeks, depending on the cell type and the inducers driving the cell into senescence, at least *in vitro* [38]. The growth arrest of senescent cells is related to increased $p16^{INK4A}$ or $p21^{CIP1}$, which are inhibitors of the cyclin/cyclin-dependent kinase (CDK) complexes that mediate cell cycle progression, and this may or may not involve activation of p53 [39]. Activation of p53 and $p21^{CIP1}$ in

senescent cells can be transient; p53 and $p21^{CIP1}$ protein levels generally decrease after growth arrest has become established [19]. $p21^{CIP1}$ expression decreases after this peak, but $p16^{INK4A}$ can increase and contribute to maintaining growth arrest in some, but not all, senescent cells [19].

Although the initial senescence-inducing signals mediate cell cycle arrest, senescent cells can continue to remodel their chromatin and transcriptional landscape to develop other features of senescence, such as the SASP, in some, but not all, senescent cells. If these senescent cells persist, they can continue evolving toward a late phase of senescence. Activation of endogenous Line-1 (L1) transpositional elements can contribute to a pro-inflammatory SASP phenotype in late senescence [19]. L1 elements can lead to chromosomal rearrangements, loss of histones, and activation of cGAS/STING in response to transposon insertion-induced DNA damage, which exacerbates the inflammatory SASP through interferon 1 (IFN-1) [40]. More investigation is needed to identify molecular and functional characteristics of early *vs*. deep senescence across various cell types to understand potential beneficial *vs*. detrimental effects on health-span, and if targeting transposon-related reverse transcriptases impacts viability of pro-inflammatory late senescent cells of the D-subtype and their impact on other cells, tissues, and systemic function.

3. Differences in the SASP

3.1. The SASP is modifiable by the microenvironment

An intracellular IL-1α/ miR-146a/b/ IL-6/ C/EBP-β loop [41] and related p38/NF-κB [42], cGAS/STING [43], and mTOR-mediated pathways [44] appear to contribute to the changes in gene expression that result in the SASP [2]. Expression and characteristics of the SASP vary considerably with the location of the senescent cell, cell type of origin, cause of senescence, hormonal *milieu*, drugs, presence of pathogens, and the passage of time. Endogenous or pharmacological glucocorticoids can attenuate the release of a subset of SASP factors [45]. Further, the SASP can be downregulated or upregulated by circulating micro-RNAs as well as mt-DNA [12, 46, 47]. Inhibitors of pathways involved in SASP expression, such as metformin and rapamycin, attenuate the SASP, partly explaining their beneficial effects on age-related tissue dysfunction [48, 49]. JAK-STAT3 inhibition leads to reprogramming of the SASP, which decreases frailty and bone loss in old animals [50]. Even intermittent application of some SASP inhibitors appears to alleviate conditions related to aging [51]. However, drugs that are SASP inhibitors can act through other mechanisms, making it difficult to disentangle their effects on age-related phenotypes that are due to SASP modulation from other effects. These other mechanisms can also contribute to side effects that may not be related to SASP suppression. For example, rapamycin can induce insulin resistance by directly acting on TORC2 [52].

3.2. Endocrine and paracrine impact of the SASP

Accumulation of senescent cells can cause local and systemic inflammation, tissue destruction, immune system inhibition, and stem and progenitor cell dysfunction due in part to the SASP. SASP factors can spread senescence to normal, non-senescent cells both

locally and systemically: intraperitoneal transplantation of small numbers of senescent ear fibroblasts or preadipocytes in young mice was sufficient to cause early onset of physical dysfunction [53]. The transplanted cells remained within the peritonaeum, yet the recipient mice developed senescent cells in distant organs including the spleen, intestine, and fat depots that originated from the recipients' own, previously normal cells, illustrating the autocrine and paracrine nature of the SASP [53]. This was linked to development of premature aging-like phenotypes, including decreased activity, reduced grip strength, and premature death from a range of conditions similar to those causing death in naturally-aged mice.

The SASP is regulated both at the transcriptional and post-translational levels and can intensify over time. Positive feedback loops that reinforce the SASP and other complex regulatory mechanisms may contribute to enhanced SASP factor secretion. An example of such a self-amplifying positive feedback loop involves regulation of IL-1α secretion [54]. IL-1α activates the transcription factors, C/EBPβ and NF-κB, leading to enhanced production of IL-6 and IL-8 and IL-1α itself. Similarly, the chemokine receptor CXCR2 and its ligand, GRO-α, can be upregulated in senescent cells, contributing to the formation of a network that reinforces growth arrest in a p53-dependent manner [55]. The SASP can also be regulated at an epigenetic level. For example, SASP induction involves recruitment of BRD4 to senescence-activated super-enhancers adjacent to SASP factor genes [56]. Among other pathways, mTOR and autophagy are also SASP regulators and can reinforce senescence [44, 57].

Through the SASP, senescent cells can signal and influence their surrounding environment and distant tissues, which can be beneficial or detrimental. For example, keratinocytes transiently exposed to SASP factors can have increased regenerative capacity. However, sustained exposure to the SASP can lead to the induction of senescence in these cells [58].

The SASP is the best-studied mechanism by which senescent cells influence their neighbors, but it is not the only one. For example, senescent cells can signal and influence adjacent cells through juxtacrine NOTCH/JAG1 signaling [59], ROS production [26], or by cargo transfer, which occurs through the formation of cytoplasmic bridges or release of exosomes [60]. Factors contributing to the spread of senescence vary among cell types and need to be explored further.

3.3. Anti-apoptotic defenses vary among senescent cells

Like cancer cells such those as in some types of B-lymphomas or lymphocytic leukemias, senescent cells can resist the pro-apoptotic effects of their own SASP through upregulating pro-survival/ anti-apoptotic defenses. The first identification of the responsible Senescent Cell Anti-apoptotic Pathways (SCAPs) was achieved by comparing the transcriptomic and proteomic profiles of senescent to non-senescent human cells (preadipocytes and Human Umbilical Vein Endothelial Cells [HUVECs]) [5]. This indicated higher expression of anti-apoptotic networks related to BCL-2/BCL-xL, PI3K/AKT, p53/$p21^{CIP1}$/serpines, dependence receptors/Src and tyrosine kinases, and HIF-1α in senescent than non-senescent cells [5, 33]. Subsequently, it was discovered that senescent cells can also have upregulated chaperones, such as HSP-90 [61], which interact with AKT or ERK to promote apoptosis

resistance. Up-regulation of these SCAPs might be related to senescence-associated mitochondrial dysfunction: mitochondria in senescent cells can have decreased membrane potential and increased resistance to fragmentation [62]. However, not all senescent cells are the same. Different types of senescent cells express different SASP factors, senescence markers, and, importantly, SCAP pathways to resist apoptosis. For example, senescent human preadipocytes do not depend on BCL- 2 family pro-survival proteins. On the other hand, senescent HUVECs display upregulation of BCL- 2 family members, particularly BCL- xL, as compared to non-senescent HUVECs [5]. Other SCAPs in senescent HUVECs include components of the PI3 kinase and HIF- 1α pathways. Senescent cells can have also increased FOXO4, which prevents cell death by sequestering p53 in the nucleus [63].

Only some senescent are pro-apoptotic and pro-inflammatory. Here, we term these cells Deleterious (D) senescent cells, which may comprise about 30 to 70 % of the senescent cell population (unpublished observations). Other senescent cells, termed here Helper- (H-) senescent cells, do not appear to release a substantial amount of inflammatory and pro-apoptotic factors (unpublished observations). H-senescent cells might promote stem cell function, wound healing, and tissue and remodelling through secretion of growth factors, such as PDGF-AA and TGF-β [13, 16, 64]. However, if H-senescent cells persist, they may promote cancer relapse (if they harbor oncogenic mutations and escape senescence-associated replicative arrest) and possibly other disorders. We speculate there could be mechanisms through which D- and H-senescent cells interconvert, but this has to be fully explored.

4. Distinct effects of senolytics on different types of senescent cells

The identification of molecular pathways that permit viability of senescent cells provided the route to developing agents that selectively induce apoptosis in senescent cells *vs.* non-senescent cells, senolytic drugs. The Src/tyrosine kinase inhibitor, dasatinib, and the flavonoid, quercetin, were shown to induce apoptosis in senescent, but not non-senescent, primary human preadipocytes and HUVECs, respectively. Dasatinib targets, among others, dependence receptor/Src kinase SCAPs, while quercetin targets the BCL-2/BCL-xL, PI3K/AKT, and p53/$p21^{CIP1}$/serpine SCAPs [5]. Based on this discovery, inhibitors of pro-survival BCL-2 family members, including navitoclax (ABT263), which inhibits BCL-xL/BCL-w/BCL-2, were later shown to induce apoptosis in senescent human fibroblasts and endothelial cells, but not senescent preadipocytes [6, 65]. The selective BCL-xL inhibitors, A1331852, and A1155463, were also shown to be senolytic against the same types of senescent cells [66]. Fisetin, which is related to quercetin, was discovered to be senolytic [66]. Several additional senolytic interventions have been identified based on the strategy of targeting SCAPs. For example, a FOXO-4-related peptide that inhibits the PI3K/AKT/$p21^{CIP1}$/serpine SCAP by blocking interaction between p53 and the transcription factor FOXO4, leads to release of p53 from the nucleus and induction of cell-intrinsic apoptosis in some types of senescent cells [63].

In addition to pathway-based senolytic development, high-throughput approaches were also used to identify new agents [61]. Such an approach was used to identify HSP-90 inhibitors as a class of senolytics. HSP-90, a family of ubiquitously expressed molecular chaperones,

can promote cell survival through stabilization of the AKT or ERK signaling pathways, that may become upregulated during senescence. Disruption of the HSP-90-AKT interaction inhibited the PI3K/AKT pathway, resulting in selective killing of senescent cells. The HSP-90 inhibitors, geldanamycin and tanespimycin (17-AAG), were senolytic in fibroblasts, endothelial cells, and mesenchymal stem cells [61]. In drug screens using models of oncogene-induced senescence in normal cells *vs*. therapy-induced senescent cells, cardiac glycosides (CGs) were identified as a class of senolytics [67, 68]. Cardiac glycosides, such as digoxin and ouabain, inhibit Na^+, K^+-ATPase, possibly contributing to their senolytic activity. Ouabain or digoxin also elevate several pro-apoptotic BCL-2 family proteins that can trigger apoptosis in senescent fibroblasts [68].

Elevated lysosomal SA β-gal activity in senescent cells has been exploited to develop drugs with selectivity for senescent cells. One example is a drug delivery system that involves encapsulating cytotoxic drugs with galacto-oligosaccharides. These capsules can be loaded with various cytotoxic compounds and take advantage of increased SA β-gal activity to release their cargo near senescent cells. A recent study used a similar strategy involving a galactose-modified prodrug [69]. Here, preferential processing of galactose-modified duocarmycin, a cytotoxic conjugate, was used to eliminate several senescent cell types. Potential problems with this approach are that not all senescent cells have increased SA β-gal activity and not every cell with high SA β-gal activity is senescent. For example, activated macrophages, which may not be truly senescent, can have increased SA β-gal activity[70]. This raises concerns about the senolytic specificity of approaches based on targeting SA β-gal activity.

Several senolytics, including dasatinib, quercetin, fisetin, navitoclax, the HSP-90 inhibitor, alvespimycin, and a peptide that targets the BCL-2- and p53-related SCAPs, have been demonstrated to be effective in reducing senescent cell burden in mice, with decreases in SA-βgal activity, $p16^{Ink4a}$, $p21^{Cip1}$, and SASP factor mRNAs, telomere-associated foci, and other senescent cell indicators [53, 71, 72]. Among the effects of senolytics in mice so far are: 1) restoration of metabolic function in obese mice [24]; 2) decreased hepatic steatosis in old mice [29]; 3) decreased frailty, osteoporosis, loss of intervertebral disc glycosaminoglycans, and spondylosis in progeroid *Ercc1*$^{-/\Delta}$ mice [73]; 4) decreased gait disturbance in mice following radiation damage to a leg and hematological dysfunction caused by whole-body radiation [5, 74]; 5) improved cognitive and behavioral functions [75, 76]; 6) improved pulmonary function and reduced pulmonary fibrosis in mice with bleomycin-induced lung damage, a model of idiopathic pulmonary fibrosis [71]; 7) improved physical function and increased median lifespan in old mice [53, 73]; 8) improved cardiac ejection fraction and fractional shortening in old mice, enhanced vascular reactivity in old mice, and decreased vascular calcification and increased vascular reactivity in hypercholesterolemic, high fat-fed *ApoE*$^{-/-}$ mice [5, 72]; and 9) reduced senescent cell-like, intimal foam cell/macrophages in vascular plaques in high fat-fed *LdlR*$^{-/-}$ mice [30];

The target of senolytic drugs is senescent cells rather than a single receptor or an enzyme *per se*. Developing senolytic drugs arguably has more in common with developing antibiotics than, for example, an antihypertensive. In the cases of senolytics and antibiotics, the strategy is to transiently target cell-specific defense networks with the goal of eliminating a

damaging cell type that depends on those networks, sometimes using agents or combinations of agents to hit multiple nodes on such networks. This can increase effectiveness. It also can reduce adverse effects by more specifically targeting pathogens or senescent cells that depend on a network than using approaches against a single, narrow target that necessitate sustained drug presence, such as a receptor or enzyme. The latter frequently can be shared across other cells types that are not the main target. Indeed, drugs with single or limited targets, such as navitoclax, have off- target apoptotic effects on multiple nonsenescent cell types, limiting clinical translation or requiring local application rather than systemic delivery, such as oral administration [77]. To most effectively target multiple senescent cell types in different diseases, a combination of senolytics or individual agents that have multiple pro-survival SCAP network targets, administered transiently in a hit-and-run fashion, may turn out to be the most effective approach, permitting oral/systemic use and minimizing adverse events from off target effects or the injections or other approaches needed for local administration.

5. Threshold Theory of Cellular Senescence

The Threshold Theory of Cellular Senescence holds that once senescent cell burden increases beyond the point that clearance by the immune system can keep pace with feed-forward generation of new senescent cells caused by the SASP, senescent cell burden becomes self-amplifying, leading to progressive tissue dysfunction and occurrence of co-morbidities [18, 78]. Several findings support this theory. 1) Senescent cell turnover slows with age in mice, and this correlates with mortality [79]. 2) Patients who receive chemotherapy for cancer have a higher burden of senescent cells and develop age-related diseases faster than age-matched individuals [80, 81]; 3) Total body clearance of senescent cells alleviates age-related brain inflammation, cognitive impairment, and psychiatric deficits in mice [75]. 4) Fewer senescent cells had to be transplanted into obese than age-matched lean mice to cause dysfunction and early death[53]; obesity is associated with increased senescent cell burden[25, 82]; 5) Transplanting 10^6 senescent preadipocytes intraperitoneally into younger mice caused decreased treadmill endurance, grip strength, daily activity, food intake, and lifespan, while transplanting $2x10^5$ senescent cells did not [53]. Hence, reducing the burden of D senescent cells to below a certain threshold may prove to have a positive impact on overall health and alleviate multiple disease conditions, a possibility that remains to be tested. Permanently reducing the ability to generate H senescent cells might impair such physiological functions as tissue repair and wound healing. Data about the abundance and characteristics of D and H senescent cells across tissues are necessary for devising interventions to selectively lower the burden of persistent, pro-inflammatory, pro-apoptotic D senescent cells, while not eliminating potentially beneficial H senescent cells, or at least preserving ability for H cells to be generated when needed.

6. Conclusions

Cellular senescence is a dynamic process that can be modified by the local microenvironment, host *milieu*, passage of time, drugs, and disease states. Senescent cells may be of helper or deleterious subtypes, depending on context and type of inducer of

cellular senescence. However, little is currently known about regulation and progression of the SASP, windows of vulnerability/resistance of senescent cells to different types of senolytics, and impact of disease states and the microenvironment on different populations of senescent cells. It is critically important to understand the diverse populations of senescent cells and their functional relevance across tissues and diseases. It will be important to compare variation in senescence markers between the D and H senescent cell subtypes *in vivo*. Such knowledge could contribute to developing strategies for selectively removing D senescent cells at the right time points, while leaving H senescent cells available for physiological functions such as tissue repair. Also, we anticipate that H senescent cells might be interconvertible with the D subtype through as yet unknown mechanisms that need to be explored further. Efforts must be taken to understand which tissues preferentially harbor D and H senescent cells to determine if local or systemic clearance of senescent cells may be the better option in aged or diseased patients.

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HIGHLIGHTS

- Cellular senescence is a dynamic process and appears to be a heterogenous cell fate.
- Senescent cells can be of helper (H) or deleterious (D) subtypes.
- Lowering the burden of the D-subtype below a threshold may alleviate co-morbidities and enhance health-span, while reducing adverse effects from targeting the H-subtype.

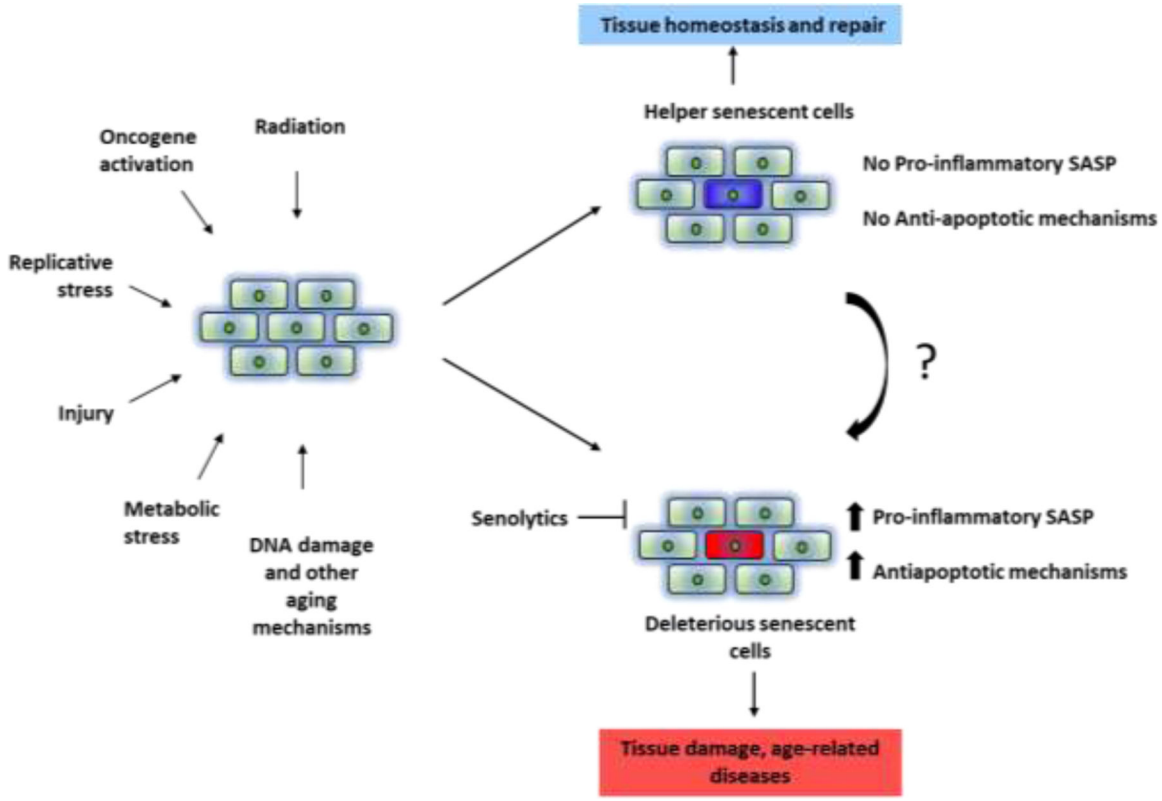

Figure 1.

Theoretical Characteristics of Helper (H) and Deleterious (D) Senescent Cells. D-senescent cells may have a pro-inflammatory, pro-apoptotic, tissue-damaging SASP and be susceptible to senolytics. H-senescent cells may not have a SASP, may not have upregulated SCAPS, and might not be cleared by senolytics. H senescent cells may be convertible into D senescent cells through as yet unknown mechanisms.