Cellular senescence and the senescent secretory phenotype: therapeutic opportunities

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Review Series

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Aging is the largest risk factor for most chronic diseases, which account for the majority of morbidity and health care expenditures in developed nations. New findings suggest that aging is a modifiable risk factor, and it may be feasible to delay age-related diseases as a group by modulating fundamental aging mechanisms. One such mechanism is cellular senescence, which can cause chronic inflammation through the senescence-associated secretory phenotype (SASP). We review the mechanisms that induce senescence and the SASP, their associations with chronic disease and frailty, therapeutic opportunities based on targeting senescent cells and the SASP, and potential paths to developing clinical interventions.

Introduction

The world's elderly population is growing rapidly. As people reach advanced age, they often face years of disability marked by multiple chronic diseases, frailty, and loss of independence. This burden of disability threatens social and economic stability. A huge challenge for modern biomedical research is to compress, if not eliminate, this period of frailty and disability and to increase health span. How can this challenge be met?

Aging is a large, if not the leading, risk factor for most of the chronic conditions that limit survival, independence, and well-being (1). These chronic disorders, including atherosclerosis, most cancers, dementias, diabetes, and many others (Figure 1), become progressively more prevalent as the elderly population grows. A prime suspected cause of these prominent age-related disorders is the chronic, nonmicrobial inflammation that develops in multiple tissues. Hallmarks of inflammation, including elevated IL-6, TNF-α, and immune cell chemokines, are associated with dementias (2), depression (3), atherosclerosis (4–8), cancers (9–11), diabetes (12–14), and mortality (2, 15, 16). Inflammation is perhaps the most important physiologic correlate of the age-related frailty syndrome (17–20), which includes heightened vulnerability to stresses (e.g., surgery, infection, or trauma), coupled with muscle wasting (sarcopenia) and cachexia/fat tissue loss, all of which become increasingly common in old age (17–19, 21–29). Frailty predisposes to chronic disease, loss of independence, and mortality and greatly increases health costs (25, 27).

Until recently, the powerful association between age and chronic disease has mainly been noted with little hope of intervention. A critical roadblock to enhancing health span is the lack of effective treatments for age-related frailty and chronic diseases as a group. Currently available treatments (social supports, mobility aides, and "Band-Aid" treatments for end-stage, downstream symptoms) are not directed at the root causes of age-related dysfunction. Treating chronic diseases one at a time does not suffice (30). Calculations based on mortality data in the United States produce surprising predictions: if cancer was eliminated as a cause of death, average human life span would increase only 3%–4% (31). The same is true were ischemic heart disease to be "cured" (30). Yet caloric restriction, which retards broad basic aging processes by as yet

incompletely understood mechanisms, extends life span in animal models, including mice, by much larger increments (32). Clearly, clinical practice would be transformed if mechanism-based treatments could be devised that break the link between fundamental aging processes and chronic diseases, making aging a modifiable risk factor. The recent awareness that age-related disorders can be driven by one or more basic aging processes has inspired efforts to identify these processes and develop strategies, preferably pharmacological in nature, to intervene.

Cellular senescence

One basic process that may contribute to age-related dysfunction and chronic sterile inflammation is cellular senescence (Figure 2). Cellular senescence refers to the essentially irreversible growth arrest that occurs when cells experience potentially oncogenic insults (33–38). There is now strong evidence that cellular senescence is a potent anticancer mechanism (39–42). In contrast, despite its name, its discovery over 50 years ago, and increasing data associating senescent cells with aging phenotypes and age-related pathology (43–50), evidence has only recently emerged showing that eliminating senescent cells can actually delay age-related dysfunction (51), at least in a progeroid mouse model. This finding still must be tested in chronologically aged models, but this is the first clear evidence that senescent cells are important drivers of multiple age-related pathologies. How cellular senescence promotes age-related diseases, frailty, and dysfunction remains one of the important questions in the biology of aging and clinical geriatrics.

The abundance of senescent cells increases in multiple tissues with chronological aging and in progeroid syndromes (43, 47, 50, 52–55). Several processes have been identified that cause or are associated with cellular senescence, all of which also increase with age (Figure 3). Senescence-causing inducers include repeated cell division and strong mitogenic signals, telomere shortening, DNA damage and mutations, protein aggregation, and increased ROS (46, 56–60). These insults activate the p53 and p16^{INK4a} tumor suppressor pathways and potentially other pathways that initiate a senescence response. Once initiated, senescence takes days to weeks to become fully established and irreversible. The process is reinforced by an intracellular signaling loop including ROS linked to DNA damage responses (DDRs), NFκB, and transforming growth factor-β, as well as an IL-1α, IL-6, and CCAAT
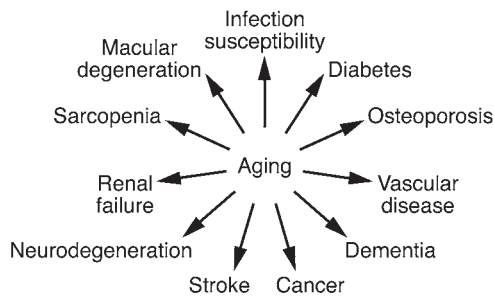
Figure 1
Aging is the leading risk factor for most serious chronic diseases and disabilities, including strokes, heart disease, cancers, dementias, osteoporosis, arthritis, diabetes, metabolic syndrome, kidney failure, blindness, and frailty.

enhancer binding protein-β (C/EBP-β) loop (59, 61–64). Senescent cells reorganize chromatin, resulting in heterochromatin formation, extensive gene expression changes, increased cell size and protein content, and changes in cell and organelle shape (55, 65, 66). Senescent cells are metabolically active, relatively resistant to apoptosis, and appear to be removed by the immune system (33, 67, 68). Senescence is effectively a cell fate that, like replication, differentiation, or apoptosis, can occur at any point in life.

A common feature of aging tissues is low-level chronic inflammation, termed sterile inflammation (indicating an absence of detectable pathogens) or inflammaging (69–72). Chronic inflammation can drive pathology by at least two mechanisms. First, infiltrating immune cells can degrade tissues because they release reactive or toxic moieties. Second, inflammatory cytokines can provoke phenotypic changes that are independent of the immune system in nearby cells. For example, IL-6 and IL-8 can stimulate angiogenesis, disrupt cell-cell communication, impede macrophage function, induce innate immune responses, and promote epithelial and endothelial cell migration and invasion (73–79).

Little is known about the source of the sterile inflammation that fuels most major age-related diseases, including degenerative pathologies, such as loss of brain function, as well as hyperproliferative pathologies, such as cancer. This chronic inflammation may derive partly from an age-related decline in homeostatic immune function or resistance to endogenous microbes. Chronic inflammation may also derive in part from senescent cells: senescent cells secrete proinflammatory cytokines, chemokines, and proteases, termed the senescence-associated secretory phenotype (SASP) (80, 81).

SASP
The SASP is primarily a DDR (82). The SASP, through the inflammatory, growth-promoting, and remodeling factors that it produces, can potentially explain how senescent cells alter tissue microenvironments, attract immune cells, and, ironically, induce malignant phenotypes in nearby cells. Proteins that are associated with the SASP, such as TNF-α, IL-6, MMPs, monocyte chemoattractant protein-1 (MCP-1), and IGF binding proteins (IGFBPs), increase in multiple tissues with chronological aging (64), and occur in conjunction with sterile inflammation. Furthermore, expression of the SASP components, IL-6, IGFBP-2,

and plasminogen activator inhibitor-1, is much higher in p16INK4a-positive senescent cells isolated directly from fat tissue of older progeroid mice compared with nonsenescent cells from the same tissue (51). This finding suggests that the SASP is the main driver of age-related inflammation, at least in fat tissue under certain conditions. Thus, selective elimination of senescent cells or their effects might be a means to reduce age-related sterile chronic inflammation, enhance health span, and interrupt the link between aging and chronic disease.

Cellular senescence is a double-edged sword. Central to understanding how senescent cells could have positive and negative effects on organismal health are two concepts. The first is antagonistic pleiotropy. This element of the evolutionary theory of aging predicts the existence of processes that were selected to ensure early life fitness (e.g., protection from cancer by promoting immune responses), but have unselected deleterious effects late in life that cause aging phenotypes and pathology (83).

The second concept is the SASP, which can have positive or negative effects, depending on context. The SASP can cause local and potentially systemic inflammation, disrupt tissue architecture, and stimulate growth of nearby malignant cells when the SASP is both pronounced or persistent, as in old age, massive obesity, or progerias (65, 84–88). Conversely, a localized, time-limited SASP may be important in resolving tissue damage, at least in younger individuals, an example of antagonistic pleiotropy. The SASP might alert nearby cells to potential danger and promote immune clearance of the damaged cells (67, 68, 89). Furthermore, SASP MMPs limit fibrosis following liver injury or during skin wound healing (68, 90, 91), which is beneficial. The SASP cytokines, IL-6 and IL-8, also reinforce the senescence growth arrest, at least in some senescent cells (62, 63), which is beneficial in defense against cancer. However, these cytokines can also cause epithelial-to-mesenchymal
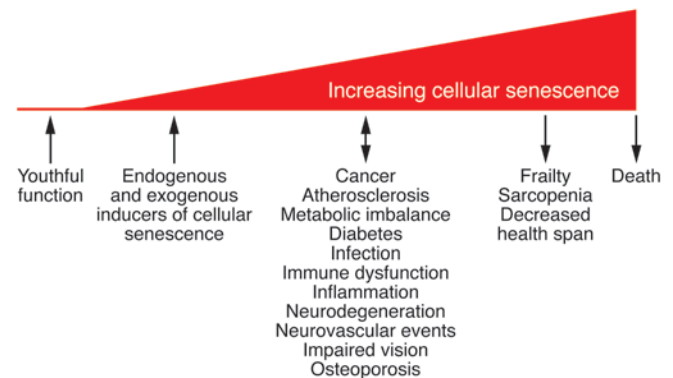
Figure 2
A disruption of the intersection between fundamental aging mechanisms and processes that lead to chronic diseases may delay age-related diseases and disabilities as a group and thereby lengthen health span. The increasing burden of senescent cells might contribute to the early etiology of age-related diseases and accelerate progression of these diseases following their initiation. Chronic disease pathology, coupled with the spread of senescence to neighboring healthy cells, might further drive cellular senescence, thus contributing to a spiral of increasing inflammation and dysfunction. Among other possibilities, chronic inflammation associated with the SASP, combined with with inflammation from preclinical and overt chronic disease, may predispose to frailty, sarcopenia, and eventually mortality.
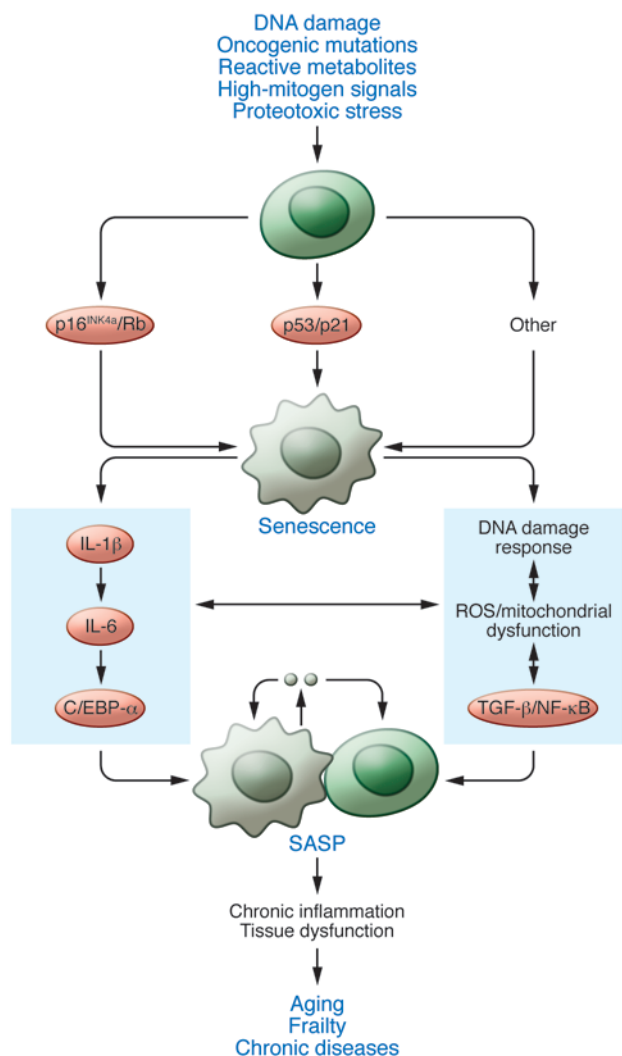
Figure 3
A number of inducers can act alone or in combination to push cells into the senescent cell fate through pathways involving p16^{INK4a}/Rb, p53/p21, and likely other pathways. Triggers may include DNA damage (e.g., telomere shortening and single- and double-strand breaks); oncogenic mutations (e.g., Ras, Myc, B-Raf); reactive metabolites (e.g., ROS, ceramides, fatty acids, high glucose); high mitogen and nutrient signals that increase mTOR activity; and proteotoxic stress (e.g., protein aggregation and unfolded proteins). These may contribute to widespread changes in gene expression and chromatin remodeling (heterochromatin formation) that underlie senescence-associated growth arrest, the SASP, and changes in morphology. In these respects, cellular senescence can be viewed as a cell fate reminiscent of differentiation, replication, or apoptosis (external and internal inducers, transcription factor cascades, gene expression changes and chromatin remodeling, leading to changes in function). Evidence is better for some of the initiators and mediators of senescence than for others, and future research is likely to uncover additional initiators and mediators. Intracellular autocrine loops reinforce progression to irreversible replicative arrest, heterochromatin formation, and initiation of the SASP over a matter of days to weeks. In addition to removing cells from the progenitor/stem cell pool, senescence may contribute to tissue dysfunction and chronic disease predisposition through the SASP and associated chronic sterile inflammation and degradation of the extracellular matrix.

transitions, which promote cancer (77, 78). Conceivably, the SASP could play a role in defense against infections or other insults, possibilities that need to be studied. Remarkably, though, continuous clearance of senescent cells for over 9 months did not have overt adverse effects, but rather enhanced health span and function, at least in progeroid mice housed in an animal facility (51).

An intracellular IL-1α / miR-146a/b / IL-6 / C/EBP-β loop as well as related p38/NF-κB– and mTOR-mediated pathways appear to contribute to the changes in gene expression that result in the SASP (Figure 2 and refs. 61–64, 92–94). SASP components include proteins that are generally conserved across cell types, although differences exist among cell types (81) and presumably across tissues, a speculation that needs further testing. Very little is known about the contribution, if any, of nonprotein factors such as nucleotides, bradykines, prostenoids, ceramides, or ROS to effects of the SASP. The composition of the SASP may vary as time progresses after the initiation of senescence and might partly depend on the mechanism through which senescence is induced. In support of this possibility, senescence induced by oncogenic RAS, p16^{INK4a} overexpression, or p53 activity is associated with variability in both the quality and extent of SASP protein secretion (81, 95). Thus, while we speak of the SASP, it is unlikely that there is a singular SASP. Rather, the amounts of SASP components released depend on cell context. For example, in senescent human fibroblastoid keratocytes in the eye, IL-6 is actually decreased (96). Studies are required to delineate upstream mechanisms causing the SASP, explore differences in the SASP among cell types, and elucidate the nature of the SASP in primary senescent cells in vivo rather than in cell culture.

Whether the SASP causes chronic age-related diseases needs to be determined in vivo. Testing of this possibility is now underway using mice from which senescent cells can be selectively removed (51) that are bred with mouse models of human age-related chronic diseases or that are treated with various dietary and other interventions. The SASP also appears to spread from cell to cell (97), potentially amplifying the senescent cell burden, sterile inflammation, and chronic disease progression, especially once the capacity of the immune system to remove senescent cells is overwhelmed. The mechanisms and consequences of spreading senescence merit further investigation.

The SASP likely has wide-ranging relationships with the immune system. Immune system elements, especially innate immune responses involving macrophage infiltration, are involved in clearing senescent cells (67, 98–100). Macrophage chemokines,

including MCP-1, are prominent components of the SASP (64, 81). However, tissue macrophage responses appear to decline with aging (101), potentially contributing to senescent cell accumulation in old age. A high burden of senescent cells could interfere with immune function. Consistent with this speculation, chronic exposure to IL-6 inhibits macrophage function (102) and SASP proteases could cleave FAS ligand or other cell surface proteins required for effective immune function. More insight is needed into how senescent cells affect the immune system and are removed by it. The speculation that reducing senescent cell burden could actually enhance immune responses to pathogens needs to be tested.

Interventions targeting cellular senescence and the SASP

Potential strategies for mitigating the deleterious effects of senescent cells include interfering with pathways that lead to senescence-associated growth arrest, eliminating senescent cells, and interfering with the adverse effects of senescent cells by targeting the SASP. The first strategy could be problematic if the mechanisms through which cellular senescence defends against cancer are compromised. Interfering with pathways that can induce senescence-associated replicative arrest are likely to promote cancer, as occurs when $p16^{INK4a}$, the retinoblastoma (Rb) protein, or p53 is diminished or inactivated (67, 103). On the other hand, strategies that delay senescent cell accumulation by reducing progenitor turnover, metabolic damage, or other processes that can cause cell damage might be beneficial. For example, caloric restriction delays cellular senescence (104) and could exert part of its beneficial effect on health span through doing so, a speculation that merits further testing.

The second approach, eliminating already-formed senescent cells, may not only reduce tissue inflammation and organ dysfunction but might also reduce cancer risk. Cells can become senescent when they harbor potentially oncogenic mutations. Furthermore, senescent cells can promote malignant transformation in neighboring cells in mouse xenografts (86), possibly through SASP-induced inflammation and tissue MMP and mitogen secretion. Indeed, health span was enhanced by clearing senescent cells in progeroid mice, without interfering with anticarcinogenic pathways upstream of senescence (51). Because terminally differentiated cells share some properties of senescent cells such as growth arrest, we were concerned that targeting senescent cells in INK-ATTAC mice might damage cells like neurons. However, terminally differentiated cells did not appear to be disrupted to the point of causing symptoms, even after over a year of continuous senescent cell removal.

The third approach, preventing development of the SASP or ameliorating its effects, could also enhance function and reduce inflammation and cancer risk, albeit without the finality of actually eliminating the senescent cells that potentially harbor cancerous lesions. Ideally, ameliorating the SASP would be achieved without interfering with senescence-associated anti-oncogenic pathways or undoing growth arrest.

Eliminating senescent cells may be possible in humans. The distinct morphology, secreted protein patterns, and gene expression profiles of senescent cells support the feasibility of doing so. At least two approaches can be envisaged for removing senescent cells: the use of antibodies to specifically target senescent cells or the development of small molecules to selectively kill them. Other approaches might include viral-based nucleotide delivery or vaccines. The antibody approach would entail developing biologicals that recognize epitopes that are more highly expressed by senescent compared with nonsenescent cells, coupled to a cytolytic agent. This approach has been difficult in the cancer field and could also be problematic with respect to eliminating senescent cells. However, unlike for cancer interventions, complete elimination of senescent cells may not be necessary for achieving beneficial effects. Thus, targeting epitopes that differ quantitatively between senescent and nonsenescent cells may be reasonable, without needing to discover epitopes that are uniquely present on senescent cells.

The small-molecule approach could be based on high-throughput, cell-based screens of chemical libraries to discover "druggable" molecules that kill senescent cells with greater selectivity than nonsenescent cells. Alternatively, molecular target–based high-throughput screens focused on pathways identified through expression, proteomic, or metabolomic analyses of senescent versus nonsenescent cells may work. These small-molecule approaches could also be adapted to targeting the SASP, as opposed to killing senescent cells. Using one of these approaches, it was discovered that glucocorticoids ameliorate the SASP (105).

Developing interventions to target senescent cells or the SASP will be a tall order. Possibly, combinations of approaches will be required. Because senescent cells do not divide, they are unlikely to develop drug resistance, a problem encountered with compounds that target dividing cancer cells or microbes. Also, finding compounds or antibodies that target 100% of senescent cells and 0% of normal cells might not be necessary to achieve clinical benefit. Thus, it may be feasible to discover agents that target senescent cells or the SASP and test them in aged animals to determine whether they reproduce the beneficial effects of removing senescent cells from genetically engineered progeroid mice.

Clinical trials strategies

Designing clinical trials to test the effectiveness of agents that target senescent cells is a challenge (106). Clearly, studying effects on human life span or health span is not feasible within a realistic time frame. Drugs that must be given early in life to have a beneficial effect in old age would also be nearly impossible to translate into clinical application. Such drugs would need to have virtually no side effects over years to decades. The time required for clinical trials would be prohibitive. Senolytic drugs would need to be developed for subjects who already have symptoms or who are guaranteed to develop them within a short time (e.g., patients with progerias or members of families who carry mutations causing early-onset Alzheimer's disease that have essentially complete penetrance). Readily measurable outcome parameters would need to be devised and accepted by regulatory authorities.

We envisage several potential clinical trial scenarios. Based on these, it may be reasonable to work backward to design drug screens and animal testing strategies. One scenario would be to target specific age-related chronic diseases in which senescence plays an etiologic role and in which eliminating senescent cells or SASP components would result in a rapidly detectable, clinically meaningful response. For example, effects of senolytics on ameliorating peripheral insulin resistance in obese subjects could be tested because massive obesity is associated with fat tissue senescent cell accumulation and elevated levels of circulating SASP-related cytokines that cause insulin resistance (56, 85). Another example would be to test whether progression of cancers that have failed conventional treatment is prevented, based on the contribution of senescent cells to cancer progression in xenograft

models (86). Other possibilities include testing whether senolytics enhance the resolution of atherosclerotic lesions, osteoarthritis, or fracture nonunion, conditions that are associated with focal senescent cell accumulation (107–109). These conditions might be amenable to local treatment, possibly by injection of senolytic agents. The effects of senolytics on the progression of dementia could be tested in longer clinical trials (lasting months rather than weeks) because Alzheimer's disease and other dementias are associated with senescent cell accumulation at sites of brain pathology (reviewed in ref. 110). Of course, all these approaches would first need to be thoroughly vetted in proof-of-principle, preclinical studies in appropriate disease-specific animal models (preferably in old rather than young genetically modified animals, so that the aging tissue microenvironment is reproduced).

Another scenario is to target the frailty syndrome, which is associated with systemic inflammation (17–20) and potentially the SASP. Trials can be envisaged in borderline frail subjects (with intermediate Fried or Rockwood scores; refs. 25, 27, 28) who are about to undergo medical procedures that increase inflammation and/or cellular senescence and that might push subjects into overt frailty or delirium. These procedures might include, for example, chemotherapy (particularly DNA-damaging agents), radiotherapy, bone marrow transplantation, and anesthesia/elective surgery. In these situations, senolytic treatment for a period before (but not during) the medical procedure might have a measurable effect on time to recovery of symptom scores, strength, or cognition. These possibilities need to be tested first in animal models such as the INK-ATTAC mouse, in which senescent cells can be quantified because of a fluorescent marker and can be selectively eliminated in an inducible manner (51).

Research issues

Issues that need to be addressed before moving senolytics into clinical application include the development of biomarkers of senescent cell burden/SASP activity, identification of any adverse consequences of targeting senescent cells, and determination of when to begin treatment and whether intermittent or continuous treatment is optimal. Blood biomarkers of senescent cell burden, perhaps based on detecting SASP products, would facilitate clinical trials. These biomarkers could be calibrated using a gold standard — for example, INK-ATTAC mice that have had senescent cells removed, senescent cell abundance in fat tissue biopsies, or whole body senescent cell counts at necropsy. Thorough testing of whether senescent cell removal impairs response to infection, wound healing, scarring, or other functions must be conducted in animals and eventually humans. Intermittent rather than continuous senescent cell elimination during periods of relatively good health could remove potentially precancerous senescent cells and reduce chronic inflammation due to the SASP, yet minimize potential adverse effects of senescent cell clearance. This approach might be more attractive than ameliorating the SASP, which may need to be done continuously, unlike removal of senescent cells.

Presumably, clinical trials of senolytics will be done in elderly subjects as well as younger subjects with diseases associated with cellular senescence and inflammation, such as diabetes in massive obesity. However, elderly subjects are not often included in clinical trials, and their study presents special clinical and logistical challenges. There are few geriatricians who have both a deep familiarity with the basic biology of aging and experience in conducting trials of investigational new drugs (INDs). Further, few clinical trial investigators with IND experience have training in geriatrics and a deep understanding about the particular needs of elderly subjects or outcomes relevant to elderly populations. There is an urgent need to build teams of basic scientists in the biology of aging, geriatricians, and clinical trial investigators. In the longer term, there is a need to train investigators who can lead efforts to translate findings from the basic biology of aging into clinical application (106).

Conclusion

If the premise is correct that targeting senescent cells or the SASP can delay or prevent multiple age-related chronic diseases as a group, rather than one at a time, and if this premise can be translated into clinical treatments, we are cautiously optimistic that health care as we know it might be transformed.

Acknowledgments

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