



The metabolic roots of senescence: mechanisms and opportunities for intervention

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Cellular senescence entails a permanent proliferative arrest, coupled to multiple phenotypic changes. Among these changes is the release of numerous biologically active molecules collectively known as the senescence-associated secretory phenotype, or SASP. A growing body of literature indicates that both senescence and the SASP are sensitive to cellular and organismal metabolic states, which in turn can drive phenotypes associated with metabolic dysfunction. Here, we review the current literature linking senescence and metabolism, with an eye toward findings at the cellular level, including both metabolic inducers of senescence and alterations in cellular metabolism associated with senescence. Additionally, we consider how interventions that target either metabolism or senescent cells might influence each other and mitigate some of the pro-aging effects of cellular senescence. We conclude that the most effective interventions will likely break a degenerative feedback cycle by which cellular senescence promotes metabolic diseases, which in turn promote senescence.

Historic perspective on cellular senescence. Cellular senescence, the non-dividing altered state into which many vertebrate cells enter when stressed, was first formally described by Hayflick and Moorhead in the early 1960s (refs. ^{1,2}). Their finding—that normal cells eventually cease dividing (in culture)—challenged the long-held idea initiated by Alexis Carrel in the early 1900s that normal cells were intrinsically ‘immortal’ (in culture)³. And, whereas Carrel suggested that organismal mortality might be a consequence of multi-cellularity, Hayflick and Moorhead suggested that the eventual cessation of cell division (subsequently termed cellular senescence) reflected organismal aging. They also noted that cells derived from malignant tumors did not undergo this form of senescence, suggesting that the senescent state existed to suppress the development of cancer. Many years later, Sager and colleagues showed that cellular senescence was a response to potential tumor-inducing stimuli, and thus formally postulated that the senescence response was a potent anti-cancer mechanism^{4,5}. Since the 1960s, our understanding of cellular senescence, including its physiological and pathological roles, has exploded. In addition, it is now apparent that senescent cells both experience and cause many aspects of metabolic reprogramming, some aspects of which we discuss below.

Cell autonomous and non-autonomous effects of senescent cells. At the core of the cellular senescence response is an essentially irreversible arrest of cell proliferation (growth). It is now clear—from multiple lines of evidence—that this arrest constitutes a potent, cell autonomous anti-cancer mechanism *in vivo*^{6,7}. Consequently, most humans and mice harboring mutations that prevent or suppress the senescence growth arrest (for example, p53 or pRB inactivating or dominant negative mutations^{8,9}) are cancer-prone and generally die an early death due to malignant tumors.

In addition, certain senescent cells become moderately resistant to cell death. In some senescent cells, this cell autonomous resistance is due to increased expression of anti-apoptotic, compared to pro-apoptotic, proteins, a characteristic shared by several cancer cells. This property has led to the repurposing of certain (mostly failed) anti-cancer drugs that were initially designed to kill tumor

cells, but now are being tested as senolytics—that is, drugs that can selectively kill senescent cells, but not non-senescent counterparts^{10–12}. However, it is not yet clear how pervasive cell death resistance is among different senescent cell types. For example, senescent endothelial cells are in general more prone to spontaneous apoptosis than senescent fibroblasts, although even fibroblasts can undergo apoptosis following senescence when faced with certain types of DNA damage (for example, transcription-blocking lesions)¹³. In any case, the mechanisms of cell death resistance in senescent cells remains incompletely understood.

Finally, all senescent cells thus far examined develop a complex, multi-component senescent-associated secretory phenotype (SASP). The SASP acts cell non-autonomously to alter the behavior of neighboring cells and the tissue microenvironment. The SASP is strikingly variable and plastic. It depends on the cell type and senescence inducer (discussed below), and is dynamic, changing characteristics over time^{14,15}. A striking, but not sole, feature of the SASP is the preponderance of pro-inflammatory molecules, including cytokines, chemokines, bioactive lipids and damage-associated molecular patterns (DAMPs; also termed Alarmins). Chronic inflammation is, of course, a major risk factor for many age-related diseases, including late-life cancer; hence the term ‘inflammaging’ has been used to describe the chronic inflammation that is a common attribute of aged tissues and might be at least partially due to the accumulation of senescent cells in aged tissues^{16–18}.

In recent years, it has become apparent that the phenotypes of senescent cells, in addition to the SASP, are remarkably variable, heterogeneous and plastic¹⁹. For example, for many—but not all²⁰—senescent cells, the growth arrest is initiated and/or enforced by the cyclin-dependent kinase and cell cycle inhibitors p16^{INK4a} and p21^{CIP1} (encoded by the *CDKN2A* and *CDKN1A* genes). Likewise, other widely used senescence markers, such as the senescence-associated β -galactosidase (SA- β gal)²¹, reduced expression of the nuclear lamina protein lamin B1 (LMNB1)²², and the relocalization and secretion of the nuclear protein HMGB1 (where, following secretion, it acts as a DAMP)²³, are neither invariable nor universal senescence markers. Thus, assessing the ability of specific stimuli to induce a

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senescence response—and assessing the burden of senescent cells in tissues *in vivo*—requires the use of multiple markers²⁴.

Adaptive vs maladaptive effects of senescent cells. The senescence response can be beneficial or deleterious, depending on the physiological context. This dualism is consistent with the evolutionary theory of antagonistic pleiotropy. Antagonistic pleiotropy postulates that traits selected to ensure the survival of young organisms in natural environments, in which life spans are short, can become deleterious in modern protected environments, in which life spans are significantly longer²⁵. Thus, aging is likely a consequence of the declining force of natural selection with age.

With regard to the beneficial effects of cellular senescence, as noted above, the senescence growth arrest protects young organisms from developing cancer. In addition, SASP factors can optimize the morphogenesis of certain structures in the embryo^{26,27}, and initiate parturition in the placenta^{28,29}. Finally, senescent cells occur transiently at sites of tissue damage where they contribute to wound healing, tissue repair and regeneration, most likely through specific SASP factors^{30–34}.

In contrast, senescent cells increase with age in most mammalian tissues, where they appear to persist. Whether this increase is due to increased production or decreased clearance, for example by the immune system, is unclear. More importantly, experiments using human cells and tissues, transgenic mouse models and pharmacological interventions in cells and mice strongly implicate senescent cells in a large number of age-related pathologies, ranging from neurodegeneration to, ironically, age-related cancer^{6,35–38}. Most of the detrimental effects of senescent cells can be attributed to the SASP, which, as noted above, is rich in pro-inflammatory molecules.

What initiates a senescence response? Little is known about how senescent cells are induced *in vivo*, particularly during aging. Known inducers of senescence responses—at least in cultured cells and mouse models—include, among others, DNA damage, activated oncogenes and mitochondrial dysfunction (discussed below). Because senescent cells are rare, even in old and diseased tissue, it has been difficult to determine how they were induced to senesce *in vivo*. However, single-cell profiling, at both the transcriptomic and proteomic levels, promises to help identify the major drivers of senescence during natural aging and in age-related pathologies. In all cases, senescent cells must undergo metabolic reprogramming in order to maintain their viable growth-arrested state and express the genes and proteins needed to sustain the highly complex, dynamic and heterogeneous SASP^{14,39}. The causes and consequences of these metabolic shifts are discussed below.

Metabolic drivers of senescence

Several forms of metabolic stress can both drive senescence and influence the SASP (Fig. 1). Here we describe some of these and discuss them in the context of aging and disease.

Mitochondrial dysfunction. Mitochondria are major regulators of age-related pathology. Mice that accumulate mitochondrial DNA (mtDNA) mutations at an accelerated rate age prematurely^{40,41}, whereas overexpression of mitochondrially-targeted catalase (mCAT) preserves mitochondrial function and extends lifespan in mice⁴². It is therefore not surprising that senescence and the SASP are similarly responsive to the function of mitochondria within the cell.

Many drivers of mitochondrial dysfunction also result in cellular senescence. These drivers include mtDNA depletion and mutations, inhibitors of the electron transport chain, loss of the mitochondrial chaperone protein HSPA9, loss of the mitochondrial protein deacetylase SIRT3 and the de-acylase SIRT5⁴³, and disruptions in complex I assembly⁴⁴. This mitochondrial dysfunction-associated senescence (MiDAS) phenotype lacks several of the proinflammatory parts of

the SASP, but instead includes its own distinct set of SASP factors. MiDAS is primarily driven by the accumulation of cytosolic NADH, which is normally oxidized by the mitochondria to NAD⁺ (Fig. 2). This lowering of the NAD⁺/NADH ratio inhibits the key glycolytic enzyme, GAPDH, resulting in ATP depletion, AMPK activation and cell cycle arrest⁴³.

Additionally, mitochondria are a source of reactive oxygen species (ROS). Loss of mitochondrial superoxide dismutase (SOD2) in mice drives cellular senescence⁴⁵. A transgenic cell culture model of mitochondrial elimination by mitophagy protects against senescence and the expression of many pro-inflammatory SASP factors; this protection is associated with reductions in both ROS and nuclear DNA damage foci, especially telomere-associated foci⁴⁶. Similarly, mitochondrial ROS can activate the JUN N-terminal kinase, which in turn promotes release of cytosolic chromatin fragments and activation of proinflammatory components of the SASP⁴⁷. These and many other studies suggest that mitochondria-derived ROS may be a key driver of cellular senescence.

Notably, despite evidence that senescent cells drive aging and limit both health span and median lifespan^{48,49}, few interventions that directly antagonize ROS extend lifespan⁵⁰. Moreover, none of these interventions have demonstrated a reduction in age-related accumulation of senescent cells. Rather, studies that target mitochondria and prevent senescence or SASP modules *in vivo* also target other aspects of mitochondrial biology. Thus, it is an open question as to whether mitochondrial ROS are actually causal for the accumulation of senescent cells with age. For example, PGC-1B depletion lowers mitochondrial biogenesis, and rapamycin increases mitophagy⁴⁶. Although both manipulations decrease ROS, they also alter many other aspects of mitochondrial metabolism and function. Additionally, ROS are essential signaling molecules for several processes, such as cell proliferation⁵¹, innate immunity⁵², stem cell renewal^{53,54}, differentiation⁵⁵ and survival⁵⁶. The relationship between ROS, senescence and aging *in vivo* is therefore likely to be more complex than culture models currently indicate.

More recent studies also showed that lowering endoplasmic reticulum (ER)–mitochondria contact by ablating the *Itpr2* calcium channel in mice lowers the number of age-related senescent cells and the levels of several SASP factors, resulting in an extension of health span and lifespan⁵⁷. However, it is unclear if these effects are due to reduced mitochondrial calcium overload, diminished ER–mitochondrial contacts, or some combination of both. These strategies demonstrate a definitive role for mitochondria in the age-related accumulation of senescent cells, but are not specifically informative about whether ROS, *per se*, are to blame. The role mitochondrial function in cellular senescence and its associated phenotypes is also the subject of several recent reviews^{58–61}.

An important caveat to many cell culture studies, especially those using mouse cells, is that while ROS—including mitochondrial ROS—can certainly induce senescence in culture, a majority of these studies were performed in atmospheric, rather than physiological, oxygen concentrations, which alters senescence responses⁶². Thus, many of the observed phenomena are potential artifacts of a hyperoxic state. Next, we discuss the role of oxygen levels in the development of senescence.

Oxygen. Phenotypes of cellular senescence are highly dependent on the levels of oxygen available to the cell. This dependence is often inversely correlated, as more oxygen tends to accelerate senescence. With over 60 oxygen-consuming enzymes in the mammalian genome⁶³, it is not surprising that oxygen levels are a key modulator of many biological processes, including senescence. Murine cells are more sensitive to oxygen levels, as murine fibroblasts cultured at atmospheric oxygen levels undergo rapid senescence, but, due to endogenous telomerase activity, will proliferate extensively if oxygen levels are lowered to 3%—closer to levels observed in the stroma

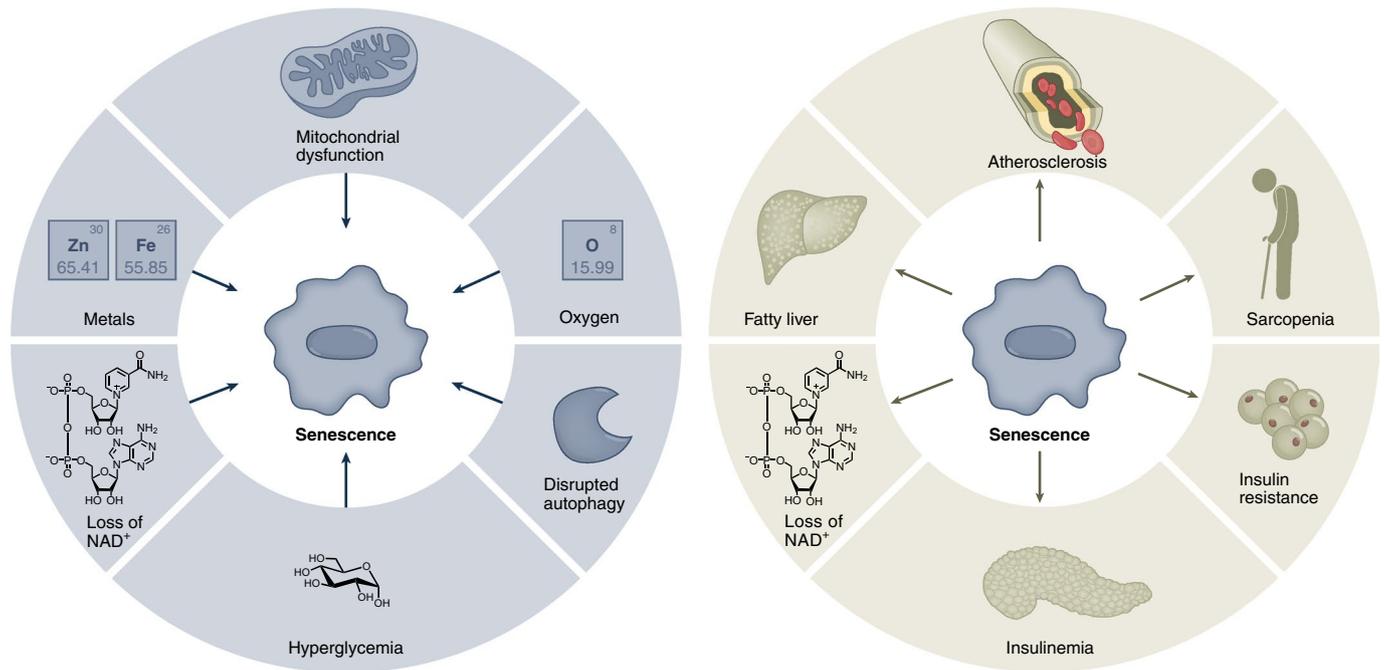


Fig. 1 | Relationships between metabolism and cellular senescence. Left: Metabolic drivers of senescence. Mitochondrial dysfunction can drive senescence through disruption of cytosolic NAD⁺/NADH ratios, production of reactive oxygen species, and potentially other mechanisms. Accumulation of excess metals (especially transition metals) also promotes senescence. Loss of NAD⁺ results in senescence through loss of sirtuin or PARP activities and changes in cellular redox states. Hyperglycemia can drive senescence, although mechanistic detail is still needed. Disrupted autophagy can drive senescence in some contexts, but also prevent it in others. Non-physiological oxygen levels influence the development of senescent cells, with higher oxygen generally favoring senescence. Right: Senescent cells as drivers of metabolic disease. Senescent cells and/or the SASP can drive both formation of atherosclerotic plaques as well as plaque instability. In the liver, senescent cells can promote steatosis. The SASP also activates macrophages, which elevate CD38 and lower tissue NAD⁺ levels. In the pancreas, senescent β -cells promote hyperinsulinemia, but as β -cells are attacked by the immune system, this can become hypoinsulinemia. In peripheral tissues (for example, fat) senescent cells can promote insulin resistance—so senescent cells can drive diabetes and metabolic disease in multiple ways. Finally, senescent cells promote sarcopenia in muscle tissue, which can influence basal metabolism, activity levels, and frailty.

in vivo⁶². Extension of replicative lifespan and stemness are also observed in a subset of human cell lines, such as human amniotic fluid stem cells⁶⁴. Additionally, atmospheric O₂ levels are required for senescence reinforcement following treatment with drugs that activate cell cycle arrest, such as nutlin-3a^{65,66}. Furthermore, lower O₂ levels are required for a human-like SASP in cultured murine fibroblasts⁶⁷, and this SASP is closer to that observed with age. Despite these findings, sub-physiological levels of oxygen (that is, hypoxia) can also activate AMPK, which can suppress the SASP by mTOR inhibition⁶⁸. More broadly, these data signal the potential for artifacts when studying senescence (and likely many other conditions) using atmospheric rather than physiological oxygen levels. Oxygen concentrations vary strongly depending on the tissue and species⁶⁹, so physiological levels for relevant cell types is an important consideration when studying senescence. Several recent articles review the role of oxygen in diverse biological phenomena, including senescence^{63,69–71}.

Unfortunately, hyperoxia is a medical necessity under certain conditions. For example, premature infants, whose lungs have not yet developed, often require supplemental oxygen but later experience increased risks of respiratory conditions, such as asthma, in early childhood⁷². Similarly, individuals that experience the severe symptoms of COVID-19 often require supplemental oxygen⁷³. Treatment of fetal human airway smooth muscle cells and lung fibroblasts with moderate hyperoxia (40% O₂) increased markers of senescence in as short as 7 days^{72,74}, suggesting that senescent cells may play a role in these disorders. Conversely, hyperbaric 100% oxygen was recently found to lower markers of senescence

in specific populations of peripheral blood mononuclear cells⁷⁵, so cell type may play an important role in senescent cell responses to oxygen. Additionally, a murine cardiac ischemia–reperfusion model accumulates senescent cells, and elimination of these cells improves recovery⁷⁶, indicating that fluctuations in oxygen can also drive senescence and pathology. Oxygen level is therefore an important consideration both in cell culture settings, and potentially for the formation of senescence under specific circumstances in animal models and humans.

Disrupted NAD⁺ metabolism. NAD⁺ plays a major role in the regulation of both the cell cycle arrest and the SASP during senescence (Fig. 2). NAD⁺ is a major cofactor for both poly-ADP-ribose polymerase (PARP) and sirtuin family proteins (SIRT). PARP antagonizes senescence by protecting against genotoxic stress⁷⁷, but also promotes NF- κ B activation and secretory phenotypes in senescent cells⁷⁸. Indeed, loss of PARP1 during epithelial cell senescence is associated with increased levels of unrepaired single strand breaks, which in turn reinforce cell cycle arrest through p38MAPK activation⁷⁹. Similarly, inhibition of nicotinamide phosphoribosyltransferase (NAMPT)—which prevents NAD salvage and lowers total NAD⁺ levels—both promotes a senescence arrest and suppresses proinflammatory aspects of the SASP^{43,80}; this effect resembles the phenotype of MiDAS. Importantly, NAD⁺ levels decline with age in many tissues, and this decline is associated with multiple degenerative conditions, including age-related muscle loss and diabetes^{81–83}.

Several sirtuins play key roles in senescence response and the SASP. Beyond SIRT3 and SIRT5, which antagonize MiDAS⁴³, SIRT5

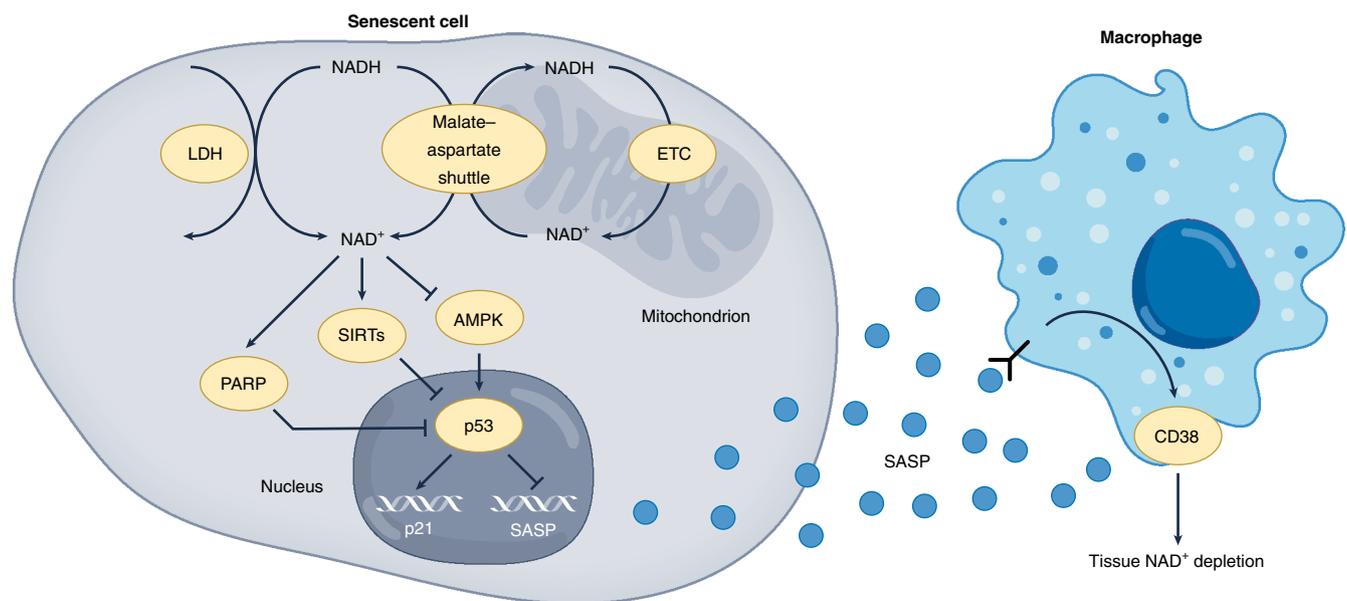


Fig. 2 | NAD Metabolism and Cellular Senescence. NAD⁺ levels and NAD⁺/NADH ratios are controlled by multiple pathways during senescence. The NAD⁺/NADH ratio is maintained by conversion of pyruvate to lactate in the cytosol, or by transfer of reducing equivalent of NADH to the mitochondrion by the malate-aspartate shuttle. NADH is then oxidized back to NAD⁺ in the mitochondrion by the activity of Complex I of the electron transport chain (ETC). Disruption of any of these processes leads to increased cytosolic NADH, AMPK activation, and senescence. NAD⁺ acts through PARPs and sirtuins to prevent genotoxic stress and p53 activation, which promotes senescence, but antagonizes the SASP. Once released by senescent cells, SASP factors can bind their cognate receptors on macrophages, which then elevate CD38. CD38 then lowers tissue levels of NAD⁺ in the surrounding tissue as part of cyclic ADP ribose (cADPR) synthesis.

1, 2 and 6 have known roles in senescence. SIRT2 deacetylates and stabilizes the mitotic checkpoint kinase BUBR1, which declines during aging⁸⁴, and protects against aneuploidy—a potent driver of senescence⁸⁵. Notably, supplementation with the NAD⁺ precursor nicotinamide mononucleotide (NMN) extends the median lifespan of progeric BUBR1 hypomorphic mice⁸⁶. SIRT6 knockout mice show premature aging and a hyperinflammatory state that is driven at least in part by de-repression of retrotransposons⁸⁷, which also occurs with senescence⁸⁸. Furthermore, overexpression of SIRT6 prevents the loss of homologous recombination observed during senescence⁸⁹. Finally, SIRT1 is degraded by autophagy during senescence⁹⁰. Loss of SIRT1 activity is associated with multiple aspects of senescence, including SASP activation⁹¹ and cell cycle arrest⁹², as well as several senescence-associated degenerative pathologies including neurodegeneration, cachexia, fatty liver and atherosclerosis^{93,94}. Together, the sirtuins and PARP demonstrate the importance of NAD⁺-consuming enzymes in senescence and help explain why loss of NAD⁺ levels with age can be so deleterious to a tissue.

NAD⁺ is not always beneficial in the context of senescence. We hypothesized that since loss of NAD⁺ antagonizes the SASP, increased NAD⁺ might have the opposite effect and promote segments of the SASP³⁹. This proved to be the case as NAMPT and NAD⁺ are elevated in senescent cells. This rise is associated with decreased AMPK and p53 activation, leading to increased p38MAPK and NF- κ B activity and the secretion of proinflammatory SASP factors⁸⁰. Thus, NAD⁺ may antagonize senescence, but once senescence is established, NAD⁺ increases may instead promote degenerative pathologies through the SASP.

Cellular senescence also plays a role in tissue-level NAD⁺ metabolism. The SASP promotes CD38 activation in macrophages. CD38 consumes NAD⁺ during the synthesis of cyclic-ADP ribose (cADPR)^{95,96}, and is a primary driver of NAD⁺ depletion with age⁹⁷. Therefore, senescent cells may drive NAD⁺ loss, which in turn could lead to more senescent cells (Fig. 1). This degenerative feedback loop could therefore drive multiple NAD⁺ and senescence-dependent

age-related conditions (for example, diabetes⁸²). In contrast, eliminating senescent cells or suppressing the SASP might protect NAD⁺ homeostasis, and CD38 inhibition in turn might be protective and prevent increased senescence with age.

As noted above, MiDAS results from lowered cytosolic NAD⁺/NADH ratios⁴³, but reduced NAD⁺/NADH ratios can occur without mitochondrial dysfunction. For example, inhibition of the malate-aspartate shuttle, which transports reducing equivalents of NADH from the cytosol to the mitochondria, by aminooxyacetate (AOA) drives senescence⁴³. Loss of the shuttle enzyme malate dehydrogenase 1 (MDH1) has a similar phenotype⁹⁸. Similar results occur when malic enzymes, which catalyze NAD⁺-dependent conversion malate to pyruvate in the cytosol (ME1) and mitochondria (ME2) are inhibited⁹⁹. These enzymes are reciprocally regulated by p53; depletion of either drives p53-dependent senescence, but p53 in turn represses malic enzymes⁹⁹. These studies show that the interplay between NAD⁺/NADH ratios and p53 is a key regulator of senescence under conditions of metabolic stress.

Hyperglycemia. Numerous studies demonstrate that culturing cells in high levels of glucose accelerates cellular senescence^{100,101}. However, because multiple pathways by which this acceleration occurs have been identified, no unifying model currently explains all forms of hyperglycemia-associated senescence. Most studies focus on endothelial cells, including retinal endothelial cells, but fibroblasts and renal epithelial cells have also been studied^{100–104}. Nonetheless, the few mechanisms ascribed to senescence induction seem unlikely to be common to multiple cell lineages. For example, arginase 1 (ARG1) expression and activity are induced in retinal endothelial cells cultured in high glucose, resulting in reduced nitric oxide (NO) and elevated oxidative stress¹⁰¹. Retinal endothelial cells in a mouse model of streptozotocin (STZ)-induced diabetic retinopathy undergo senescence in an ARG1-dependent manner¹⁰⁵, and inhibition of ARG1 activity prevents high glucose-induced senescence in cultured retinal epithelial cells and diabetes-induced

retinal senescence in mice. Ironically, prior reports in human umbilical vein endothelial cells (HUVECs) showed that arginase activity declined during high-glucose-induced senescence, and this decline was linked to decreased NO synthesis¹⁰⁶. Supplementation with arginine prevented these effects and antagonized senescence in high-glucose-treated HUVECs. Generally, NO antagonizes endothelial cell senescence¹⁰⁶, so the key effector of glucose-induced senescence in these cells is likely related to the level of cellular NO production. This mechanism is unlikely to be universal because ARG1 is expressed in only a few cell types, and NO is not produced at appreciable levels by all cells.

High glucose also drives senescence in fibroblasts^{100,104}, and glucose restriction can extend replicative lifespan¹⁰⁷. Although fibroblasts produce NO, they do not express ARG1, so it is unclear whether the phenotypes observed with endothelial cells translate to this cell type. For example, inhibition of nitric oxide synthases (NOS) with N(G)-nitro-L-arginine methyl ester (L-NAME) drives senescence in endothelial cells¹⁰⁶, but can antagonize senescence when fibroblasts are co-cultured with macrophages¹⁰⁸. Thus, the role of NO in the senescence responses of different cell types requires further investigation.

In another study, high glucose promoted senescence in fibroblasts by lowering the levels of SIRT3, and transgenic overexpression of SIRT3 prevented this high glucose-induced senescence¹⁰⁴. Loss of SIRT3 can drive MiDAS⁴³, so lowering of SIRT3 by excess glucose is an appealing link between these processes. Furthermore, high glucose accelerates the loss of sirutin expression in endothelial cells¹⁰³, and this loss is also associated with increased senescence. Since SIRT3 deacetylates mitochondrial proteins, and high-sugar diets can increase mitochondrial protein acetylation^{109,110} and decrease NAD⁺/NADH ratios¹¹¹, it is possible that hyperglycemia drives senescence by MiDAS or a MiDAS-like phenotype.

Hyperglycemia can also result in the formation of advanced glycation end-products (AGEs), which can drive senescence in some models. For example, treatment of kidney proximal tubule epithelial cells with exogenous AGEs (for example, glycated albumin) triggered increased expression of p21 (ref. ¹¹²) or p16 (ref. ¹¹³), coupled to activation of SA- β gal, in a receptor for advanced glycation end-products receptor (RAGE)-dependent manner. Notably, these studies focused only on effects observed 48 hours after treatment, so chronic exposure and effects of AGE removal were not considered. Further, these effects may not extend to other cell types. Conversely, activation of RAGE extends the replicative lifespan of murine preadipocytes by binding to p53 and antagonizing p21 expression¹¹⁴. More research is therefore required to fully elucidate the relationship between AGEs and cellular senescence. Furthermore, studies linking high glucose to senescence have not addressed the SASP, and therefore the consequences of this form of senescence are still somewhat unclear. Despite these needs, the links between diabetes and senescence are growing, and increases in senescent cells at sites of diabetic complications indicate that this is an important area for future research.

Metabolism of senescent cells

Senescent cells show several alterations in their metabolic states (Fig. 3). Here we review some of these alterations and their significance in the development of senescence phenotypes.

Lipid metabolism. A growing body of evidence indicates that senescent cells alter lipid metabolism. One of the first observations along these lines came from the finding that the sphingomyelin–ceramide pathway, and especially sphingomyelinase, was significantly more active in senescent cells¹¹⁵. Addition of ceramides at concentrations that approximate those found in senescent cells lowers markers of cell proliferation and increases markers of senescence in both fibroblasts¹¹⁵ and endothelial cells¹¹⁶. Overall, ceramide and sphingosine

promote senescence, whereas sphingosine-1-phosphate (S1P) promotes cell proliferation via its cognate receptors. Ceramide-activated protein phosphatases (PP1 and PP2A) can promote a senescence arrest by dephosphorylating CDK2 and specifically up-regulating p21 (ref. ¹¹⁷). Sphingosine has similar effects, but the mechanisms are less understood¹¹⁸. The relationships among ceramides, sphingolipids and cellular senescence were recently reviewed¹¹⁹.

Additional studies indicate that senescent cells upregulate β -oxidation—the metabolic breakdown of lipids¹²⁰—and that this activity is necessary for the secretion of many proinflammatory components of the SASP in oncogene-induced senescence. In addition, fatty acid synthase (FASN) was elevated in senescent cells and required for both the cell cycle arrest and parts of the SASP¹²¹. The expression of several enzymes related to both fatty acid synthesis (for example FASN) and oxidation (for example ACADS, ACADL, ACADSB, ACADVL) increase in senescent cells¹²², whereas stearoyl-CoA desaturase—which converts saturated fatty acids into monounsaturated fatty acids (MUFAs)—declines¹²³. These data suggest that saturated fatty acids might be an important substrate for energy production in senescent cells.

Beyond energy use and storage, fatty acids play additional roles in senescence responses. Notably, lipid droplets increase and accumulate with at least some forms of cellular senescence. Exogenous lipids are preferentially incorporated into triacylglycerols, which form the lipid droplets in senescent cells¹²³. Furthermore, free polyunsaturated fatty acid (PUFA) levels increase in senescent cells¹²⁴, likely via p38-dependent activation of phospholipase A2, which cleaves PUFAs from cellular membranes¹²⁵. Senescent cells incorporate higher levels of PUFAs into triglycerides¹²⁶, which ultimately accumulate in lipid droplets. Notably, animal models of obesity show increased numbers of senescent cells in the brain, and these cells show increased numbers of lipid droplets. Importantly, clearance of senescent cells lowered the accumulation of these droplets¹²⁷. Lowering exogenous lipid levels in cell culture media prevented both lipid droplet accumulation and the upregulation of many SASP factors¹²⁷. These data suggest that lipid droplets, or at least the presence of lipids, is required for some key aspects of cellular senescence and the SASP.

The release of free PUFAs from the plasma membrane is associated with multiple forms of senescence^{124,126}. Additionally, levels of several PUFAs, such as eicosapentanoic acid (EPA), arachidonic acid (AA), dihomo- γ -linolenic acid (DGLA) and docosahexanoic acid (DHA), are highly elevated during MiDAS¹²⁴, above other forms of senescence. This elevation may reflect an attempt by cells to preserve the NAD redox balance by using PUFA desaturation to convert NADH into NAD⁺, as described¹²⁸. Beyond accumulating as triglycerides in lipid droplets, free PUFAs are also converted to oxygenated signaling molecules collectively known as oxylipins¹²⁴. Oxylipins associated with cellular senescence include leukotrienes^{125,129} and prostaglandins, including dihydro-prostaglandins^{124,130}. Senescence-associated leukotriene release drives profibrotic features of pulmonary fibrosis, and eliminating senescent cells ameliorates these effects^{125,131}. The synthesis of prostaglandins is essential for reinforcing the senescence-associated cell cycle arrest, as inhibitors of the inducible prostaglandin synthase 2 (PTGS2 or COX-2) extend the replicative lifespan of cultured cells¹³² and allow increased numbers of dividing cells in response to genotoxic stress-induced and oncogene-induced senescence¹²⁴.

Surprisingly, it does not appear that prostaglandin synthesis promotes senescence through interactions with prostaglandin receptors. Rather, senescent cells upregulate expression of the prostaglandin transporter (PGT/SLCO2A1), but without a commensurate elevation in expression of prostaglandin dehydrogenase (PGDH), which inactivates prostaglandins by converting them to 15-keto derivatives. These metabolic changes result in the intracellular accumulation of 15-deoxy prostaglandins, especially 15d-PGJ2

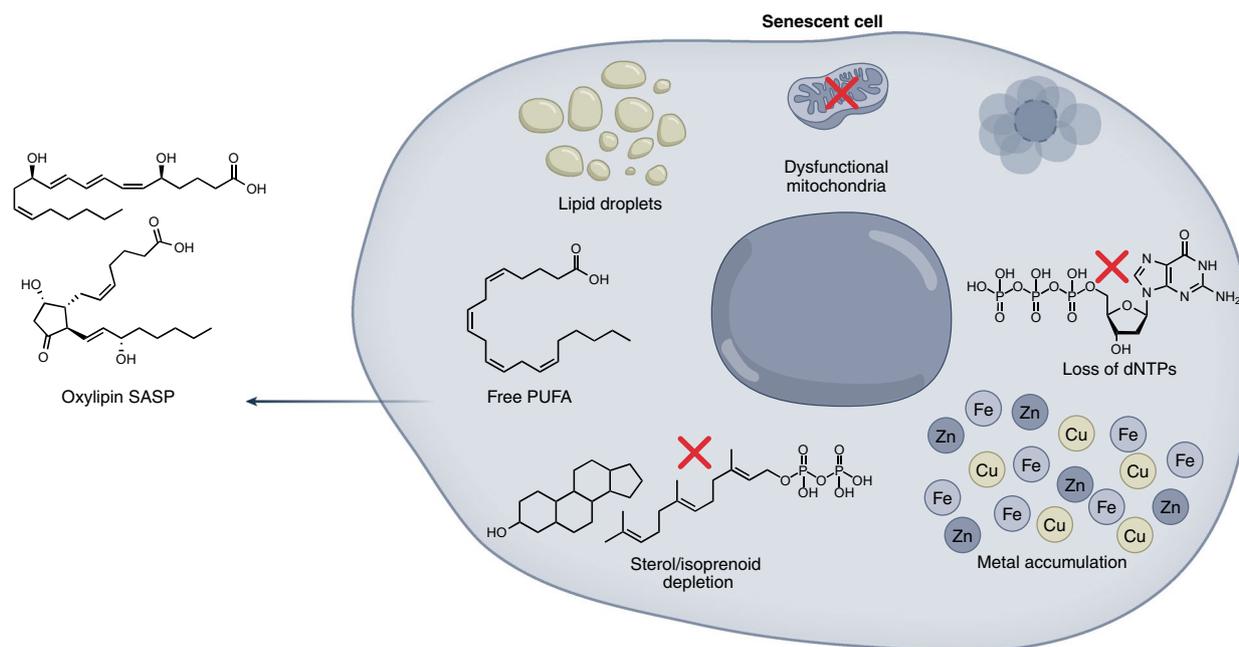


Fig. 3 | Altered metabolic states of senescent cells. Senescent cells have increased free cytosolic PUFAs, which in turn can be converted into oxylipins as part of a lipid-based SASP. Fatty acids also accumulate in lipid droplets during senescence. Sterols preferentially accumulate in the ER of some senescent cells in a p53-dependent manner, inhibiting sterol response element-binding protein 2 (SREBP2) activation and lowering sterol synthesis in the cell as a whole. Senescent cells often have increased mitochondrial mass or mtDNA, but this is coupled to altered membrane potential and ROS production. Lysosomes of senescent cells can become permeable, acidifying the cytosol and disrupting some forms of autophagy. This can lead to accumulation of transition metals inside senescent cells. Finally, senescent cells can have lower dNTP levels due to loss of ribonucleotide reductase 2. Note that senescence is a complex and varied phenomenon, and it is likely that individual metabolic alterations may be present in some, but not all, forms of senescence.

and senescence-associated diholmo-15d-PGJ2. These electrophilic prostaglandins have multiple activities inside cells. Primarily, they change the structure of proteins by Michael addition to exposed cysteines¹³³—that is, the nucleophilic addition of a carbanion or other nucleophile to an α,β -unsaturated carbonyl compound containing an electron withdrawing group.

The intracellular accumulation of diholmo-15d-PGJ2 in senescent cells promotes the activation of endogenous HRAS, thereby reinforcing senescence in a manner analogous to oncogene activation¹²⁴. Notably, this property of senescent cells allows the detection of senolysis because diholmo-15d-PGJ2 is only ~343 Da in size and thus escapes the cytosol during apoptosis. Consequently, it can be detected in culture media or biological fluids upon senolysis¹²⁴. Together, these studies demonstrate that senescence and lipid metabolism are intricately linked (Fig. 4), but also highlight the importance of future work, as many areas of lipid metabolism are still underexplored in the context of senescence.

Lysosomes and autophagy. Autophagy, the process by which a cell breaks down its organelles and macromolecules, is dysregulated in senescent cells. Specific factors show selective microautophagic degradation in senescent cells, whereas macroautophagy appears to be less active in senescent cells^{134,135}. Inhibition of autophagy by depletion of ATG7, ATG12 or LAMP2 induces senescence¹³⁶, implying that loss of autophagic homeostasis during senescence may reinforce the senescence arrest and maintain the senescent state. However, activation of autophagy is associated with oncogene-induced senescence, and overexpression of ULK3 also promotes senescence¹³⁷. The complex nature of the relationship between senescence and autophagy are the subject of recent reviews^{135,138}.

Notably, many senescent cells have dysfunctional lysosomes. Indeed, SA-gal is a lysosomal β -galactosidase that is markedly

upregulated in senescent cells^{139,140}, and SA- β gal may reflect lysosomal dysfunction. Recent findings show that lysosomal permeability increases in senescent cells. This increase results in the previously observed loss of lysosomal acidity^{141,142}, but also elevates cytosolic acidification¹⁴³. To combat this increase, senescent cells upregulate glutaminolysis by increasing glutaminase 1 (GLS1) expression, which increases ammonia production and neutralizes the cytosolic acidity¹⁴³. Inhibition of GLS1 kills senescent cells both in culture and in vivo and ameliorates several indicators of age-related organ dysfunction¹⁴³. Therefore, lysosomal dysfunction, while potentially deleterious, is an exploitable weakness for targeted elimination of senescent cells.

Disruption of transition metal homeostasis. Transition metals—divalent cations that occupy a central block in the periodic table—play a key role in several cellular processes, but the role of these metals in senescence is only beginning to be understood. Surprisingly, senescent cells were found to accumulate transition metals at much higher levels than non-senescent or immortalized cells¹⁴⁴. These transition metals included iron, manganese and zinc. Later, other transition metals, such as copper¹⁴⁵, were found to accumulate in senescent cells.

Iron. Iron levels are tightly controlled in most cells. Iron is required for oxygen transport, respiration and oxidative phosphorylation¹⁴⁶. However, too much unbound or 'labile' iron can catalyze the production of ROS and peroxidized lipids, which can drive toxic cell processes, including a form of iron-dependent cell death known as ferroptosis¹⁴⁷. Iron overload is antagonized by the protein ferritin, which binds and sequesters iron in an inactive form. Senescent cells import increased levels of iron by elevating the transferrin receptor, which then endocytoses iron-bound transferrin¹⁴⁸.

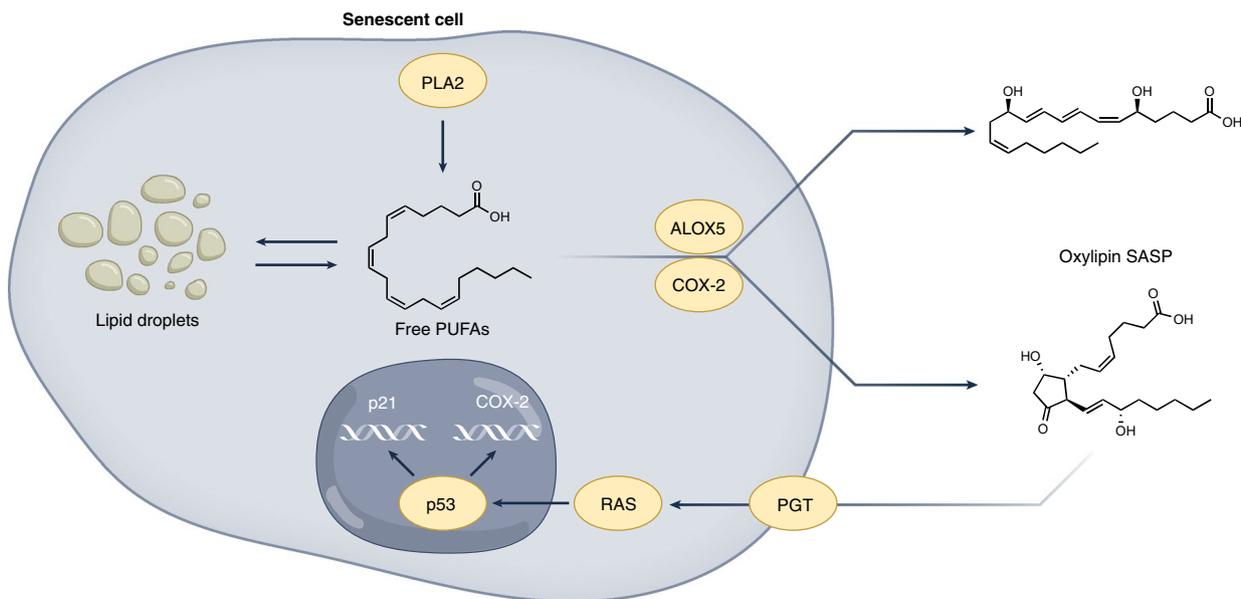


Fig. 4 | Lipid metabolism in senescent cells. Activation of phospholipase A2 (PLA2) frees PUFAs from the plasma membrane, which then accumulate in the form of triglycerides in lipid droplets but are also used as substrates for oxylipin synthases such as arachidonate 5-lipoxygenase (ALOX5) and cyclooxygenase 2 (COX-2), resulting in release of an oxylipin SASP. PGT imports prostaglandins into the cytosol, where cyclopentenone prostaglandins activate RAS, leading to p53 activation, leading to both cell cycle arrest p21 and elevation of COX-2, reinforcing oxylipin biosynthesis.

Ferritin levels increase in senescent cells, sequestering much of the imported iron in an inactive state¹⁴⁸. Because iron is released from ferritin by targeted autophagy (ferritinophagy), and senescent cells display compromised autophagy and lysosomal dysfunction, senescent cells likely accumulate iron at least in part due to failure to degrade ferritin. Activation of autophagy by rapamycin partially restored cellular iron levels¹⁴⁸. While iron-metabolizing protein levels suggest that labile iron may actually decrease during senescence¹⁴⁸, no reports of direct measurement of the labile iron pool in senescent cells exist to date. If the labile iron pool does indeed decrease during senescence, this finding might explain the observation that senescent cells are resistant to ferroptosis, as lack of labile iron would prevent iron-catalyzed lipid peroxidation. However, rapamycin had no effect on ferroptosis resistance in senescent cells, emphasizing the need for more research in this area.

Copper. Copper is an essential trace element required for the activity of multiple enzymes, including superoxide dismutase, neurotransmitter synthases, amine oxidases, ceruloplasmin and lysyl oxidase¹⁴⁹. Excess copper can promote senescence in human fibroblasts¹⁵⁰ and human glioblastoma cells by downregulating the polycomb protein Bmi-1 (ref. ¹⁵¹), although both copper and oxygen levels were supraphysiological in these studies. Similar to iron, copper accumulates in senescent cells¹⁴⁵, and, much like iron, this accumulation appears due to defective lysosomal autophagic degradation of copper chaperones. However, since copper-dependent antioxidant enzymes (for example, superoxide dismutase and glutathione reductase) are elevated during senescence¹⁴⁵, and prevent senescence¹⁵², this elevation may be a compensatory mechanism to counteract the more oxidative environment observed in senescent cells. Much like iron, rapamycin partially abrogates copper accumulation in senescent cells¹⁴⁵, suggesting regulation by autophagy, but the consequences of this elevation are unclear.

Zinc. Zinc is notable for its essential use by many organisms. One reason for this use is that, unlike iron and copper, zinc does not catalyze the formation of radicals from lipid peroxides¹⁵³. Thus, zinc is less toxic and more redox inactive than iron and copper.

Nonetheless, zinc levels are inversely associated with lifespan in *Caenorhabditis elegans*¹⁵⁴. Zinc levels also increase in senescent cells, and are generally higher than iron or copper levels¹⁴⁴. Excess zinc promotes senescence in cultured vascular smooth muscle cells¹⁵⁵, endothelial cells¹⁵⁶ and fibroblasts¹⁵⁷. Despite these studies showing an elevation of transition metals during senescence, no functional significance has yet been ascribed to the iron, copper or zinc accumulation in senescent cells. Since overload of these metals drives senescence, this accumulation may enforce a cell cycle arrest program that maintains senescent cells in an essentially irreversible growth-arrested state.

Senescence and dNTP synthesis. As senescent cells do not divide, their principal uses for deoxynucleoside triphosphates (dNTPs) are for DNA repair and mitochondria DNA synthesis. Ribose-linked ATP, GTP and CTP are converted to dNTPs by ribonucleotide reductase (RRM2). Unsurprisingly, RRM2 declines during senescence¹⁵⁸. However, when senescence is induced by oncogenic RAS, RRM2 depletion actually precedes the growth arrest and occurs during the hyperproliferative phase prior to cell cycle arrest¹⁵⁸. Increased proliferation in the absence of dNTPs causes replication fork collapse, DNA double-strand breaks and cell cycle arrest. Addition of exogenous dNTPs allows continued proliferation in this model¹⁵⁸. Eliminating p53 or p16 allows cells to bypass oncogenic RAS-induced senescence by increasing nucleotide synthesis via shunting metabolites through the pentose phosphate pathway^{159,160}. Notably, RAS activation antagonizes the hippo pathway transducers YAP/TAZ, and activation of this pathway restores RRM2 expression and allows escape from RAS-induced senescence¹⁶¹. These findings suggest that senescent cells integrate external cues to control DNA metabolism.

Interventions that reciprocally target senescence and metabolism

As described above, senescence and metabolism are intricately linked. It would therefore follow that interventions that target metabolism might influence the accumulation of senescent cells—or properties of senescent cells, such as the SASP. Conversely,

targeting senescent cells with, for example, senolytic drugs, should influence the development of metabolic disorders, as shown in Fig. 1 (right). In this section, we explore some of these links.

Senolytics as regulators of metabolism. Transgenic mice and drugs that allow the selective killing of senescent cells (senolysis/senolytic drugs) reveal that senescent cells promote many age-related conditions, thereby limiting both lifespan and health span^{48,49}. Senolysis prevents several pathologies, including chronic kidney disease^{48,162}, osteopenia¹⁶³ and osteoarthritis¹⁶⁴. Senescent cells also promote tumorigenesis^{48,165}, including both cancer relapse and several side effects of anti-cancer chemotherapy^{166,167}. It is therefore not surprising that senescent cells also promote aspects of metabolic disease.

Senescent cells promote both diabetes and the degenerative complications of diabetes. Diabetes can be driven by pancreatic cell senescence, promoting insulinitis and Type 1 diabetes¹⁶⁸. Diabetes can also be driven by peripheral senescent cells in obese mice, which drive insulin resistance and Type 2 diabetes¹⁶⁹. Surprisingly, increasing the presence of senescent cells by transgenically overexpressing *p16^{INK4a}* in pancreatic β -cells of mice actually increases insulin secretion¹⁷⁰, so the development of senescence may reflect metabolic compensation for high blood glucose levels. Similarly, insulin resistance can drive hyperinsulinemia and promote β -cell senescence¹⁷¹, implicating peripheral senescent cells as feedback drivers of senescence in the pancreas. Furthermore, complications of diabetes, including diabetic kidney disease^{172,173} and diabetic retinopathy¹⁷⁴, potentially result from senescent cells driven by increased blood glucose levels, as described above. In each case, senolytic therapies improve health outcomes in terms of either blood glucose levels^{168,169,171} or development of diabetes-driven complications^{172,174}. Together, these studies implicate senescent cells as a major nexus for interventions into diabetes and its associated disorders.

Dietary interventions and senescence. Multiple dietary interventions can limit the accumulation of senescent cells with age. Calorie restriction (CR) was one of the first lifespan-extending interventions to be identified¹⁷⁵. Thus, when it was found that senescent cells can limit lifespan⁴⁸, it seemed that links between CR and senescence were likely. CR lowers markers of senescence in the colons of mice and humans¹⁷⁶ and in inguinal white adipose tissue¹⁷⁷. CR prevents the development of senescence in the kidney of aged animals, whereas high-calorie diets increase senescence¹⁷⁸.

Other dietary interventions can similarly extend health span and either or both median and maximum lifespan. These interventions include methionine restriction¹⁷⁹ and ketogenic diets^{180,181}. Methionine restriction lowers markers of senescence and the SASP¹⁸², while injection of mice with β -hydroxybutyrate, a major ketone body produced by ketogenic diets, lowers markers of senescence in vascular smooth muscle and endothelial cells¹⁸³. These results indicate that dietary interventions that create a favorable metabolic state that can limit the accumulation of senescent cells.

Metabolic interventions that influence senescent cells. As described above, hyperglycemia induces senescence. It therefore reasons that diabetes could be pro-senescence, and interventions that lower blood sugar should antagonize the formation of senescent cells. Indeed, kidney proximal tubule cells become senescent within 4 weeks of induction of diabetes in mice, and this rise in senescent cells can be prevented by lowering glucose levels with insulin or inhibiting glucose transport into the cells by inhibiting the activity of the sodium-glucose co-transporter SGLT2¹⁰². Similarly, acarbose—which lowers blood sugar by antagonizing intestinal carbohydrate absorption—extends lifespan in mice¹⁸⁴ and prevents diet-induced atherosclerosis-associated senescence in rabbits¹⁸⁵.

Another antidiabetic medication, metformin, extends both lifespan and health span in mice¹⁸⁶, and is a drug being studied for the

first clinical trial to monitor general aspects of aging (under review; see <https://www.afar.org/tame-trial>). Metformin antagonizes the development of senescence in response to ceramide in immortal mouse myoblast cells¹⁸⁷ and extends the replicative lifespan of human fibroblasts and mesenchymal stem cells¹⁸⁸. Additionally, metformin lowers several SASP factor levels, including many pro-inflammatory cytokines, by interfering with NF- κ B activation¹⁸⁹. Furthermore, metformin protects against the development of senescence in murine models of intervertebral disc degeneration¹⁹⁰ and chronic kidney disease¹⁹¹. Metformin therefore interferes with senescence at several levels and is perhaps the best-characterized metabolic intervention that antagonizes senescence and the SASP.

Atherosclerosis, statins and senescence. Statins, such as simvastatin, are commonly used among older adults as preventives for elevated cholesterol levels and associated atherosclerosis-related complications, such as stroke and heart attack. They prevent cholesterol synthesis by inhibiting β -hydroxy β -methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the mevalonate pathway that leads to cholesterol biosynthesis¹⁹². Statins prevent the senescence growth arrest, with lowered markers of senescence, in endothelial progenitor cells¹⁹³, which is dependent on lowered levels of farnesylpyrophosphate and geranylgeranylpyrophosphate¹⁹³. Statins also inhibit many aspects of the proinflammatory SASP of senescent fibroblasts, but, as with endothelial progenitor senescence, this effect is independent of the cholesterol-lowering properties of the drug¹⁹⁴. Rather, HMG-CoA reductase inhibition lowers farnesyl-CoA and geranylgeranyl-CoA levels, which in turn are required for membrane localization and activation of rho-type GTPases, including RAS and RAC, which are required for the SASP. Since p16-positive (presumably senescent) foamy intimal cells promote deleterious features of atherosclerosis¹⁹⁵, it is possible that statins may have anti-atherogenic properties beyond regulating cholesterol. However, statins can also act as radiosensitizers—increasing cancer cell senescence in response to irradiation¹⁹⁶. So, the effects of statins are likely to be context-specific. Surprisingly, p53 activation during senescence limits both the mevalonate and cholesterol synthesis pathways¹⁹⁷, and therefore may be a mechanism by which p53 activation limits aspects of the SASP^{15,66}. The relationship between senescence, cholesterol and atherosclerosis is therefore likely to be more complex than previously thought.

Exercise as an intervention for senescence. While numerous studies highlight the health benefits of exercise and its improvement of health span with age, few studies to date have demonstrated lifespan extension due to exercise in mice^{198,199}; just one late-life exercise study showed lifespan extension²⁰⁰. There is evidence that exercise can prevent senescence and segments of the proinflammatory SASP in the sera and hearts of aged mice¹⁹⁹. More notably, exercise offsets diet-induced senescence and the SASP in mouse adipose and liver tissues²⁰¹, suggesting that the most beneficial aspects of exercise may not pertain to already healthy animals, but rather those likely to develop metabolic disease. In humans, exercise and activity are associated with reduced markers of endothelial and leukocyte cell senescence^{202,203}. These data suggest that exercise can offset deleterious aspects of senescence brought about by poor diet and sedentary lifestyles.

Conclusions

As noted throughout this article, the interplay between senescent cells and systemic metabolism is dynamic. Senescent cells can disrupt metabolic homeostasis in multiple tissues, and these changes in turn can promote senescence, creating a feedback loop that can drive pathology. This loop occurs in the context of NAD⁺, where the SASP promotes CD38-dependent NAD⁺ breakdown by immune cells^{95,96}, while lowered NAD⁺ levels in turn can promote senescence³⁹ (Fig. 2). Similarly, peripheral senescence can result in

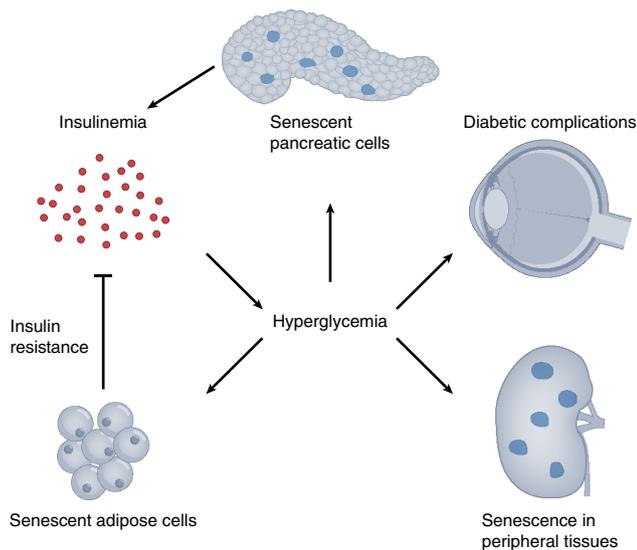


Fig. 5 | Multiple roles for senescence in diabetes and its complications.

Senescent adipose cells can result in insulin resistance, which results in hyperglycemia, which can in turn promote additional adipose tissue senescence. Hyperglycemia also forces pancreatic β -cells to over-produce insulin. This stress results in β -cell senescence, leading to insulinemia, resulting in further hyperglycemia. Hyperglycemia also results in senescence in peripheral tissues such as the retina and the kidney, fueling diabetic complications.

insulin resistance¹⁶⁹, while insulin resistance in turn drives stress and senescence in pancreatic β -cells¹⁷¹. This degenerative cycle leads to elevated blood glucose and senescence in the eye and kidney, ultimately driving diabetic complications^{172,174} (Fig. 5). These feedback loops require interventions that break the cycle in order to be efficacious.

There are three types of interventions that target senescent cells and their degenerative pathologies. First, we can slow the formation of senescent cells, as observed during dietary restriction and similar interventions. Second, we can allow senescent cells to accumulate, but prevent them from causing harm, as observed during metformin-mediated SASP suppression or after CD38 inhibition. Finally, we can use senolysis to remove senescent cells. Unlike the first two interventions, senolysis can be used intermittently—allowing for a ‘hit and run’ approach that might be easier to implement in humans—whereas dietary and suppressive drug regimens require regular adherence to maintain benefits.

The metabolic changes observed in senescent cells also imply that additional disorders might be driven by senescent cells. For example, it was recently shown that senescent cells occur in the chorioamniotic membrane prior to birth in mammals, and that these cells send signals to promote parturition³⁸. Given that parturition is promoted by prostaglandins E₂ (PGE₂) and F_{2 α} (PGF_{2 α}), it may be that senescence-associated prostaglandins are part of normal (and potentially premature) labor in humans. Prostaglandins also have additional roles, such as the essential role of PGD₂ in sleep initiation^{204,205}. Given that many people experience increased sleep disruption with age²⁰⁶, it is tempting to speculate that senescent cells may produce additional PGD₂ that drives age-related sleep disruption.

Finally, age is a major outcome of human nutrition, while nutritional interventions can prevent many age-associated diseases²⁰⁷. Much is known about the role of macronutrient stress in longevity and senescence, but less is known about how malnutrition of key micronutrients controls the development of senescence. For example, choline deficiency is common in the elderly in the United

States²⁰⁷, and is a driver of hepatic steatosis—a condition also promoted during age by senescent cells²⁰⁸. The relationship between nutrition and senescence is therefore fertile ground for future exploration and will undoubtedly be the subject of multiple studies moving forward.

Clearly, many aspects of the interplay between metabolism and cellular senescence need additional research. Encouragingly, however, emerging tools of modern biology, including single cell and systems biology and artificial intelligence, are making it easier to obtain deep insights into this complex interplay, which will undoubtedly lead to more efficacious interventions into age-related metabolic pathologies in the future.

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Author contributions

C.D.W. and J.C. conceived of the concepts described in this review. C.D.W. wrote the metabolic sections with assistance from J.C. J.C. wrote the introduction with assistance from C.D.W. Both authors wrote the conclusions section.

Competing interests

J.C. is a scientific founder of Unity Biotechnology, which develops senolytic therapies. C.D.W. and J.C. hold patents for induction and detection of senolysis using metabolic targets.

Additional information

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