HHS Public Access

Author manuscript

Nat Rev Endocrinol. Author manuscript; available in PMC 2020 May 15.

Published in final edited form as:

Nat Rev Endocrinol. 2020 May ; 16(5): 263–275. doi:10.1038/s41574-020-0335-y.

The role of cellular senescence in ageing and endocrine disease

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Abstract

With the ageing of the global population, interest is growing in the 'geroscience hypothesis', which posits that manipulation of fundamental ageing mechanisms will delay (in parallel) the appearance or severity of multiple chronic, non-communicable diseases, as these diseases share the same underlying risk factor—namely, ageing. In this context, cellular senescence has received considerable attention as a potential target in preventing or treating multiple age-related diseases and increasing healthspan. Here we review mechanisms of cellular senescence and approaches to target this pathway therapeutically using 'senolytic' drugs that kill senescent cells or inhibitors of the senescence-associated secretory phenotype (SASP). Furthermore, we highlight the evidence that cellular senescence has a causative role in multiple diseases associated with ageing. Finally, we focus on the role of cellular senescence in a number of endocrine diseases, including osteoporosis, metabolic syndrome and type 2 diabetes mellitus, as well as other endocrine conditions. Although much remains to be done, considerable preclinical evidence is now leading to the initiation of proof-of-concept clinical trials using senolytics for several endocrine and non-endocrine diseases.

Ageing is now generally accepted as the single largest risk factor for many of the major chronic diseases (for example, type 2 diabetes mellitus (T2DM), cardiovascular disease and cancer) that account for the bulk of morbidity, deaths and health costs in the USA and other developed countries[1]. For these conditions, the predictive ability of advanced chronological age exceeds the predictive ability of all other risk factors combined. Enormous progress has been made over the years in the development of specific drugs to treat individual diseases associated with ageing, including metabolic dysfunction and T2DM (for example, GLP1R agonists and DPP4 inhibitors), skeletal fragility and osteoporosis (for example,

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

T.T. and J.L.K. have a financial interest related to this research. Patents on senolytic drugs are held by Mayo Clinic. Mayo Clinic has licensed patents on dasatinib and quercetin as senolytics to Unity Biotechnology. This research was reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic conflict of interest policies. All other authors declare no competing interests.

Peer review information

Nature Reviews Endocrinology thanks L. Hofbauer, D. Towler and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note

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bisphosphonates and denosumab) and vascular dysfunction and disease (for example, statins), with potential new drugs on the horizon to treat skeletal muscle loss and frailty (for example, the activin type 2 receptor antibody bimagrumab); however, the combined effect of these drugs on reducing morbidity and mortality has been modest.

Age-associated chronic diseases rarely, if ever, exist in isolation in elderly individuals. Rather, they tend to occur in synchrony as multimorbidities, the prevalence of which increases exponentially after age 70 years[2]. This multimorbidity leads to substantial barriers to the appropriate treatment of each disease, including prioritization of each condition by the busy primary care physician and perhaps most importantly the growing problem of polypharmacy in older (aged >60 years) patients. Indeed, the most recent estimates indicate that 36–39% of adults aged 62 years or older take five or more prescription medicines[3]. When over-the-counter medication use is included, the prevalence of such adults taking five or more medications increases to 67% (REF.[3]). Fundamentally, polypharmacy occurs because most treatment strategies for chronic age-related morbidities are disease specific. This specificity inevitably leads to polypharmacy, which can result in problems related to adverse effects, unpredictable drug interactions and poor adherence.

Osteoporosis provides a good case study of the reasons why a disease-specific approach fails in older (aged >60 years) patients with multimorbidities. Currently, numerous options are available for the treatment of osteoporosis, including a selective oestrogen receptor modulator (raloxifene), four bisphosphonates (alendronate, risedronate, ibandronate and zoledronic acid), a human monoclonal antibody to receptor activator of nuclear factor-κB ligand (RANKL; denosumab), analogues for parathyroid hormone and parathyroid hormone-related protein (teriparatide and abaloparatide) and a monoclonal antibody to sclerostin (romosozumab)[4]. Despite these treatment options, most patients with osteoporosis, including following an event as devastating as hip fracture, remain untreated[5]. A number of reasons could explain the lack of appropriate treatment for age-associated co-morbidities, including osteoporosis, some of which are condition specific. For example, in osteoporosis, the fear of rare bisphosphonate-related adverse effects, such as osteonecrosis of the jaw or atypical femur fractures[6], can reduce treatment uptake.

Within the context of multiple co-morbidities of ageing and the growing recognition by the gerontology community that ageing in itself is the largest risk factor for most age-related chronic diseases, the ‘geroscience hypothesis’ has gained accelerating momentum. This hypothesis posits that manipulation of fundamental mechanisms of ageing will delay (in parallel) the appearance or severity of multiple chronic diseases because these diseases share the same underlying risk factor — namely, ageing[7] (FIG. 1). The potential efficiency of targeting fundamental ageing mechanisms, as opposed to treating each age-associated condition separately, is truly remarkable. Indeed, by one estimate, a 2% delay in the progression of ageing processes would lead to an increase often million healthy (as opposed to disabled) elderly people in the USA by 2060 compared with doing nothing, which would delay the onset of cancer or heart disease[8]. This increase would correspond to savings in US health costs of $7.1 trillion over 50 years[8].

A number of common ageing mechanisms that influence lifespan and healthspan have been identified on the basis of studies across a range of species[9]. These ageing mechanisms can be categorized into nine hallmarks, which are genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication[9] (FIG. 2). Although it is useful to consider these hallmarks individually in mechanistic studies related to ageing, they are highly interconnected, and, ultimately, systems biology approaches will be needed to fully understand interactions among these pathways as they contribute to ageing. Of these fundamental ageing mechanisms, cellular senescence has received considerable attention as a process that is potentially druggable to prevent or treat multiple ageing comorbidities.

In this Review, we discuss the biological mechanisms of cellular senescence and the evidence that this physiological process has a causative role in multiple diseases associated with ageing. Subsequently, we focus on the role of cellular senescence in a number of endocrine diseases, as well as approaches to target this mechanism to prevent or alleviate age-associated endocrine diseases.

Mechanisms of senescence

Cellular senescence, originally described by Hayflick[10], is a cell fate that involves essentially irreversible replicative arrest, tumour suppressor activation, profound chromatin changes, apoptosis resistance and frequently increased protein synthesis[11]. The increase in protein synthesis includes excessive production of proinflammatory cytokines (the senescence-associated secretory phenotype (SASP)), which is responsible for tissue damage and contributes to ageing across tissues[11].

Signals for senescence.

Multiple signals can induce a cell to enter senescence (FIG. 3). These include external signals, such as damage and/or danger signals (for example, damage-associated molecular patterns or pathogen-associated molecular patterns), metabolic signals (for example, high levels of glucose, ceramides, certain fatty acids, prostanoids, hypoxia and reactive oxygen species (ROS)), factors that induce repeated cell replication (for example, growth hormone or IGF1), inflammatory factors, radiation, shear or mechanical stress, certain extracellular vesicles and other factors (for example, signals originating from other senescent cells that lead to spread of senescence)[11-23]. Internal factors that can drive cells into senescence include telomeric dysfunction from repeated cell replication, DNA damage, aneuploidy, oncogene activation, mitochondrial dysfunction, intracellular metabolites (for example, ROS), nuclear envelope dysfunction and certain genetic variants or mutations (for example, those involving lamin A, DBC1 or p53)[11,15,16,22,24-31]. These senescence-inducing signals activate transcription factor cascades, including pathways involving the cell cycle inhibitors pl6^{INK4A}/RB andp53/p21^{CIP1}.

The senescent phenotype.

At least in cell culture, 2 weeks or more after the initiation of senescence, the senescent cell phenotype can become established and essentially irreversible. Cellular senescence is characterized by loss of proliferative potential, changes in expression of multiple genes, altered histone binding, changes in the levels of heterochromatin and/or euchromatin, resistance to apoptosis, increased metabolic activity, increased protein production and frequently a SASP[11]. The SASP involves secretion of inflammatory cytokines, chemokines that attract and anchor immune cells, tissue-destroying proteases, factors that affect stem and progenitor cell function (such as activin A), haemostatic factors (such as plasminogen-activated inhibitor 1), vasopressors, ceramides, prostanoids, bradykinins, nucleotides (such as microRNAs, mitochondrial DNA and nuclear DNA) and growth factors. These factors can act in a paracrine or endocrine manner to induce local and systemic inflammation, immune system activation, fibrosis, apoptosis, tissue damage, and stem and progenitor cell dysfunction and can induce the spread of senescence to other cells both locally and systemically[11,15,16,26,32-34] (FIG. 3).

Senescent cells can undergo a partial Warburg shift with increased glycolytic metabolism, reduced fatty acid utilization, intracellular accumulation of lipids and increased ROS generation, which perturbs nearby cells and contributes to the spread of senescence to non-senescent cells in vitro and in vivo[34,35]. Several months after cells have entered senescence, transposons (such as LINE1 and other elements) can be produced within senescent cells along with reverse transcriptase. These elements lead to DNA rearrangements within the cell and further activation of DNA damage signals that can induce intracellular cGAS–STING damage-response pathways, thereby reinforcing and further exacerbating the proinflammatory proapoptotic SASP[36].

The composition of the SASP differs depending on the cell type from which the senescent cells originated, the specific inducers that caused senescence and the hormonal milieu. For example, transcriptome analysis of senescent fibroblasts from different tissues showed considerable heterogeneity in the SASP[37]. Furthermore, the SASP varies over time and seems to be modifiable: hormones and drugs such as glucocorticoids, rapamycin, metformin or JAK1/JAK2 inhibitors can inhibit components of the SASP both in vitro and in vivo[26,38-41]. In addition, external signals can upregulate the SASP For example, at least in vitro, tumour necrosis factor (TNF) can exacerbate and reinforce the proinflammatory nature of the SASP[14].

Markers of cellular senescence.

Much more research needs to be conducted on developing and optimizing sensitive and specific assays that track changes in the burden of senescent cells using biopsy samples, blood assays, assays of other body fluids or imaging[42]. Complicating such work, the definition of cellular senescence is somewhat vague: several potentially proinflammatory cell types, such as macrophages, osteoclasts or precancerous or cancer cells, share many features with senescent cells[43]. Furthermore, these proinflammatory ‘senescent-like’ cells could arguably even be the same as what are currently regarded as being senescent cells[43]. Few cellular or tissue markers are highly sensitive or specific for senescent cells, so

combinations of these markers generally need to be analysed to draw firm conclusions about the effects of diseases or interventions on the burden of senescent cells. These in vitro and in vivo markers of senescent cells include increased cell size and intracellular protein content, accumulation of lipofuscin, increased expression of $p16^{INK4A}$, $p21^{CIP1}$ and SASP factors (for example IL-6, IL-1α, IL-8, monocyte chemoattractant protein 1, plasminogen-activated inhibitor 1 and plasminogen-activated inhibitor 2), increased cellular senescence-associated β-galactosidase (SA-βgal) activity, cytoplasmic HMGB1, the presence of senescence-associated distension of satellites (SADS) and telomere-associated DNA damage foci, among other markers[44-48].

These markers of cellular senescence have their limitations. For example, both SA-βgal activity and $p16^{INK4a}$ expression can be high in activated macrophages, and $p16^{INK4A}$ expression can also be elevated in certain differentiated cell types in vitro and in vivo[43,49]. Furthermore, levels of $p16^{INK4A}$ are not elevated in all cells that could be considered to be senescent[50]. Thus, SA-βgal activity and $p16^{INK4a}$ expression are neither fully sensitive nor specific markers of senescent cells. A combination of assays is needed to determine senescent cell numbers in tissue samples.

Currently, it is unclear if circulating SASP factors or local senescent cell numbers in biopsy samples of skin, adipose tissue or other tissues reflect the overall burden of senescent cells. New or composite assays are needed for use in clinical trials, for example assays of circulating or urinary microvesicles originating from senescent cells, senescence-specific microRNAs or other nucleotides, or epigenetic profiles. Imaging methods also need to be developed to follow localized populations of senescent cells, for example in trials of senolytic drugs for idiopathic pulmonary fibrosis[51] or osteoarthritis[52].

Physiological relevance of cellular senescence.

Consistent with the notion of cellular senescence being a terminal cell fate that occurs in response to certain stimuli, senescent cells can develop at any point during life. For example, senescent cells can even occur in 32-cell-stage human blastocysts in response to hypoxia[53]. However, studies conducted using combinations of senescence markers suggest that the abundance of senescent cells increases with ageing in multiple tissues of mice, monkeys and humans[20,54-56]. In otherwise healthy humans, the abundance of senescent cells can increase from low or even nearly undetectable levels in skin or adipose tissue biopsy samples from young individuals (that is, aged 20–30 years) to as many as 5% of cells or even more in individuals aged 40–80 years; however, more research using a broader range of markers of cellular senescence needs to be undertaken[57-60]. Consistent with a relationship between cellular senescence and ageing, interventions that increase healthspan and lifespan, including caloric restriction or mutations in the growth hormone axis (for example, those in Ames dwarf mice (which lack growth hormone) and growth hormone receptor geneknockout mice) are associated with decreased burden of senescent cells in mice[54,61].

Senescent cells that have a proinflammatory SASP can cause substantial pathogenic effects, resulting in phenotypes that are associated with ageing. For example, transplanting small numbers of senescent cells (mouse fibroblasts induced to become senescent by radiation) around the knee joints of young mice induces an age-related osteoarthritis-like condition[52].

Furthermore, transplanting autologous ear fibroblasts or syngeneic (genetically compatible) preadipocytes induced to become senescent by radiation or chemotherapeutic drug exposure intraperitoneally into lean middle-aged mice is sufficient to cause frailty, early onset of all age-related diseases and premature death compared with transplanting similar numbers of non-senescent cells. This effect occurs even though only 1 in 10,000 of the total number of cells in the recipient mice is a transplanted senescent cell[34]. Moreover, transplanting only half the number of senescent cells was sufficient to cause an early ageing phenotype and increased mortality in middle-aged diet-induced obese mice or lean old mice as opposed to lean middle-aged mice[34]. Thus, even small numbers of senescent cells can cause considerable dysfunction.

Targeting cellular senescence

Senescent cells can be beneficial in certain circumstances: for example in the defence against cancers, in the course of wound healing or in placental regulation of parturition[62-64]. Hence, interfering with the generation of new senescent cells is probably not a viable overall therapeutic strategy. For example, systemically reducing the expression of $p16^{INK4A}$ or p53 might delay the development of senescent cells but might also increase the risk of cancer[63]. However, once formed, senescent cells harbour oncogenic mutations and cause dysfunction owing to their SASP. Removing these already formed and persistent senescent cells might decrease cancer risk, decrease the spread of senescence and reduce tissue dysfunction.

SASP inhibitors.

SASP inhibitors (otherwise known as senomorphics) target processes or proteins in senescent cells that regulate or exacerbate the SASP, including mechanistic target of rapamycin complex 1 (mTORC1), JAK1/JAK2, STAT3, inflammatory mediators and mitochondrial dysfunction. SASP inhibitors include rapamycin and related mTORC1 inhibitors, ruxolitinib, glucocorticoids and metformin[26,39,40,59,65,66]. Most of these SASP inhibitors are segmental; that is, they reduce some sets of SASP components but not the entire range of SASP factors. Of note, these agents also have many other effects in addition to being SASP inhibitors.

Among other effects, rapamycin and related agents can reduce frailty in old mice, decrease heart failure, cancers, cognitive impairment and immune dysfunction in mouse models, and delay age-related adipose tissue loss and increase lifespan in mice[67-72]. Administering rapamycin at doses associated with lifespan extension in mice did not seemingly reduce the levels of at least some of the SASP factors in adipose tissue of old mice[73]. This finding indicates that much more remains to be learned about potential relationships among adipose tissue inflammation, SASP factors and the lifespan-extending effects of rapamycin, which is used in humans as an immunosuppressive agent following organ transplantation[74].

Ruxolitinib is a JAK1/JAK2–STAT3 inhibitor in clinical use for various disorders (for example, polycythaemia vera, myelofibrosis and graft-versus-host disease) that also inhibits the SASP in vitro and in vivo in ageing mice[59]. Of note, in old mice this drug attenuates age-related adipose tissue dysfunction, decreases insulin resistance, reduces age-related osteoporosis and decreases stem cell dysfunction[59,75]. Furthermore, even in very old mice

the drug reduces frailty, a condition once believed to be untreatable[59]. In older patients (median age, 65 years) with myeloproliferative syndrome, ruxolitinib partially attenuates the features of frailty, including reduced body weight, strength and appetite; however, no effect was seen on their underlying haematological condition, which was the original target of the drug[76].

Of note, metformin, which among other mechanisms of action is a weak SASP inhibitor[40], alleviates a number of age-related conditions in experimental animals and in humans; these disorders include metabolic dysfunction, insulin resistance, T2DM, cardiovascular disease, cognitive dysfunction and cancer development and spread[42]. In individuals with T2DM, one study has suggested that metformin could increase 5-year survival[77].

On the basis of animal studies, although SASP inhibitors could help to alleviate conditions related to ageing and cellular senescence when administered intermittently[41], generally these drugs would need to be administered continuously to maintain SASP suppression. Notably, SASP inhibitors act through a range of mechanisms, which makes disentangling effects on age-related phenotypes due to SASP modulation from other ‘off-target’ age-related processes difficult. This fact is especially true if continuous dosing of these inhibitors is used. Off-target effects that occur during continuous dosing could contribute to adverse effects that are unrelated to suppressing the SASP. For example, rapamycin can induce insulin resistance by inhibiting mTORC2 (REF[78]) and can cause cataract formation perhaps unrelated to mTORC2 inhibition[70], among other effects in mice and humans. SASP inhibitors could see clinical application for age-related conditions, especially in situations where the short-term suppression of the effects of senescent cells is indicated. For example, SASP inhibitors could be used to promote recovery after myocardial infarction, to increase the effectiveness of immunizations or to decrease ventilator-induced myopathy; however, clinical studies specifically addressing these indications need to be done[79,80].

Senolytics.

SASP inhibitors attenuate the SASP without killing senescent cells. To decrease the burden of senescent cells pharmacologically, small molecules, peptides and antibodies that selectively eliminate senescent cells, referred to as ‘senolytics’, are being developed[15,32,81]. Since the discovery of senolytic agents in 2015 (REF.[82]), considerable progress has been made in identifying additional small molecule senolytic drugs and their effects. In that first article[82], a hypothesis-driven senolytic agent discovery paradigm was used. This system was based on the resistance of senescent cells to apoptosis[83], despite their production of proapoptotic SASP factors, which should trigger their own death. Indeed, proapoptotic pathways were found to be upregulated in senescent cells[82]. Therefore, the hypothesis was tested that senescent cells depend on senescence-associated antiapoptotic pathways (SCAPs), which permit senescent cells to survive their own SASP.

SCAPs were identified by bioinformatics approaches based on senescent cell expression profiles of human preadipocytes induced to become senescent by radiation[82]. The reliance of senescent cells on SCAPs was verified in vitro by RNA interference studies, which identified SCAPs as the Achilles heel of senescent cells[82,84]. This finding led to the discovery of potential senolytic targets within these SCAP networks and the first senolytic

drugs[82], including the combination of dasatinib, which is an FDA-approved tyrosine kinase inhibitor[85], and quercetin, which is a flavanol present in many fruits and vegetables[86] (D+Q)[82]. Of note, a protein of the BCL-2 family that antagonizes apoptosis (BCL-XL) was identified as a SASP component[82]. Following this discovery, the third senolytic drug, navitoclax, which is a BCL-2 family inhibitor, was identified[87,88]. Researchers have now identified an increasing number of senolytics, including additional synthetic small molecules, compounds that are derived from natural products and peptide inhibitors that target known SCAPs. In addition, further SCAPs are being investigated as potential senolytic targets[84,89,90].

SCAPs that are required for senescent cell survival differ among cell types. For example, the SCAPs needed for survival of senescent human primary adipose progenitors are different from those in senescent human embryonic venous endothelial cells (HUVECs)[82]. This difference means drugs that target a single SCAP will probably not be able to eliminate a wide range of senescent cell types. Indeed, most senolytics tested are effective against only a limited repertoire of senescent cell types. For example, navitoclax targets HUVECs; however, it is ineffective against senescent primary human adipocyte progenitors[51,87]. Evidence suggests that senolytics might have differing effectiveness even within one particular type of cell. For example, in human lung fibroblasts, navitoclax targets and kills senescent cells in the culture-acclimated IMR-90 lung fibroblast-like cell strain but is less effective against senescent primary human lung fibroblasts[51,87]. Thus, without extensive testing across a range of cell types, it is difficult to contend that any particular senolytic is universally effective.

Senolytics can act synergistically. For example, D+Q is effective in targeting senescent cultured mouse embryonic fibroblasts, whereas dasatinib or quercetin used alone are not[82]. Thus, different senolytics could prove to be effective for different indications. Moreover, combining different senolytics could extend the range of senescent cell types that can be eliminated.

The continuous presence of senolytics is not required for the drugs to be effective, as senescent cells take weeks or months to develop and acquire a SASP, at least in cell culture[15,81,91]. A brief disruption of prosurvival pathways is adequate to kill senescent cells in human adipose tissue explants, with senescent cells being eliminated within 18 hours of continuous exposure to the senolytic drug[34]. Thus, senolytics are seemingly effective in human tissue when administered intermittently[75,82]. Although D+Q has an elimination half-life of a few hours, a single dose of this combination decreases the effects of exposure of a leg to a 10-Gy dose of radiation for at least 7 months in mice[82]. Furthermore, as discussed later, intermittent administration of D+Q in doses that are weeks apart is effective in alleviating age-related osteoporosis in mice[75].

The frequency of senolytic treatment for particular conditions will probably depend on the rates of senescent cell accumulation, which might differ among circumstances in which cellular senescence occurs. For example, repeated exposure to DNA-damaging cancer therapies or continued high-fat feeding could cause a more rapid reaccumulation of senescent cells after senolytic therapy than chronological ageing does. The intermittent

administration of senolytics might reduce the risk of patients developing adverse effects and could permit the administration of senolytics during periods of good health. Moreover, intermittent dosing could decrease the risk of off-target effects and reduce the likelihood of patients developing drug resistance. Indeed, the development of proliferation-dependent drug resistance to senolytics is unlikely, as senescent cells do not divide (FIG. 3) and therefore cannot acquire advantageous mutations, which is in contrast to the situation with anticancer drugs or antibiotics.

Cellular senescence in ageing

Ageing is the leading risk factor for most of the chronic non-communicable diseases that account for the bulk of morbidity, death and health-care costs[8,92]. Most chronic diseases become more prevalent with increasing age, including T2DM, kidney disease, atherosclerosis, blindness, osteoporosis, osteoarthritis and dementias. The risk of various geriatric conditions, including frailty, immobility, mild cognitive impairment and incontinence, also increases with ageing. Furthermore, a loss of physiological resilience, with reduced capacity to recover following stresses such as surgery or pneumonia, occurs with advancing age[93]. These chronic diseases, geriatric syndromes and losses of resilience tend to cluster within older individuals (aged >60 years)[94], which is consistent with strong links to upstream fundamental ageing processes, such as cellular senescence[92].

The aforementioned observations led to the formulation of the geroscience hypothesis. If this hypothesis is true, then therapeutics that target fundamental ageing processes might be predicted to delay, prevent or alleviate age-related diseases and disorders as a group, as opposed to specific therapies that target one condition at a time. Moreover, if cellular senescence is indeed a key fundamental mechanism of ageing, then senolytics should be effective against a range of age-related conditions and chronic diseases.

Obesity, T2DM and their complications, including hepatic steatosis, diabetic kidney disease and obesity-related neuropsychiatric dysfunction, as well as age-related osteoporosis and growth hormone and/or IGF1 excess are among the endocrine conditions that have been associated with increases in senescent cells in pathogenically relevant tissues in mice and humans, as will be discussed later. In addition to these endocrine conditions, accelerated local or systemic senescent cell accumulation has been observed across a broad range of disorders and diseases in humans (BOX 1).

Evidencefrom mouse models.

In mouse models, a number of senescence-associated disorders analogous to those seen in humans (BOX 1) can be alleviated by treatment with senolytic agents, including D+Q, fisetin and navitoclax. For example, bleomycin inhalation results in a condition that models human idiopathic pulmonary fibrosis, and treatment with D+Q results in improved pulmonary function[51]. In mouse models of neurodegeneration and cognitive dysfunction generated by tau overexpression and amyloid-β overexpression, treatment with D+Q resulted in a reduction in markers of neuroinflammation and cognitive deficits[95,96]. Moreover, mouse models of myocardial damage repair and cardiac progenitor dysfunction[24,97], as well as age-induced or high-fat diet-induced vascular calcification and

hyporeactivity[82,98], showed improvements in cardiac and vascular function following clearance of senescent cells with D+Q treatment. In addition, mouse models of arteriovenous fistula occlusion show upregulation of senescent cell markers in the fistula that are reduced following D+Q treatment[17].

Other common age-related conditions that are characterized by senescent cell accumulation have been modelled in mice. For example, in an injury-induced osteoarthritis mouse model[99], treatment with the senolytic drug UBX0101, which targets the BCL-2 family of antiapoptotic factors, attenuated the development of post-traumatic osteoarthritis. Furthermore, frailty and vertebral disc degeneration were reduced by D+Q in progeroid *Ercc1*$^{-/\Delta}$ mice[82] and cirrhosis was attenuated by another senolytic drug targeting the BCL-2 pathway (A-1331852) in *Mdr2*$^{-/-}$ mice[100] (*Mdr2* is also known as *Abcb4*). In old mice, physical dysfunction and frailty were decreased by treatment with D+Q[34]. Moreover, treatment with D+Q reduced frailty, early onset of all age-related diseases and premature death in mice into which senescent cells had been transplanted[34]. As described earlier, impaired gait in mice after radiation-induced damage of a leg was improved after treatment with D+Q[82]. Finally, mortality associated with a range of diseases was decreased in aged mice that were treated with D+Q or another flavonoid senolytic drug, fisetin[34,101]. Thus, as predicted for agents that target fundamental ageing processes, senolytics seemingly hold promise for multiple disorders if these findings in mice can be translated into clinical application.

Ex vivo human studies and causality studies.

In addition to showing effects in mouse models of human diseases, the efficacy of senolytic agents can be evaluated by treating tissue explants from patients with specific diseases using different candidate senolytic drugs. This approach has been used for T2DM by treating adipose tissue explants from patients with T2DM undergoing bariatric surgery with senolytics[34]. Similar ex vivo human studies are ongoing for other diseases.

Before proceeding to clinical trials, it is important to establish whether the effects of a candidate agent for a disease state are due to actual senolytic effects or are due to off-target effects. Such evidence can be gained from preclinical models by fulfilling a modified set of Koch's postulates to establish causality (BOX 2). Such proof of causality is provided for D+Q by a study of physical dysfunction (that is, frailty) in mice[34]. Moreover, studies are under way to establish causality regarding treatment of osteoarthritis with fisetin in mice and attenuation of insulin resistance, age-related osteoporosis and cognitive dysfunction by D+Q in mouse models. We are not aware of work nearing completion that attempts to prove that navitoclax, nutlins or other potential senolytic agents alleviate disorders in preclinical animal models principally by clearing senescent cells as opposed to through off-target effects. Studies addressing this question would be a valuable direction for future research.

Clinical trials of senolytic drugs.

Clinical trials of senolytics are currently under way for several chronic non-communicable diseases. In the first clinical trial of senolytic agents, D+Q alleviated physical dysfunction defined as low gait speed, decreased gait distance, difficulty arising from a chair and

impaired function assessed by the short physical performance battery in a small trial in patients with idiopathic pulmonary fibrosis[102]. A second trial indicated that the senolytic agent dasatinib might decrease the number of senescent skin cells in patients with scleroderma, as skin senescence signatures and SASP signatures decreased after a 6-week trial of dasatinib[103]. Preliminary data from an ongoing trial of senolytics to alleviate dysfunction in patients with diabetic kidney disease[57] showed that a single 3-day course of D+Q is sufficient to reduce the burden of senescent cells in adipose tissue and SASP factors in the circulation; measurements were taken 11 days after the last dose. This finding suggests that, similarly to mice, in humans an intermittent 'hit-and-run' senolytic drug administration strategy might be effective, which reduces the risks of adverse effects compared with continuous treatment.

Other clinical studies currently under way include trials of senolytic drugs for frailty in older women (aged >60 years)[104] and for the premature ageing-like state in bone marrow transplant recipients[105]. In addition, clinical trials of senolytics are about to start for several disorders, for example age-related osteoporosis. These trials will contribute to testing whether the geroscience hypothesis holds in humans. So far, it seems that targeting senescent cells might be a promising potential approach for delaying, preventing or alleviating multiple age-associated and cellular senescence-associated conditions.

Senescence in endocrine diseases

As in other diseases, it is becoming clear that fundamental ageing mechanisms, in particular cellular senescence, have an important role in a number of endocrine diseases. Our current state of understanding of the role of cellular senescence in endocrine diseases and endocrine physiology is discussed in this section.

Osteoporosis.

For many years in the bone field, the question as to whether senescent cells accumulate within bone in the context of natural, chronological ageing as they do in other tissues remained unanswered. The type (or types) of cells in the bone microenvironment that become senescent with ageing and the nature of their SASP was also unclear. In the first study to address these questions[106], our group measured in vivo senescence and SASP markers in young versus old male and female mice in highly enriched populations of various cell lineages, all of which were rapidly isolated from the bone microenvironment without extensive manipulation. This survey revealed that expression of the senescent cell biomarker $p16^{Ink4a}$ (also known as *Cdkn2a*) increased consistently (approximately fivefold to tenfold) in both sexes with ageing in bone-derived enriched populations of B cells and T cells, myeloid cells, osteoprogenitors, osteoblasts and osteocytes. Despite the viewpoint held by some researchers at the time that postmitotic cells (such as osteocytes) cannot undergo cellular senescence, we found that osteocytes from old mice also displayed senescent cell features, including higher expression not only of $p16^{Ink4a}$ but also of $p21^{Cip1}$ (also known as *Cdkn1a*) as well as an accumulation of SADS[106].

Although the SASP can be variable depending on the cellular senescence inducer or type of senescent cell[37,38,107], our analysis revealed that with ageing, senescent myeloid cells and

senescent osteocytes in particular upregulate their production of multiple key SASP factors in mice[106]. Several of these SASP factors, including *Hmgb1, Il1a, Il6, Il8, Mmp9, Mmp12, Nfkb1* and *Pai2* (also known as *Serpinb2*), seem to be conserved across different types of senescent cells with ageing[37,108,109]. Our observation of an accumulation of senescent osteocytes in the bones of old mice has since been independently confirmed[110]. These findings have added to mounting evidence demonstrating that differentiated, non-dividing postmitotic cells, including adipocytes[111], neurons[112,113], hepatocytes[114] and cardiomyocytes[24], can acquire multiple key features of cellular senescence with ageing, such as high expression of $p16^{Ink4a}$ and $p21^{Cip1}$ as well as increased levels of SADS and SASP factors compared with normal cells of the same type.

Consistent with these observations in mice, our group additionally found that the expression levels of senescence biomarkers $p16^{INK4A}$ and $p21^{CIP1}$ were statistically significantly increased in small needle bone biopsy samples isolated from the iliac crest of older postmenopausal women compared with younger premenopausal women[106]. Despite the heterogeneous nature of the biopsy samples, which contained various populations of bone marrow cells and bone cell lineages, multiple SASP factors were detected at statistically significantly higher levels in the bones of older women than in those of younger women[106]. Collectively, these data establish that senescent cells accumulate in old bone in mice and humans, thereby implicating a potential role for these cells in the pathogenesis of osteoporosis.

To determine whether cellular senescence has a causative role in mediating age-related bone loss, our group targeted senescent cells in old mice by genetic clearance using *INK-ATTAC* mice; this model allows inducible systemic elimination of $p16^{Ink4a}$-expressing cells, many but not all of which are senescent, on administration of a drug (AP20187)[115]. This approach was compared with systemic elimination of senescent cells by intermittent administration of senolytics (D+Q) or with treatment with a SASP inhibitor (ruxolitinib)[75]. Each of these approaches in old mice (20 months or older) improved bone microarchitecture and increased strength[75]. Reducing the overall burden of senescent cells resulted in suppressed bone resorption on both trabecular and cortical surfaces, as well as bone formation that was increased on endocortical surfaces or maintained on trabecular surfaces[75]. Several lines of evidence indicate that these favourable effects on bone remodelling and metabolism were mediated partly by eliminating a subset of senescent osteocytes. For example, osteocyte-enriched bone samples from old *INK-ATTAC* mice treated with AP20187 (the drug used to activate the suicide transgene in *INK-ATTAC* mice) had reduced $p16^{Ink4a}$ expression[115] as well as decreased SADS. Furthermore, intermittent senolytic therapy in old wild-type mice reduced $p16^{Ink4a}$ expression as well as SADS in osteocyte-enriched bone samples[75].

Further evidence supporting the favourable effects of senescent cell clearance on bone formation included our findings of increased osteoblast numbers and bone formation rates in combination with a reduction in bone marrow adipose tissue in old *INK-ATTAC* mice treated with AP20187 compared with vehicle-treated mice[75]. This finding is consistent with an alteration in the lineage commitment of stem cells towards the osteoblast and away from the adipocyte lineage following senescent cell clearance. Thus, several hallmarks of ageing

bone, including reduced bone formation and increased bone marrow adiposity, were rescued at least partially by reducing the senescent cell burden in old mice[75].

Currently available treatments for elderly patients with osteoporosis and the associated fragility fractures are suboptimal. Furthermore, the fear of very rare adverse effects (for example, osteonecrosis of the jaw or atypical femur fractures) and complications has led to a crisis whereby many patients who are in need of pharmacological therapy are either not being prescribed any available medications or simply not adhering to their therapy[6]. Moreover, from a therapeutic perspective, targeting senescent cells using the intermittent administration of senolytics seems to offer substantial advantages over conventional antiresorptive therapy[116-118]. Indeed, with antiresorptive therapy (for example, bisphosphonates, denosumab, oestrogen or raloxifene), bone resorption is decreased as a result of the elimination of osteoclasts, which in turn (owing to the coupling of bone resorption to bone formation[119]) leads to a reduction in bone formation (FIG. 4).

As described earlier, senescent cells accumulate in the bone microenvironment with ageing[106], where they act to increase bone resorption and decrease bone formation[75]. Intermittent senolytic therapy reduces the burden of senescent cells, which in turn suppresses bone resorption with either an increase (cortical bone) or maintenance (trabecular bone) of bone formation, leading to favourable ‘uncoupling’ between osteoclasts and osteoblasts[75] (FIG. 4). However, as with any novel therapeutic paradigm, a need exists for the discovery, development and optimization of interventions that target senescent cells to alleviate age-related bone loss. As such, further research is needed to better understand the underlying mechanisms that are altered in response to reducing the senescent cell burden in the setting of ageing. This knowledge is required to guide development and translation of senotherapeutics for treatment of osteoporosis in humans.

In addition to having a role in the pathophysiology of osteoporosis, cellular senescence might also be important in bone physiology in the growth plate and might be regulated by a local modulator of the growth plate, parathyroid hormone-related protein (BOX 3).

Metabolic syndrome and T2DM.

The major risk factors for the development of T2DM are increasing age and obesity, each of which is associated with an increased burden of senescent cells. Senescent cells accumulate in adipose tissue of humans and mice in T2DM, obesity (primarily hypertrophic obesity) and age-related metabolic dysfunction[18,20,23,26,111120-123]. In normoglycaemic individuals, adipocyte size is positively related to the number of markers of senescence present in subcutaneous fat[20,124]. Of note, in individuals with genetic predisposition for T2DM, increased senescent cell burden can occur even before T2DM develops[124]. Consistent with this finding, polymorphisms in genetic markers of cellular senescence (for example, $p16^{INK4}$ (*CDKN2A*)) are associated with increased risk of developing both T2DM and cardiovascular disease[125].

As described earlier, the cell cycle regulator p53 is also a senescence-associated regulator, particularly in adipose tissue. Furthermore, p53 expression is glucose responsive and a key mediator of adipogenesis[126]; p53 activity must be downregulated before adipose progenitors

can undergo differentiation into insulin-responsive adipocytes. Activation of p53 and accumulation of ROS occur in adipose tissue early during the development of obesity, which prevents the normal differentiation of progenitors into adipocytes. This change can precede the appearance of insulin resistance, adipose tissue inflammation and glucose intolerance[127]. Additionally, activation of p53 reduces insulin-induced glucose transport and increases lipolysis in adipocytes, thereby contributing to both inflammation and insulin resistance[127]. In both ageing and T2DM, p53 activity and expression in adipose tissue is increased, and genetic activation of p53 in adipose tissue of mice leads to insulin resistance[111].

Cellular senescence in adipose progenitors is a key negative regulator of adipogenesis, both through cell-autonomous mechanisms and by affecting neighbouring cells via the SASP[23]. For example, components of the SASP secreted by adipose-derived senescent cells can contribute to insulin resistance in metabolic tissues in vivo in mouse models by interfering with insulin signalling, impeding adipogenesis and attracting immune cells, each of which is a mechanism through which senescent cells can contribute to the risk of T2DM and metabolic syndrome[26,34,121]. Once formed, senescent adipose progenitors can affect the function of neighbouring cells by inhibiting adipogenesis, as demonstrated in co-culture experiments[26]. Additionally, senescent cells in general can directly promote insulin resistance through the secretion of SASP factors such as activin A, IL-6 and TNF[59,128]. In diet-induced obese (DIO) mice, senescent adipocytes can also chemoattract macrophages into visceral adipose tissue[121]. Chemokines released by these macrophages can further exacerbate T2DM.

Although cellular senescence can contribute to development of T2DM, the pathophysiological processes occurring in T2DM can themselves increase the burden of senescent cells. For example, elevated glucose and lipid levels, similarly to inflammation, can induce cellular senescence, which potentially amplifies the accumulation of senescent cells not only in adipose tissue but also systemically, particularly in uncontrolled T2DM[18,129]. Moreover, senescent-like pancreatic β-cells can accumulate in vivo in mice with T2DM and in ageing[130]. These cells might be capable of increased, rather than decreased, insulin secretion[130]. Perhaps paradoxically, type 1 diabetes mellitus in mouse models is partially alleviated and insulin production is increased in association with decreases in the abundance of these senescent-like β-cells following the administration of navitoclax (ABT263), a senolytic that targets senescent-like β-cells but not senescent adipocyte progenitors[87,131]. Thus, navitoclax acts by increasing insulin release and has little direct effect on peripheral tissue insulin sensitivity.

The senolytic combination therapy D+Q decreases the abundance of human senescent cultured adipocyte progenitors, a key cell type involved in the pathogenesis of T2DM and its complications[82,121]. Furthermore, D+Q is more effective in doing so than navitoclax[131]. In vivo, the abundance of senescent cells declines after intermittent administration of D+Q to DIO mice[121]. Mass cytometry analyses conducted after a single course of D+Q in these mice showed that adipose tissue senescent cells were statistically significantly reduced in number. Moreover, the main cell type targeted was adipose progenitor cells, rather than endothelial cells, macrophages or T cells. After D+Q administration, these DIO mice became more insulin sensitive, as indicated by insulin tolerance testing and an increased

glucose infusion rate during hyperinsulinaemic clamping. In addition, AKT Ser473 phosphorylation (a marker of cellular insulin sensitivity) increased in response to insulin stimulation of adipose tissue freshly isolated from these mice[121].

In addition to improved metabolic parameters, DIO mice treated intermittently with D+Q show decreased abundance of adipose tissue macrophages, as well as reductions in the crown-like structures that are associated with macrophage infiltration of adipose tissue in obesity and T2DM[121]. Notably, this decline in macrophage abundance was not due to the killing of macrophages by D+Q[34]. Rather, D+Q administration and associated reductions in the burden of senescent adipose progenitors caused decreased chemoattraction of monocytes into adipose tissue, despite the absence of changes in diet or weight. This finding is consistent with the hypothesis that senescent cells in adipose tissue attract, anchor and activate immune cells in T2DM associated with obesity[23].

T2DM is associated with risk of complications involving the liver, heart, brain, nerves, eyes and kidneys. For example, T2DM is associated with an increased risk of non-alcoholic fatty liver disease (NAFLD), which is associated with metabolic syndrome. In humans, NAFLD is linked with increased abundance of senescent cells in the liver, and the extent of steatosis correlates with the senescent cell burden[114]. In mice, the induction of senescence in hepatocytes causes increased lipid deposition in the liver, suggesting a direct role of senescent hepatocytes in the pathogenesis of NAFLD. Furthermore, steatosis in these mice can be alleviated by administering D+Q[114].

Cells in the media of the aorta and atherosclerotic plaques of both hypercholesterolaemic (*Apoe*$^{-/-}$) and ageing mice have increased levels of senescence markers. Furthermore, the removal of senescent cells from these mice with D+Q leads to increased vascular smooth muscle reactivity in response to nitric oxide donors and decreased calcium deposition[82,98], indicating that senescent cells have a role in vascular dysfunction associated with metabolic syndrome. Senolytic treatment of obese or ageing mice improves cardiac function, which has translational implications for patients with T2DM, in whom heart failure is common[23,97]. With ageing, cardiac progenitor cells develop a hypertrophic, profibrotic phenotype and undergo loss of replicative capacity. Senescent cell removal from mice alleviates the age-related dysfunction of cardiac progenitor cells and decreases the fibrotic area formed after myocardial infarction[24,97,132].

Cellular senescence contributes to cognitive dysfunction in both DIO insulin-resistant mice and old mice[113]. Intermittent treatment with D+Q reduces the burden of senescent cells in the brain, restores neurogenesis and alleviates neuropsychiatric dysfunction in DIO mice[113]. Moreover, D+Q treatment also reduces neuroinflammation, restores neurogenesis, partly reverses brain atrophy and alleviates cognitive dysfunction in old mice with Alzheimer-like states due to tau or amyloid-β protein overexpression[95,96].

Cellular senescence is increased in kidney cells from individuals with T2DM[23,133]. In addition, in DIO mice compared with lean mice, renal function is impaired, cortical oxygenation reduced and glomerulomegaly induced, which occur together with increases in markers of senescence (p16^{INK4A}, p19 and p53) and the SASP in renal tubular cells.

Quercetin improves renal function (decreases plasma levels of creatinine), improves oxygenation, decreases glomerulomegaly and reduces renal fibrosis in DIO mice[133]. Furthermore, D+Q treatment reduces proteinuria in DIO insulin-resistant mice[121]. In summary, the administration of senolytics alleviates a number of the complications of metabolic disease in mice.

Importantly, in patients with diabetic kidney disease, a 3-day course of oral D+Q treatment decreased the burden of senescent cells in adipose tissue and decreased adipose tissue macrophage infiltration and crown-like structures, together with decreased circulating levels of SASP factors[57]. Taken together, these findings in humans and mice support the importance of cellular senescence in the development of T2DM and its complications. They suggest that further clinical trials of reducing the senescent cell burden in both T2DM and its complications could be of value.

Cellular senescence in reproductive ageing.

A survey across tissues in rodent models of ageing showed increased *p16*^{Ink4a} expression in multiple tissues, including the ovaries in old mice and rats and the testes in old rats but not old mice[54]. Caloric restriction markedly attenuated the age-related increase in *p16*^{Ink4a} expression across many tissues, including the ovary and testes. To date, however, more data are available on the role of cellular senescence in ovarian versus testicular ageing. Accumulating evidence, principally from rodent models, suggests that cellular senescence has an important role in ovarian ageing. For example, shortened telomeres in ovarian cells can lead to replicative senescence and impair female reproduction. In addition, cellular senescence might limit the proliferation of primordial germ cells[134].

Evidence suggests that cellular senescence could have a physiologically beneficial role during pregnancy by contributing to normal placental development. Furthermore, in the developing fetus, programmed cell senescence has been identified at multiple locations during embryonic development, where it facilitates tissue remodelling[135,136]. In addition, the SASP of senescent cells seems to contribute to the initiation of parturition. However, cellular senescence might also have a role in the impaired angiogenesis associated with pre-eclampsia[137]. Thus, for reproductive function, cellular senescence could represent an example of antagonistic pleiotropy, whereby it contributes to proper fetal development and timely parturition during the reproductive years but also to the risk of pre-eclampsia and a decline in ovarian function with ageing[134]. In addition, chemotherapy is a potent trigger of cellular senescence in multiple tissues[64]; therefore, cellular senescence might have an important role in the premature ovarian failure associated with chemotherapy, although further studies are needed to evaluate this.

Oncogene-induced cellular senescence in pituitary tumours.

Pituitary tumours, which are present at autopsy in ~20% of the population[138], generally remain benign and rarely progress to carcinomas; indeed, malignant transformation occurs in less than 2% of all clinical pituitary tumours[139]. A number of mechanisms have been hypothesized to contribute to cessation of pituitary tumour cell proliferation, including G1 cell cycle arrest associated with DNA damage and p53-mediated induction of *p21*, leading to

cellular senescence[140]. This process has been described as ‘oncogene-induced senescence’ and is believed to have a role in preventing malignant transformation by causing growth arrest of tumour cells. However, clinical evidence supporting this hypothesis is conflicting. One study found increased SA-βgal staining in pituitary adenomas compared with normal pituitary tissue in specimens from pituitary surgical operations[141]. By contrast, a second study found similar SA-βgal staining in both pituitary adenomas and carcinomas obtained from surgical operations, arguing against loss of cellular senescence as contributing to malignant transformation[142]. Thus, the role of cellular senescence in maintaining pituitary or other endocrine tumours in a cell cycle-arrested, benign state warrants further study.

Conclusions

It is now increasingly clear that cellular senescence has a key role in the pathogenesis of a number of age-associated disorders, including osteoporosis, metabolic syndrome, T2DM and probably several other endocrine diseases. Targeting senescent cells has emerged as an attractive therapeutic strategy to simultaneously treat several diseases, thereby reducing polypharmacy and the attendant risks of adverse events and drug interactions. Senolytic drugs are particularly attractive, as they seem to be effective even when given only intermittently, thus minimizing potential toxic effects. However, given the potential role of cellular senescence in reducing uncontrolled proliferation and cancer[11], and potentially in wound healing[64], carefully conducted clinical trials in humans are needed to better define the benefits and potential risks of these drugs.

Acknowledgements

The authors acknowledge the support of the US National Institutes of Health through grants AG062413 (project 1, J.L.K.; project 2, S.K., J.N.F.), AG004875 (S.K.), AR027065 (S.K.), AR070241 (J.N.F.) and AG013925 (J.L.K.), the Translational Geroscience Network (AG061456; J.L.K.), Robert and Arlene Kogod, the Connor Group (J.L.K.), Robert J. and Theresa W. Ryan (J.L.K.) the Ted Nash Long Life Foundation (J.L.K.) and the Noaber Foundation (J.L.K.).

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Key points

- The ‘geroscience hypothesis’ posits that manipulation of fundamental ageing mechanisms will delay the appearance or severity of multiple chronic diseases because these diseases share the same underlying risk factor — namely, ageing.
- Cellular senescence is a fundamental ageing mechanism that can contribute to or cause age-related phenotypes as well as multiple diseases, including endocrine disease, even in younger individuals (aged <40 years).
- Some senescent cells, which accumulate with ageing, develop a proinflammatory, tissue-destructive and stem cell-disrupting or progenitor cell-disrupting senescence-associated secretory phenotype (SASP), which can spread senescence to nearby and distant non-senescent cells.
- Senolytic agents have been discovered that selectively eliminate senescent cells by transiently disabling the survival pathways (senescent cell antiapoptotic pathways) that protect senescent cells against their own SASP.
- Preclinical studies suggest that senolytics hold promise for delaying, preventing or treating many age-associated disorders.
- Despite the growing experimental support for targeting cellular senescence to treat multiple age-associated diseases simultaneously, carefully conducted clinical trials in humans are needed to better define the benefits and risks of senolytic drugs.

Box 1 |

Senescent cell accumulation in non-endocrine disorders

- Frailty and impaired ambulation in elderly women[143]
- Progeroid syndromes in children[82,101,144]
- Pre-eclampsia[137]
- Macular degeneration and other eye diseases[145]
- Skin conditions, including psoriasis, scleroderma, melanocytic naevi, actinic keratosis, non-healing skin wounds and other skin disorders[146]
- Idiopathic pulmonary fibrosis[51,102]
- Chronic obstructive pulmonary disease[147]
- Dementias[95,96,148]
- Parkinson disease[149]
- Sites damaged by radiation[150]
- Cardiac dysfunction after myocardial infarction[151]
- Atherosclerosis[152]
- Sclerosis-related occlusion of arteriovenous fistulae used for dialysis[17]
- Chronic kidney disease[57]
- Cirrhosis[153]
- Cancers[154]
- Osteoarthritis[155]
- Degenerated vertebral discs[156]

Box 2 |

Modified Koch's postulates to prove the senolytic properties of a drug

- Senescent cells are present in association with the phenotype.
- Individuals without senescent cells do not have the phenotype.
- Inducing accumulation of senescent cells causes the phenotype.
- Clearing these induced senescent cells alleviates the phenotype.
- Clearing naturally occurring senescent cells alleviates the phenotype.
- The drug has few or no effects related to the phenotype in young individuals without senescent cells.
- Administering senolytics intermittently is effective.
- Senolytics should alleviate multiple age-related conditions.

Box 3 |

Parathyroid hormone-related protein and regulation of the growth plate

A series of studies[157-159] have demonstrated that mice that express only a nuclear localization signal (NLS)-deficient form of parathyroid hormone-related protein (PTHrP) have growth retardation and an accelerated ageing phenotype in multiple tissues, which is characterized by increased cellular senescence in multiple tissues. Loss of the PTHrP NLS results in reduced expression of *Bmi1,* a member of the Polycomb–Trithorax gene family that regulates gene expression through chromatin remodelling[157]. Decreased *Bmi1* expression leads, in turn, to increased expression of $p16^{Ink4a}$ and $p21^{Cip1}$, triggering cellular senescence. Thus, PTHrP, through nuclear localization, serves to prevent senescence not only in bone but also in multiple other tissues.

These findings are perhaps related to a separate line of work showing that mesenchymal stem-progenitor cells (MSPCs) in the primary spongiosa of the long bones in mice at late puberty undergo programmed senescence, which is controlled by another member of the Polycomb family, the histone methyltransferase enhancer of zeste homologue 2 (EZH2)[160]. Temporally, this senescence of MSPCs is associated with cessation of longitudinal growth and fusion of the growth plates. The Indian hedgehog protein (IHH)–PTHrP signalling pathway is critical for maintaining the growth plate in an open phase[161]; therefore, nuclear PTHrP signalling could have a physiological role in preventing senescence of MSPCs in the primary spongiosa and thereby maintaining longitudinal growth. These actions would be complementary to the known effects of IHH-PTHrP signalling in delaying the differentiation of columnar chondrocytes into hypertrophic chondrocytes that also serve to keep the growth plate open[161]. However, further studies are needed to test this hypothesis.

Damage-associated molecular patterns

Extracellular nucleotides, Other cellular debris or proteins that are released by damaged cells (for example, HMCB1).

Pathogen-associated molecular patterns

Factors released by viral, fungal or bacterial pathogens.

Warburg shift

The preferential utilization of glycolysis rather than oxidative phosphorylation by a cell.

Lipofuscin

Lipid-containing pigment granules found in cells and associated with ageing.

Senescence-associated distension of satellites (SADS).

The distention of satellite DNA found in senescent cells.

Myeloproliferative syndrome

Disorders of bone marrow and blood associated with the clonal proliferation of cells that may progress to leukaemia.

Koch's postulates

A set of criteria used to establish the cause of a disease.

Hypertrophic obesity

Refers to enlarged adipocytes, typically found in abdominal obesity.

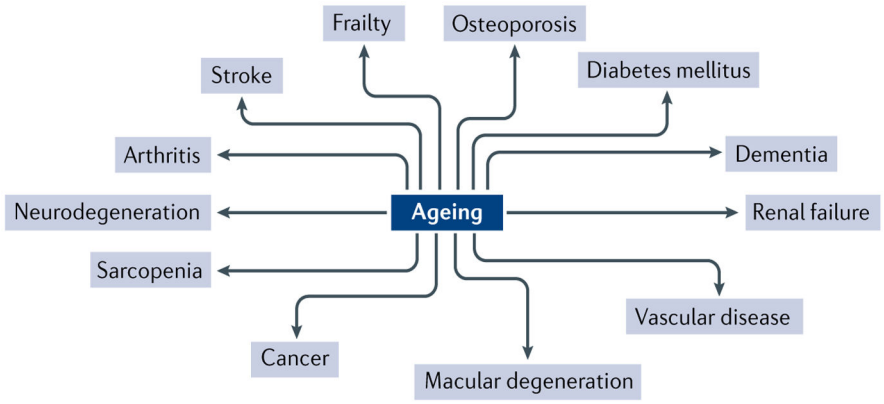

Fig. 1 |. The central role of ageing in chronic diseases.

This figure shows examples of chronic non-communicable diseases that have ageing as one of the main risk factors. Reprinted with permission from REF.[11], JCI.

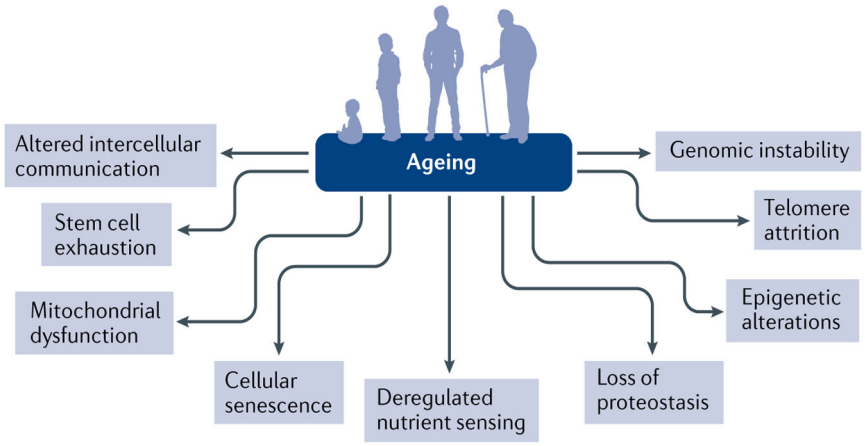

Fig. 2 |. Nine fundamental hallmarks of ageing.

Nine fundamental physiological, cellular and molecular hallmarks of ageing that provide a useful framework for mechanistic studies. Reprinted with permission from REF.[9], Elsevier.

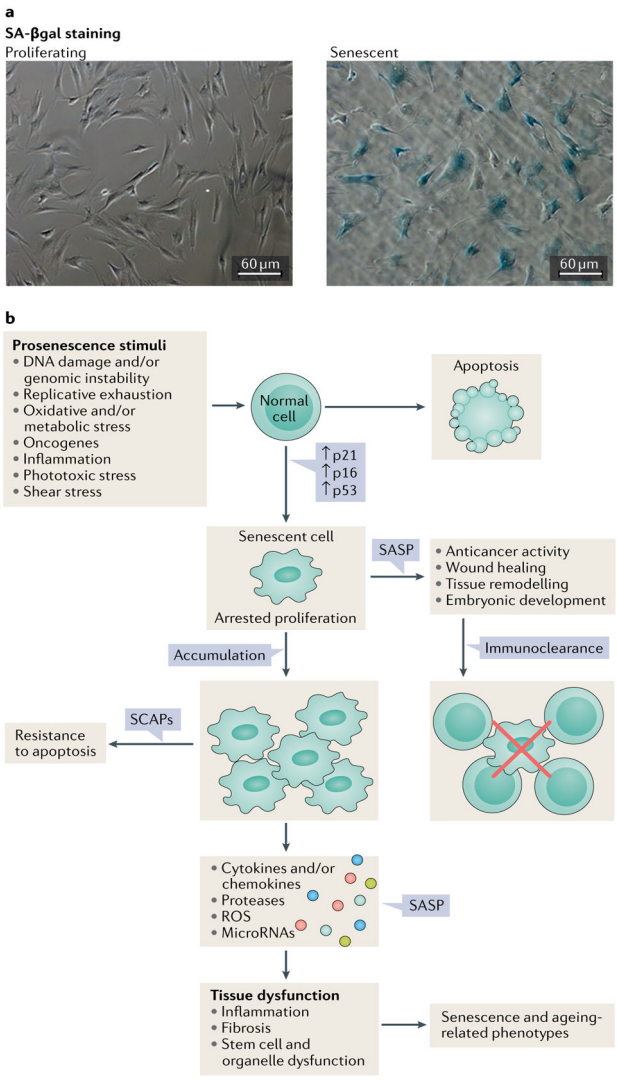

Fig. 3 |. Causes and consequences of cellular senescence.

a | Images of senescence-associated β-galactosidase (SA-βgal) staining of proliferating and senescent human preadipocytic cells. Bars represent 60 μm. **b** | Different stimuli can induce cellular senescence. The phenotypes of senescent cells are cell type and context dependent; however, cells can develop a senescence-associated secretory phenotype (SASP), arrested proliferation and resistance to proapoptotic pathways through senescence-associated antiapoptotic pathways (SCAPs). The formation of senescent cells in response to damage has an important role in tumour suppression and is also necessary for tissue repair (for instance during wound healing). By contrast, the persistent presence of fairly small numbers of senescent cells can promote a protumorigenic milieu and impair the function of multiple tissues. ROS, reactive oxygen species.

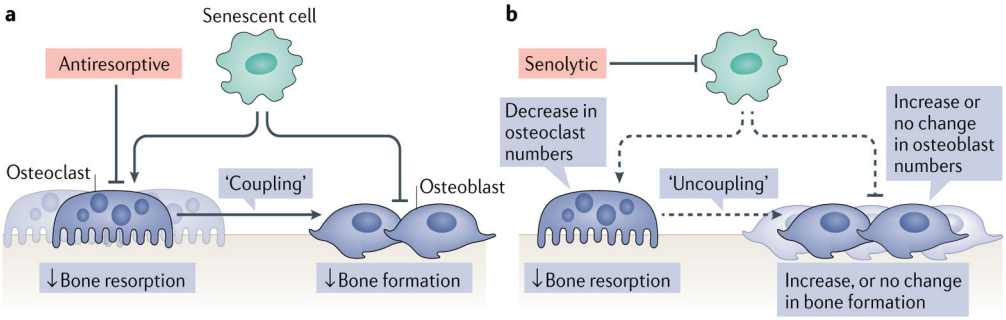

Fig. 4 |. The effects of antiresorptive versus senolytic therapies on bone metabolism.

a | Senescent cells accumulate in the bone microenvironment with ageing, where they increase bone resorption by osteoclasts and reduce bone formation by osteoblasts. Antiresorptive drugs inhibit or eliminate osteoclasts and decrease bone resorption. Owing to coupling between osteoclasts and osteoblasts in the bone remodelling cycle, bone formation is also reduced. **b** | Senolytic therapy reduces the burden of senescent cells, which leads to a reduction in bone resorption with either increased (cortical bone) or maintained (trabecular bone) bone formation, resulting in a beneficial 'uncoupling' between bone resorption and bone formation. Dashed lines indicate a reduction in the adverse effects of senescent cells on osteoclasts, osteoblasts, and the coupling between osteoclasts and osteoblasts. Reprinted from REF.[75], Springer Nature Limited.