HHS Public Access

Author manuscript

Nat Rev Mol Cell Biol. Author manuscript; available in PMC 2021 August 06.

Published in final edited form as:

Nat Rev Mol Cell Biol. 2021 February ; 22(2): 75–95. doi:10.1038/s41580-020-00314-w.

Cellular senescence in ageing: from mechanisms to therapeutic opportunities

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Abstract

Cellular senescence, first described in vitro in 1961, has become a focus for biotech companies that target it to ameliorate a variety of human conditions. Eminently characterized by a permanent proliferation arrest, cellular senescence occurs in response to endogenous and exogenous stresses, including telomere dysfunction, oncogene activation and persistent DNA damage. Cellular senescence can also be a controlled programme occurring in diverse biological processes, including embryonic development. Senescent cell extrinsic activities, broadly related to the activation of a senescence-associated secretory phenotype, amplify the impact of cell-intrinsic proliferative arrest and contribute to impaired tissue regeneration, chronic age-associated diseases and organismal ageing. This Review discusses the mechanisms and modulators of cellular senescence establishment and induction of a senescence-associated secretory phenotype, and provides an overview of cellular senescence as an emerging opportunity to intervene through senolytic and senomorphic therapies in ageing and ageing-associated diseases.

Cellular senescence occurs in response to many different triggers, including DNA damage, telomere dysfunction, oncogene activation and organelle stress, and has been linked to processes such as tumour suppression, tissue repair, embryogenesis and organismal ageing.

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Author contributions

The authors contributed equally to the writing and revision of the article.

Competing interests

D.B. is a co-inventor on patent applications licensed to or filed by Unity Biotechnology, a company developing senolytic medicines, including small molecules that selectively eliminate senescent cells. Research in the Baker laboratory has been reviewed by the Mayo Clinic Conflict of Interest Review Board and is being conducted in compliance with Mayo Clinic conflict of interest policies. V.K. is a co-inventor on patent applications in the field of senolytics, some of which are licensed to Sentaur Bio. F.d'A.d.F. is among the inventors on patent applications for the use of antisense oligonucleotides to target DNA damage-induced transcripts. R.D.M. declares no competing interests.

Hayflick and Moorhead demonstrated in 1961 that normal cultured human fibroblasts display a finite capacity for cell division before entering an irreversible growth arrest known as replicative senescence[1]. This led to the hypothesis that tissue ageing is caused by cells progressively losing their ability to proliferate, which is essential to replace damaged cells that naturally accumulate over time[2]. However, it took several decades to develop tools to prove that senescent cell accumulation promotes organismal ageing and dysfunction.

The first hurdle involved identifying selective markers to detect these cells in living tissues. Senescence-associated-β-galactosidase (SA-β-gal) activity, a simple colorimetric assay, was one of the first biomarkers described, and became instrumental in demonstrating that cells with features of senescence accumulate at sites of ageing-associated diseases and in aged tissues in a variety of mammals. A distinctive feature of senescent cells is the increased expression of cell cycle-inhibitory proteins, collectively known as cyclin-dependent kinase inhibitors. The cyclin-dependent kinase inhibitor with the most prominent role in senescent cell accumulation during ageing is $p16^{INK4A}$ — hereafter referred to as p16 (REFS[3,4]) — as it is crucial for durably maintaining the state of proliferative arrest. Indeed, mice lacking p16 are predisposed to spontaneous tumour formation[5]. In the late 1990s, it was found that cellular senescence is prematurely induced by excessive oncogenic signalling or loss of tumour suppression[6]. Senescence induction was later shown to be caused by aberrant DNA replication and DNA damage accumulation[7,8], thus restricting the proliferation of damaged precancerous cells. However, none of these features is universal for senescent cells, and it is important to test several biomarkers simultaneously to define the senescence state.

To reconcile the seemingly opposing pro-ageing and anticancer roles of senescent cells from an evolutionary perspective, senescent cells were proposed to fit the antagonistic pleiotropy theory of ageing, which posits that natural selection favours genes that promote reproductive fitness early in life, which may be accompanied by unselected consequences with negative effects later in life[9], although this has not been proven[10]. Alternatively, it is conceivable that evolutionary cost and benefit theory is relevant for senescence. This theory implies that senescent cells have beneficial effects throughout life (for example, limiting tissue damage and suppressing tumorigenesis), but the cost of these effects overcomes the benefits in old age. Several approaches have recently enabled the establishment of a causative role for senescent cells in many diseases. These include the development of INK-ATTAC[11] and p16-3Mr[12] transgenic mouse models in which p16-expressing cells can be selectively eliminated, and of senolytic and senomorphic pharmacological agents. Senolytics target senescent cells for elimination, whereas senomorphics modulate the properties of senescent cells without eliminating them[13].

INK-ATTAC

Transgenic mouse model with drug-inducible caspase 8 under the control of a minimal p16 promoter element active in senescent cells to allow selective elimination of p16-expressing senescent cells.

p16-3MR

Transgenic mouse model expressing a trimodal reporter of red fluorescent protein, luciferase and herpes simplex virus thymidine kinase under the control of the p16 promoter to allow tracking and elimination of p16-expressing senescent cells.

In this Review, we first describe the properties of senescent cells and the mechanisms that promote this phenotype. We then discuss the implication of senescent cells in diverse biological processes, and how their removal or the attenuation of their properties could be exploited for therapeutic intervention and to increase healthspan.

Inducers and features of cellular senescence

Cellular senescence is a stable and terminal state of growth arrest in which cells are unable to proliferate despite optimal growth conditions and mitogenic stimuli (BOXES 1,2; FIG. 1). Senescent cells have increased resistance to apoptotic cell death owing to upregulation of cell survival pathways, including the BCL-2 family of antiapoptotic proteins, even on exogenous stress exposure[14,15]. Whether this prolonged viability is the result of selection for the most death-resistant cells or whether it is an intrinsic property of the senescence programme, especially in vivo, remains to be established. The molecular mechanisms that determine the choice between apoptosis and senescence remain unclear, but it is possible that cell fate depends on the intensity and duration of the initial stimulus, as well as the nature of the damage and the cell type[16]. Because senescence and apoptosis programmes converge on crucial components, including activation of the p53 pathway, it is also possible that senescent cell resistance to apoptosis depends on p53 levels and activity[17]. Although senescence was believed to be a permanent condition of cell cycle arrest, recent evidence indicates that, at least in the context of tumour formation and anticancer therapies, the establishment of cellular senescence might involve epigenetic mechanisms that reprogramme cancer cells towards a certain degree of stemness in a cell-autonomous fashion[18]. Of note, the establishment of senescence is a dynamic process, whereby overlapping but distinct molecular pathways are engaged at different stages, from immediately on cell cycle exit to late, senescence stages[19].

Cellular senescence and the DNA damage response.

Several stressors can induce cellular senescence. Nuclear DNA damage is often reported as a commonly underlying cause of senescence, mainly in the form of DNA double-strand breaks (DSBs)[20] that activate the DNA damage response (DDR) pathway (FIG. 1). DDR exerts checkpoint functions to block cell cycle progression and prevent the propagation of corrupted genetic information to daughter cells. Some DDR factors accumulate at sites of DNA damage and form cytologically detectable nuclear foci composed of extended chromatin modification events, such as the phosphorylation of histone H2AX, and the proteins associated with them, including MDC1, 53BP1 and the activated form of the kinase ataxia telangiectasia mutated (ATM)[21]. These foci mark individual sites of DNA damage and contribute to checkpoint enforcement and cell cycle arrest, until damage has been repaired. If DNA damage persists, it causes prolonged DDR signalling and protracted

proliferative arrest in the form of cellular senescence[22]. The recent demonstration that persistent DDR foci observed in cultured senescent cells contain unrepaired DSBs[23] supports the notion that cellular senescence is akin to prolonged checkpoint activation. Inhibition of DDR signalling kinases (ATM, ATR, CHK1 and CHK2) allows senescent cells to re-enter the cell cycle[7,24,25]. At the bottom of the DDR cascade, the tumour suppressor p53, which is a target of ATM and its paralogue ATR, is activated and stimulates the expression of the cyclin-dependent kinase inhibitor p21, an essential mediator of senescence-associated cell cycle arrest. p16, an inhibitor of CDK4 and CDK6, is also key in several types of senescence[26]; p21 is activated early on senescence entry and p16 is activated later, probably to maintain the senescence phenotype[27]. In addition to the DDR cascade being activated, the tumour suppressor ARF stabilizes p53, which contributes to the induction of senescence[28]. Efforts have been devoted to assessing the contribution of these two major routes, the DDR and ARF pathways, to p53-dependent senescence establishment especially in response to oncogenic challenges. The original view, based mainly on murine studies, was that the DDR and ARF play antagonistic roles, as ARF was transcriptionally activated during tumorigenesis in a DDR-independent manner[29,30]. More recently, a tight regulatory network in human cancer models was reported, whereby ATM suppresses ARF levels and ARF acts as a secondary barrier to cancer progression when ATM is inactivated[31]. Consistent with this temporal regulation, DDR precedes ARF engagement, whose activation is detected at later stages of cancer progression and less frequently than DDR.

Telomere shortening and damage.

One of the first and best characterized mechanisms of cellular senescence induction is telomere shortening. As the standard DNA replication apparatus is unable to fully duplicate chromosomal DNA ends, in the absence of telomere maintenance mechanisms such as the expression of telomerase or recombination among telomeres, telomeres shorten with each round of DNA replication. Below a certain length, the loss of telomere-capping factors or protective structures makes critically short telomeres resemble one-ended DSBs and thus triggers a DDR that is very similar to that triggered by DNA DSBs[24,32] (FIG. 1). One or a few DDR signalling telomeres are sufficient to trigger replicative cellular senescence[33], and forced expression of telomerase prevents cellular senescence and promotes unlimited cell proliferation[34].

Persistent DDR activation occurs also at telomeres that are not critically shortened, in non-dividing cells exposed to exogenous genotoxic treatments and in non-dividing ageing cells, because repair is much less efficient when DSBs are localized within telomeres[35–37]. As telomeric DSBs persist, cellular senescence is established and maintained. Thus, persistent DDR activation at telomeres, which is a trigger of cellular senescence, can occur both upon telomere shortening in proliferating cells and upon telomeric DNA damage also in non-proliferating (quiescent or terminally differentiated) cells, independently of telomere length[38].

Oncogene-induced senescence.

Oncogene activation is a powerful inducer of cellular senescence. Oncogene expression triggers an initial hyperproliferative phase that is intrinsically associated with altered DNA

replication, which eventually engages DDR pathways and causes senescence[7,8,39]. This process is known as oncogene-induced senescence (OIS). Loss of tumour suppressor expression can also induce proliferation arrest, as exemplified by PTEN loss-induced cellular senescence (PICS). Although initially PTEN loss-induced cellular senescence was not associated with DDR activation[40], it was later found to be associated with hyperproliferation, DDR engagement and cellular senescence in vivo[41]. Noteworthy, unlike oncogenic RAS or BRAF, activation of the PI3K–AKT pathway promotes p53-dependent senescence often in the absence of detectable hyperproliferation and strong DNA damage accumulation[42,43], suggesting distinct underlying mechanisms.

Telomeres are hypersensitive to DNA replication stress, including that induced by oncogenes and accumulation of oncogene-induced telomeric dysfunction, and a DDR has been observed in hyperplastic cancer lesions in humans[44]. Reactive oxygen species (ROS) accumulate in tumours, and in this context, in addition to their recognized role as DNA-damaging agents, they can act as signalling molecules that mediate pro-mitogenic oncogene functions. Recently, this paradoxical role of ROS in promoting cell proliferation and senescence-associated DNA damage was partly solved by the unexpected discovery that oncogene-induced ROS, generated by NADPH oxidases, can induce cellular senescence by boosting the initial hyperproliferative phase associated with altered DNA replication and DNA damage accumulation[45] (FIG. 1).

Mitochondrial dysfunctions and cellular senescence.

Increased oxidative stress in senescent cells has been linked to the accumulation of dysfunctional mitochondria. Indeed, senescent cells are characterized by changes in mitochondrial mass, membrane potential and mitochondrial morphology[46]. Dysfunctional mitochondria may play an important role in senescence establishment, as depletion of mitochondrial sirtuins, a group of evolutionarily conserved proteins that regulate ageing across different species, as well as selective chemical inhibition of mitochondrial function, triggers senescence[47]. There is evidence in support of a reciprocal influence between nuclear DNA damage and mitochondrial dysfunction[48]. Of note, mitochondrial dysfunction-associated senescence (MiDAs), which is characterized by a distinct phenotype, exhibits a unique cell-non-autonomous programme that is potentially responsible for the altered metabolism and aberrant adipocyte differentiation observed in aged animals[47].

Sirtuins

Nicotinamide dinucleotide (NAD+)-dependent deacylases that regulate diverse cellular processes, including DNA repair, inflammation, metabolism and ageing.

Mitochondrial dysfunction-associated senescence

(MiDAs). Mitochondrial damage triggers senescence with a distinct secretory phenotype that lacks IL-1-dependent inflammation.

Chromatin changes in senescent cells.

Most senescent cells display profound changes in the epigenome and chromatin organization. These changes have been linked to both the cell-autonomous and paracrine aspects (that is, the effect on surrounding cells) of senescence-associated proliferation arrest. Senescence-associated heterochromatin foci (SAHF) are spatially organized heterochromatic domains that can be detected as dense 4′,6-diamidino-2-phenylindole (DAPI)-positive nuclear structures that are enriched in repressive chromatin marks and proteins, including trimethylated histone H3 Lys9 (H3K9me3), heterochromatin protein 1 (HP1), high mobility group protein A (HMGA) factors, histone variant macroH2A and histone co-chaperones HIRA and ASF1A[49–51]. However, SAHF are not universal markers of senescence; they are most robustly observed upon oncogene activation and form in a DNA replication- and ATR-dependent manner[52]. SAHF were originally proposed to repress genes promoting cell cycle progression[49,53,54]. Rather, SAHF enforce a DDR-resistant heterochromatin structure that restrains DDR signalling[52]. Indeed, treatment with histone deacetylase (HDAC) inhibitors, which induce chromatin relaxation, boosts DDR signalling with consequent cell death by apoptosis. This treatment is probably the first reported example of a successful senolytic approach[52], which was later supported by the reported senolytic activity of the HDAC inhibitor panobinostat[55]. HDAC inhibitors can also induce cellular senescence in normal human fibroblasts, which may be related to their impact on the DDR[56].

Another chromatin feature of senescent cells is the unfolding of constitutive heterochromatin domains characterized mainly by distension of pericentromeric satellite sequences, which was observed in different species and following different modes of senescence induction[57]. These changes in chromatin structure were not linked to the selective removal of repressive histone marks, but were associated with changes in nuclear structural proteins, including breakdown of the nuclear lamina[57]. Loss of nuclear lamina can lead to the release of cytosolic chromatin fragments (CCFs) in the cytoplasm of senescent cells[58–60]. Although yet unprobed, in the context of oncogene-induced DNA replication, hard-to-replicate genomic regions such as fragile sites[61], telomeric sequences[44] and repetitive DNA[62] probably contribute to CCFs. It remains unclear whether CCFs are formed only in deeply senescent cells. Importantly, CCFs dictate senescence-associated paracrine functions through the activation of the cyclic GMP–AMP synthase (cGAS) and the adaptor stimulator of interferon genes (STING) pathway (discussed later). Low doses of HDAC inhibitors have been reported to reduce CCFs and suppress the senescence-associated secretory phenotype (SASP)[63].

Recent technological advances in genome-wide mapping of chromatin modifications led to the generation of a molecular blueprint of senescence establishment and maintenance. During replicative senescence, late-replicating, gene-poor regions display widespread DNA hypomethylation, whereas focal hypermethylation is seen at tumour suppressor genes. These observations led to the hypothesis that senescent cells may be epigenetically primed for malignant transformation[64]. But this hypothesis was recently challenged by the observation that cells with OIS display only limited changes in methylation patterns compared with cells that have bypassed OIS, indicating that tumour-associated methy-lome changes may arise stochastically and independently of the senescence state[65]. In contrast to what would be

observed in the case of DNA methylation changes, oncogene-induced senescent cells and late replicatively senescent fibroblasts exhibit a marked increase in chromatin accessibility at the nucleosomal level, with most of the open chromatin regions mapping to regulatory elements and repeats[66,67]. Chromatin loosening at genomic repeats results in increased expression levels of transposable elements, which are normally epigenetically silenced and dormant in unstressed cells[67]. Despite the well-accepted role of transposable elements in triggering genomic instability via transposition, the reactivation of transposable elements also contributes to mediate the non-cell-autonomous functions of senescent cells as detailed later[62]. Genome-wide analysis of H3 Lys4 trimethylation, H3 Lys27 trimethylation and H3 Lys27 acetylation in senescent cells has also revealed the dynamic acquisition and depletion of large-scale chromatin domains that have been proposed to regulate the expression of key senescence downstream effectors[68–72].

SASP composition and regulation

One potential mechanism through which senescent cells exert their pleiotropic biological functions is the transcriptional activation of a SASP programme characterized by cytokines, chemokines, growth factors and extracellular matrix (ECM) proteases, which may self-reinforce senescence or affect the local tissue microenvironment of senescent cells and possibly the entire organism (FIG. 2). SASP activation is a dynamic process that accompanies senescence establishment. SASP was originally defined as a robust secretory programme comprising dozens if not hundreds of bioactive factors[73–76].

SASP composition varies depending on the cell type and the nature of the initial stimulus, with the oncogenic trigger greatly amplifying protein secretion compared with replicative or irradiation-induced senescence[75]. Despite some qualitative and quantitative differences among the SASP in different tissues and senescence models, a core SASP programme comprising mainly proinflammatory interleukin-6 (IL-6), CXC chemokine ligand 8 (CXCL8, hereafter named IL-8) and monocyte chemoattractant protein 1 (MCP1; also known as CCL2) was reported in all types of in vitro-generated senescent cells[75]. SASP not only includes proinflammatory molecules but also enzymes involved in ECM remodelling, such as matrix metalloproteinases (MMPs)[77], serine/cysteine proteinase inhibitors (SERPINs)[78] and tissue inhibitors of metalloproteinases (TIMPs)[79]. More recently, a comprehensive unbiased quantitative proteomic characterization of SASP led to the identification of additional and diverse SASP effectors, released as soluble molecules or in exosomes[79–81] with a set of components previously reported to be enriched in human plasma during ageing and age-associated diseases[82]. Exosomes were recently identified as key mediators of the paracrine senescence effects of SASP as well as of its protumorigenic properties[83,84].

Exosomes

Extracellular vesicles produced by the endosomal compartment involved in intercellular communication.

Interplay between the DDR and SASP.

p16 induction can arrest the proliferation of normal cells and drive cellular senescence but is not sufficient to induce a complete SASP[85]. Of note, persistent DDR signalling is often required to initiate inflammatory cytokine secretion[86]. Consistent with a role for upstream DDR elements in promoting both cell-autonomous and paracrine functions of senescent cells, ATM, NBS1 and CHK2 prime SASP genes for activation, as depletion of these DDR upstream regulators dampens cytokine production in response to genotoxic stress[86]. Inhibition of p53 has the opposite effect, as it further enhances SASP following a senescence-inducing insult[86], which may contribute to the generation of an inflammatory microenvironment that favours senescence escape and malignant transformation.

Recently, ATM was found to regulate expression of SASP genes indirectly by mediating the removal of the histone variant macroH2A1.1 from SASP genes in response to DNA damage and oncogenic stress[87]. However, because DDR activation is a quick response and SASP establishment is slow, additional pathways must control SASP. Indeed, the activation of the stress-inducible MAPK p38 was proven both necessary and sufficient to trigger growth arrest and SASP even in the absence of DNA damage[88]. Like ATM, p38 induces the expression of SASP transcripts by increasing the activity of nuclear factor-κB (NF-κB), suggesting that although DDR and p38 activation are independent, they can converge on SASP activation.

Ageing is one of the factors that is consistently associated with an increase in DDR in proliferating and non-proliferating cells, thus contributing significantly to the accumulation of senescent cells with age[35,89]. Incomplete DNA repair may further contribute to the accumulation of DNA lesions and DDR activation as well as to the widespread chromatin changes seen in different cell types and at the organismal level during ageing[90,91]. Moreover, DDR is a driver of metabolic reprogramming, which can enhance SASP[92]. Therefore, regulation of SASP by the DDR via multiple pathways can be one of the routes by which the DDR drives age-related inflammation.

Transcriptional and epigenetic control of SASP.

Several transcription factors and chromatin regulators have been implicated in the regulation of SASP, which is controlled mainly at the transcriptional level. NF-κB and the transcription factor CCAAT/enhancer-binding protein-β (C/EBPβ) bind promoters of SASP genes and regulate their activation[74,93,94]. Moreover, the transcription factor GATA4 activates a plethora of genes involved in immune response and inflammation, including those encoding IL-6, IL-8, CXCL1 (also known as GROα), granulocyte–macrophage colony-stimulating factor, and ECM proteases and their inhibitors. Because GATA4 levels increase following DDR activation and in senescent cells, it was proposed that GATA4 acts as the molecular link between DDR signalling and the subsequent NF-κB activation for full SASP establishment[95,96], although GATA4 regulation of the NF-κB pathway happens indirectly through increased expression and secretion of IL-1α, an upstream regulator of NF-κB[97].

The JAK2–STAT3 pathway activates a subset of SASP factors with immunosuppressive properties in a model of senescence induced by PTEN loss. In this context, JAK/STAT

inhibitors were effective in reprogramming the SASP to enhance chemotherapy and T cell-mediated clearance of cancer senescent cells[98]. Furthermore, JAK inhibitors alleviated frailty in aged mice[99]. SASP gene expression is temporally dynamic[100], and temporal changes in NOTCH1 activity during senescence have been reported to modulate the composition of SASP. NOTCH1 levels increase early on, and NOTCH1 activates transforming growth factor-β and its effectors, while keeping under control the proinflammatory arm of the SASP cascade by repressing its positive regulator C/EBPβ. At later stages, in deeply senescent cells, NOTCH1 levels are lower, and the SASP proinflammatory cytokines IL-1, IL-6 and IL-8 are induced[94]. Whether this function of NOTCH1 is linked to its recently reported role in direct ATM inhibition[101,102] remains unknown.

The epigenetic reader bromodomain-containing protein 4 (BRD4), an acetylated histone-binding protein involved in oncogenesis, is recruited to superenhancers adjacent to SASP genes in OIS[103]. BRD4 contributes to the proper execution of cellular senescence and acts, unexpectedly, as a tumour suppressor. Indeed, chemical and genetic inactivation of BRD4 blunts the SASP, limits OIS immune-mediated cell clearance and may thus fail to remove damaged cells that are prone to senescence escape[71]. However, a recent chemical screen identified a small molecule that degrades BRD4 and has senolytic activity[104]. Moreover, BRD4 was shown to positively regulate telomere elongation in murine and human cultures[105]. Thus, BRD4 inhibitors may limit SASP activation but also cause more cellular senescence by promoting telomeric shortening. BRD4 binds to acetylated histone H3 Lys27, thus competing with Polycomb repressor complex 2 (PRC2), which methylates the same histone residue (producing trimethylated H3Lys27). Consistent with BRD4 and EZH2 (the catalytic core subunit of PRC2) competing for the same residue and having antagonistic functions, overexpression of EZH2 prevents entry into OIS through a variety of mechanisms, including DDR regulation and inhibition of the SASP gene expression programme[106]. The transcription-associated histone methyltransferase and oncoprotein MLL1 was also reported to be essential for SASP activation, but mainly through oncogene-induced hyper-replication and DDR engagement rather than direct transcriptional control of SASP genes[107].

HMGB proteins also regulate the SASP. HMGB2 directly binds and specifically regulates SASP gene expression in oncogene-induced senescent cells, and its depletion diminishes SASP without affecting the senescence growth arrest[108]. HMGB1 functions mainly as one of the damage-associated molecular patterns, also known as alarmins[109], that are released extracellularly to induce SASP-mediated paracrine senescence and alert the immune system[110]. Furthermore, increased nuclear pore density during OIS, which is key to establish SAHF, regulates SASP expression by mediating heterochromatin reorganization[111].

HMGB proteins

Non-histone molecules that bind DNA and affect chromatin compaction.

SASP and innate immunity.

The DNA sensor cGAS and the adaptor protein STING have been reported to be major regulators of the SASP programme across species, presumably by activating NF-κB and the interferon response factor IRF3 (REFS[60,112,113]). cGAS–STING activation occurs mainly through recognition of 'self' double-stranded DNA or chromatin fragments in the cytosol of senescent cells[114]. cGAS–STING genetic depletion reduces the proinflammatory SASP and mitigates senescence immunosurveillance in senescence models in vivo. Adding to the complexity of SASP regulation, it was reported that aberrant activation of the cGAS–STING pathway could be linked to the downregulation of DNases (for example, DNase 2 and TREX1) that are normally devoted to cytoplasmic DNA degradation, resulting in cytosolic accumulation of nuclear DNA during senescence establishment[115]. While the mechanisms causing the release of cytosolic chromatin in senescent cells are still under intense scientific investigation, these discoveries suggest that STING inhibitors could potentially be used for the treatment of age-related chronic inflammation[116].

cGAS–STING is not the only innate immunity pathway involved in SASP initiation and execution. The inflammasome, a multiprotein complex comprising caspase 1 and key regulators of the defence mechanisms against pathogens[117], and Toll-like receptors, which regulate the inflammasome, have been reported to promote maturation and secretion of SASP factors during OIS[73,118].

Senescent cells, especially when persisting for extended periods in culture or in vivo, display a profound activation of type I interferon response and downstream targets. This distinctively strong induction of type I interferon was found to be partly caused by reactivation of transposable elements and consequent to cGAS–STING activation[62]. Treatment with nucleoside reverse-transcriptase inhibitors, which inhibit retrotransposition of transposable elements, limits the senescence-associated detrimental effects of SASP and ameliorates chronic inflammation in aged animals[62,119].

Cellular senescence of stem cells

Stem and progenitor cells are key to maintain tissue homeostasis and organization during physiological turnover and following tissue and organ injury. The functional capacity of stem cells, but not necessarily their number, declines with age[120]. DNA damage and markers of DDR activation have been observed in various stem cell types in different tissues and species, during normal and pathological ageing[121,122], suggesting that stem cells are not immune to DNA damage accumulation and DDR activation[123].

Although the activation of DDR pathways is expected to preserve genome stability and stemness, there is evidence that, following DNA damage, events regulated by the DDR lead to permanent cell cycle arrest with features of cellular senescence and cell differentiation. Indeed, exposure of mice to ionizing radiation leads to fur greying, which is due to damage-induced differentiation of hair bulb melanocyte stem cells after one round of cell division[124]. Although some markers of cellular senescence, such as p16 and SA-β-gal activity, were not detected, differentiation of melanocyte stem cells was associated with persistent DDR activation and was enhanced in *Atm*-knockout mice[124]. Similarly, self-renewal of

haematopoietic stem cells (HSCs) is inhibited by telomere dysfunction or exogenous DNA damage followed by the induction of lymphoid differentiation[125]. Single-cell transcriptomic analysis of human HSCs revealed a dose-dependent activation of senescence-like programmes in response to DSBs, which were characterized by activation of p53 and the induction of proinflammatory programmes that resulted in reduced clonogenic potential, engraftment capacity and lineage output on transplantation[126]. Similarly, accumulation of DNA replication stress in HSCs from aged mice was linked to limited yet detectable HSC senescence, unbalanced haematopoietic differentiation and myeloid skewing[127]. However, especially in humans, it remains to be clarified whether myeloid-restricted haematopoiesis in elderly people results from increased myeloid-primed HSC differentiation or impaired lymphoid differentiation.

Myeloid skewing

An age-related proportional increase in myeloid cells at the expense of other lineages as observed in the bone marrow and blood.

DNA damage, for example induced by ionizing radiation, has been shown to promote differentiation and induce cellular senescence in mouse neural stem cells[128]. DNA damage led to a loss of expression of stemness genes and a general induction of a transcriptional profile indicative of differentiation into astrocytes. This cell differentiation programme was controlled by ATM and by soluble factors, in particular through BMP2 signalling[128]. Lineage tracing experiments in vivo in irradiated mice confirmed the induction of expression of differentiation markers in the subventricular zone of the brain, normally populated by neural stem cells[128]. Thus, DNA damage-induced cellular senescence can coincide with cell differentiation[128,129].

Notably, in mouse embryonic stem cells, a p53-induced programme is associated with transcriptional activation of a differentiation programme and the repression of pluripotent stem cell genes[130]. Consistent with this observation, it was reported that senescence occurs in a programmed manner and contributes to mammalian embryonic development and tissue patterning, although mainly via induction of p21, p15 and mediators of the SASP rather than through overt DDR signalling[131,132].

Altogether, these independent observations in melanocyte stem cells, HSCs, neural stem cells, embryonic stem cells and whole embryos suggest that persistent genotoxic stress in stem cells, and possibly more broadly in the context of progenitors and less differentiated cells, can lead to cellular senescence with features of cell differentiation. Although cellular senescence is not commonly considered a form of cell differentiation, as it is often the outcome of macromolecular damage, whereas cell differentiation is not, the commonalities are striking: they both involve cell cycle exit with a distinct transcriptional programme often controlled by soluble factors. It is possible that the common use, from the very beginning, of fairly differentiated cells (most typically fibroblasts) for senescence studies may have prevented the discovery of cellular senescence as a stress-induced differentiation programme, and perhaps with a different research history, cellular senescence could be known as a form of DNA damage-induced cell differentiation.

Can postmitotic cells become ‘senescent’?

The observation that during ageing terminally differentiated cells can accumulate persistent DNA damage and DDR markers[35,89] prompts the question of whether persistent DNA damage signalling leads to the expression of cell cycle inhibitors and ultimately the establishment of cellular senescence, thus shifting cells from a non-dividing physiological state to a non-dividing pathological state. Although not extensively investigated, and mainly studied in neurons, evidence is emerging that cellular senescence can be associated with terminally differentiated cells[133,134]. Markers of DDR signalling, heterochromatin induction and activation of SASP, including the secretion of IL-6 and accumulation of SA-β-gal, were all detected in different types of neurons in ageing mice, and a short period of dietary restriction prevented their accumulation[134]. Such phenotypes were exacerbated in telomerase-inactivated mice, while p21 loss reduced many of these markers. Conversely, obesity in mice was associated with the expression of senescence markers in neurons of specific parts of the brain[135]. In a mouse model of Alzheimer disease, transcriptomic analyses of tau-containing neurons indicated an expression profile consistent with cellular senescence[136]. Neurons in the retina, known as retinal ganglion cells, were found to accumulate markers of DDR activation, SA-β-gal and both p16 and p21, in retinopathies[137].

tau

A protein found in neurons that is important for maintaining microtubule structure in axons. Mutants and hyperphosphorylated forms are found in a variety of neurodegenerative diseases, including Alzheimer disease.

Mature postmitotic adipocytes show strong SA-β-gal staining and SASP induction in a p53-dependent manner in mice subjected to excessive caloric intake, although other markers, such as accumulation of DNA damage, were not reported[138]. Osteocytes are postmitotic differentiated cells controlling bone homeostasis. In old mice, osteocytes show markers of telomeric dysfunction and accumulate p16. SASP activation in osteocytes was shown to promote osteoclast activity, thus impairing bone strength. In this context, senolytic drugs reduced bone loss[139].

Furthermore, persistent telomeric DNA damage in cardiomyocytes drives a senescence phenotype characterized by induction of p16 and p21 and a non-canonical SASP programme that contributes to cardiac hypertrophy and fibrosis[140]. Indeed, genetic and pharmacological clearance of p16-expressing senescent cells ameliorated heart functions in aged mice[140]. The most informative approach to determine the contribution of senescent cells to a condition is their genetic or pharmacological removal. However, as there are currently no tools to selectively target this subset of differentiated senescent cells, their role in ageing-related processes remains unclear.

Fibrosis

Pathological accumulation of extracellular matrix in diseases tissue that limits normal tissue function and leads to long-term tissue scaring.

Beneficial effects of cellular senescence

Senescence can be regarded as a stress response that evolved to perform essential and beneficial functions[141] (FIG. 3). The beneficial roles of senescent cells are evident in embryonic development. A distinct form of cellular senescence occurs in the mammalian developing embryo and in the placenta to control growth and patterning[131,132,142,143]. Similarly, in amphibians, cellular senescence occurs at specific steps during development to shape body growth[144]. Therefore, the cellular senescence early in life is important for normal development and morphogenesis, and later in life it becomes important for tissue repair and inhibition of cancer outgrowth. While tumour suppression activity is mediated mainly by cell-autonomous cell cycle arrest, most other senescence functions involve the SASP. Over time, although SASP favours proper tissue development, tissue repair and recruitment of immune cells, its persistence may generate chronic inflammation and contribute to ageing-related diseases and, paradoxically, cancer.

Cellular senescence contributes to the maintenance of the structure and the function of tissues following injury. For example, in liver fibrosis, a condition associated with scarring of the liver and decrease in its function, senescence limits the proliferation and expansion of ECM-producing activated hepatic stellate cells[145,146]. This response limits the progression of the pathology following liver damage. SASP from these cells attracts natural killer (NK) cells that eliminate senescent cells from the liver to restore liver homeostasis[145]. Cellular communication network factor 1 (CCN1; also known as CYR61), an ECM protein that mediates the induction of cellular senescence in the liver, promotes senescence in fibroblasts during cutaneous wound healing, thus limiting skin fibrosis[147]. During wound healing, the SASP component PDGF-AA accelerates wound closure[12]. Moreover, induction of a senescence programme limits fibrosis progression in the pancreas[148].

Cellular senescence is involved in tissue repair in other systems. In zebrafish, senescence impairment prevents fin regeneration following amputation[149], and in salamanders, senescent cells have been associated with limb regeneration[150]. Altogether, these observations suggest that cellular senescence is a programme that has evolved to limit tissue damage response in the organism and facilitate tissue repair and remodelling to promptly restore the tissue to a functional state.

The homeostatic function of senescent cells is dependent on their elimination by the immune system, once their beneficial functions have been performed[145,151]. Specialized SASP chemokines are able to attract distinct subsets of immune cells, including NK cells, neutrophils, dendritic cells, monocytes/macrophages, B cells and T cells[75,151,152]. Among these cell types, NK cells, T cells and macrophages can physically interact with senescent cells in pathological and physiological conditions[131,132,145,150,153–156]. This response is mediated by SASP components and by the direct interaction between immune cells and senescent cells[153,156–158]. The surveillance and clearance of senescent cells by the immune system is necessary in order to limit tumorigenesis in premalignant lesions and following cancer therapy[155,156,159]. Senescence immunosurveillance is also essential to limit pathological fibrotic conditions and ageing[20,145,151,157]. By contrast, during cancer development associated with accumulation of senescent cells, SASP can recruit immature

myeloid cells to promote tumorigenesis in a paracrine manner[160,161]. In addition, through SASP, senescent cells contribute to cancer development[162,163] and metastasis[164], treatment failure and increased risk of recurrence[165]. Thus, SASP is a component of the senescence phenotype that seems to have evolved to signal the presence of senescent cells to the immune system and promote their elimination, but when senescent cells persist, their SASP becomes detrimental.

Detrimental impacts of senescence

Senescent cells can contribute to organismal ageing through multiple mechanisms (FIG. 3). With advancing age, individuals tend to develop a proinflammatory condition, characterized by high circulating levels of inflammatory molecules, known as inflammageing[166]. Inflammageing is a risk factor for various chronic age-associated diseases, including cardiovascular diseases, some cancer types and neurodegeneration, and can be associated with premature death. Furthermore, the presence of inflammatory molecules in the blood of elderly individuals is associated with weight loss, muscle loss and weakness, chronic inflammation and depression — manifestations of a condition recently recognized as frailty. A molecular link between cellular senescence, inflammageing and frailty was unveiled during the identification of common genomic variations that contribute to chronic phenotypes associated with ageing. Genome-wide association studies have recently revealed that the INK4/ARF gene locus, encoding $p15^{INK4B}$, p16 and ARF, key effectors of the senescence growth arrest, is a genomic hotspot for susceptibility to several ageing-associated diseases, including cancer, diabetes and cardiovascular disease[167,168], and physical dysfunction in people with advancing age[169,170]. Moreover, shortened telomere length, observed during ageing, correlates with metabolic and cardiac dysfunctions[171,172]. The SASP may contribute to dysfunction of multiple aged organs. Indeed, increased blood levels of IL-6, IL-1 receptor antagonist (IL-1RA) and tumour necrosis factor (TNF) receptor, all key SASP effectors, can be predictors of chronic disease in old individuals[173]. That cellular senescence is causative for frailty and age-associated diseases was demonstrated by transplantation of relatively few senescent cells and observation of tissue dysfunction and shortened lifespan in mice[174].

Recently, atherosclerotic plaques from LDL receptor-null mice were found to accumulate high amounts of SA-β-Gal and p16-positive endothelial cells, vascular smooth muscle cells and macrophages. Removal of p16-positive cells in both p16-3MR and INK-ATTAC transgenic mice by both genetic methods and senolytics reduced plaque formation and progression, while dampening the SASP[175]. Consistent with these data in mice, human atherosclerotic plaques are highly enriched in p16-positive cells, although p16 seems to be expressed mainly by inflammatory macrophages[176], and it cannot be ruled out that the positive effects of senolysis in atherosclerosis-prone mice[175] are the consequence of elimination of inflammatory macrophages. Macrophages with senescence-like features have also been identified in lesions from patients with cell histiocytoses, haematological neoplasms associated with oncogene activation characterized by multiorgan dissemination of highly inflamed, p16-positive myeloid cells[177,178].

LDL receptor

Mediates entry of LDL into cells. Mutations in the gene encoding this receptor predispose to the development of atherosclerosis.

In addition to the contribution of SASP to inflammation and chronic diseases at the organismal level, senescent cells might affect tissue regeneration by limiting the proliferative potential of stem and progenitor cells, as discussed earlier. Muscle progenitor cells that accumulate damage, both DNA and molecular, and upregulate p16 enter senescence on stimulation and are unable to contribute to muscle regeneration after injury[179]. From the findings taken together, senescence limits proliferation of stem and progenitor cells in a cell-autonomous manner. In addition, it was recently reported that the clonogenic properties of HSCs are impaired when they are exposed to SASP factors derived from senescent stromal cells[180], suggesting that senescence may also affect regeneration in a paracrine fashion.

While the detrimental effects of senescence on adult somatic stem cell functions are well established, cellular senescence is more ambiguous in the context of somatic cell reprograming to an embryonic-like state. In vitro studies demonstrated that cellular senescence is a potent cell-autonomous barrier for transcription factor-mediated reprograming using OCT4, SOX2, KLF4 and MYC (commonly referred to as OSKM), in a manner similar to its role in tumour suppression[181–183]. However, expression of these factors in vivo induces senescence and SASP production, which promotes paracrine senescence as well as reprogramming in non-senescent cells in a cell-non-autonomous manner[184]. In these same models, exogenous tissue damage that drives cellular senescence facilitates reprogramming. Senescence induction is necessary for efficient cell reprograming, as SASP factor production promotes reprogramming into induced pluripotent stem cells in a paracrine manner[184]. IL-6 produced in the SASP appears to be crucial for the generation of induced pluripotent stem cells in this context.

The non-cell-autonomous effects of cellular senescence can differ depending on the context. In vitro, the SASP factors IL-8, GROα, IL-6, and IGFBP7 reinforce the senescent phenotype in an autocrine manner[74,93,185]. In addition, specific SASP components, either soluble or in extracellular vesicles[186], can induce senescence in a paracrine manner — which includes DDR activation[73,83]. These effects could potentially contribute to the spread of senescence in tissues and lead to tissue and organismal dysfunction. In vivo, SASP consequences are more complicated. For example, the SASP component interferon-γ induces cellular senescence and tissue ageing in mice with shortened telomeres, and abrogation of interferon-γ signalling rescues ageing-related phenotypes and extends lifespan[187]. Similarly, transforming growth factor-β improves liver regeneration on injury by blocking paracrine senescence in neighbouring uninjured hepatocytes[188]. By contrast, short-term exposure to the SASP promoted expression of stem cell markers and increased the regenerative capacity of mouse keratinocytes[189] and of skeletal muscle[190] in vivo. However, prolonged exposure to the SASP resulted in paracrine-induced senescence, indicating that, at least in vivo, the effects are dependent on the composition and length of exposure to the SASP. From an evolutionary perspective, SASP may in the short-term facilitate wound healing and tissue

damage repair by enhancing stem cell function, whereas the long-term presence of senescent cells may not be selected for and contributes to SASP deleterious effects.

Senescence as a driver of ageing

The rapidly ageing BubR1 hypomorphic mouse model has been valuable to demonstrate that cellular senescent cells cause ageing and disease[191,192]. BubR1 is part of the mitotic checkpoint machinery that ensures proper segregation of duplicated chromosomes into two identical daughter cells during mitosis. Mice expressing ~10% of normal BubR1 levels develop a variety of progeroid features, including shortened lifespan, cataracts, lordokyphosis, lipodystrophy and infertility very early in life[191]. Adipose tissue, skeletal muscle and the eyes of BubR1 hypomorphic mice express high levels of p16 and other senescence-associated features[192]. In an attempt to prevent the accumulation of these cells, BubR1-mutant mice were bred to *Cdkn2a*^{p16}-knockout mice. In the absence of p16, the age-related deterioration of adipose tissue, skeletal muscle and eye was attenuated[193]. Importantly, genetically preventing the accumulation of p19^{ARF}, a tumour suppressor that modulates the stability of p53 by influencing MDM2-mediated destruction, did not result in similar prevention[193], indicating that p16 was critical for these disorders.

Cataracts

Clouding of the lens in the eye leading to inability to have clear vision. surgical intervention to replace diseased lenses is a common medical procedure in aged humans.

Lordokyphosis

Abnormal rearward curvature of the spine, observed both in laboratory mice and in humans.

Lipodystrophy

Abnormal distribution of adipose tissue in the body, can refer to both excessive or insufficient deposition.

On the basis of these observations, two different transgenic mouse models, INK-ATTAC[11] and p16-3MR[12], have been generated to critically test whether removal of senescent cells impacts ageing and diseases associated with senescent cell accumulation. Importantly, treatment of BubR1 hypomorphic mice harbouring the INK-ATTAC transgene to remove p16-expressing cells beginning at weaning age attenuated the accumulation of senescent cells and premature ageing in skeletal muscle, eye and adipose tissue[11]. A second study using the INK-ATTAC system in naturally aged mice corroborated these findings[194] and increased median lifespan of both male and female mice in different genetic backgrounds and increased healthspan, as indicated by reduced kidney scarring, cardiomyocyte hypertrophy, cardiac stress intolerance, cataractogenesis and lipodystrophy[194].

The p16-3MR mouse model expresses a trimodality reporter fusion protein consisting of synthetic *Renilla* luciferase, monomeric red fluorescent protein and a truncated herpes simplex virus thymidine kinase under the control of an artificial promoter for p16 (REF.[12]). In this model, cells that express p16 become sensitive to elimination by ganciclovir, a nucleoside analogue that is converted into a toxic DNA chain terminator by herpes simplex virus thymidine kinase and causes cell death[195]. These two mouse models have greatly accelerated our understanding of whether senescent cells contribute to ageing and age-related diseases, at least in model organisms, for numerous diseases, including Parkinson disease[196], Alzheimer disease[197,198], atherosclerosis[175], idiopathic pulmonary fibrosis[199], chronic obstructive pulmonary disease[200] and osteoarthritis[201]. It remains unclear whether the elimination of senescent cells themselves or their SASP is the key element underlying these improvements.

Exploiting senescence for therapeutics

The literature is becoming inundated with evidence that senescent cells accumulate in a variety of age-associated diseases[13]. With the observation that elimination of senescent cells is largely beneficial and seems to lack long-term negative consequences, researchers in academia and industry have aimed to identify novel agents and strategies to eliminate senescent cells or their effects in the absence of genetical engineering to be applicable for use in humans. These ‘senotherapeutic’ strategies can be broadly categorized into two categories: pharmacological agents termed ‘senolytics’, which eliminate senescent cells, and senomorphics, which prevent the detrimental cell-extrinsic effects of senescent cells and include SASP inhibitors.

Senolytics.

Various senolysis strategies have been developed recently using a combination of in vitro models of senescence and in vivo animal models (FIG. 4; TABLE 1). Senescent cells frequently upregulate negative modulators of apoptosis, including members of the BCL-2 family (including BCL-2, BCL-W and BCL-X_L), which confers resistance to apoptosis-inducing signals[14,15]. The senolytic agents ABT-737 and ABT-263 (also known as navitoclax) inhibit the activity of the BCL-2 family members, thereby permitting senescent cells to initiate apoptosis. Additionally, A-1331852 and A-1155463, which are thought to inhibit BCL-X_L, have also been shown to exhibit senolytic activity[202]. Recently, the cardiac glycoside ouabain demonstrated senolytic activity, at least in part, through the induction of NOXA, a proapoptotic BCL-2 family protein[203,204]. Promoting proteasomal degradation of BCL-2 through EF24 treatment also results in the selective killing of senescent cells[205]. Administration of proxofim (a peptide) promotes senolysis by interfering with the binding of p53 to forkhead box protein O4 (FOXO4)[206]. In senescent cells, FOXO4 binds to p53 to localize it to the nucleus. If this interaction is disrupted by the administration of an inverse peptide, p53 is excluded from the nucleus, initiating cytochrome *c* release from the mitochondria and apoptosis[206]. The use of various natural flavonoids, including quercetin and fisetin, either alone or in combination with dasatinib, a pan-tyrosine kinase inhibitor, can stimulate senolysis in a variety of contexts in vitro and in vivo[199,207–210]. In agreement with studies performed on INK-ATTAC naturally aged mice[194], treatment of advanced-age mice

with a combination of dasatinib and quercetin improved physical function and increased lifespan[174]. Importantly, the administration of dasatinib and quercetin has shown effectiveness in reducing the expression of p16 and SA-β-gal in a phase I clinical trial among patients with diabetic kidney disease[211] and idiopathic pulmonary disease[212]. Other senolytics, including HSP90 inhibitors[213,214] and piperlongumine[215,216], have also been demonstrated to be selective towards senescent cells. More recently, clinically approved antibiotics have been reported to have senolytic activity towards DNA-damage induced senescent cells through metabolic changes[217]. Collectively, these strategies target a broad spectrum of cellular pathways, indicating that senescent cells can be removed via multiple avenues.

HSP90

A molecular chaperone that promotes proper protein folding and degradation, which also contributes to heat stress resilience.

A novel strategy to induce senolysis exploits the observed increased level of SA-β-gal activity. Nanoparticles containing either fluorophores or cytotoxic agents coated with galacto-oligosaccharides were found to preferentially deliver cytotoxic cargo to senescent cells because of the higher level of SA-β-gal activity in these cells[218]. Additionally, recent studies have further shown the potential of delivering cytotoxic factors to lysosomes of senescent cells by galactose-modifying prodrugs or cytotoxic agents[219,220].

Prodrugs

Compounds that are metabolized into an active drug to modify drug bioavailability and activity.

In support of senolytic therapies being potentially beneficial is the notion that organisms have an intrinsic senolytic system: immunosurveillance against senescent cells. Senescent cells are indeed subjected to immunosurveillance by multiple components of innate and adaptive immunity, including NK cells, T cells and macrophages[145,151,153–156]. Therefore, it is conceivable to harness the mechanisms of immunosurveillance of senescent cells, and all mechanisms that the immune system uses to target other threats, to eliminate senescent cells. Several such approaches were recently implemented (reviewed in[221]). It is possible that senescent cells accumulate in aged and diseased tissues owing to a decline in immunosurveillance. Therefore, restoring or boosting the ability of the immune system to specifically eliminate senescent cells could result in their successful clearance from tissues. Such an approach is based on our understanding of the mechanisms of immunosurveillance of senescent cells, in particular the interaction between NK and senescent cells[151,157,158]. NK cells use perforin-mediated granule exocytosis and not death receptor ligands, which induce cell death by binding to these receptors, to target senescent cells[151]. This mechanism is favoured because decoy receptor 2 (DCR2) is strongly expressed in senescent cells. DCR2 prevents targeting via the death receptors (DR4 and DR5) by the variety of cytotoxic cells that express their ligand TRAIL[151]. Therefore, blocking such an inhibitory mechanism can

lead to removal of the inhibitory effect and increased targeting of senescent cells by endogenous naturally occurring mechanisms.

Perforin

A pore forming protein expressed in cytotoxic T cells and natural killer cells. When these cells execute cytotoxicity, they secrete granules containing perforin, which binds to the target cell's membrane and forms pores on the target cell in order to allow cytotoxicity.

An alternative approach to enhance the immune clearance of senescent cells is by enhancing the activity and increasing the accumulation of immune cells that are responsible for senescent cell surveillance. Stimulation of innate immune response with poly(I:C), a simulator of viral infection, improves senescent cell clearance[145]. Although treatment with similar agents can hardly be considered in humans due to possible side effects, the subtler stimulation of the immune system with specific cytokines that boost NK cells could be a plausible approach. The cytokines IL-21 and IL-15 have been suggested to substantially boost NK cell-mediated immunity against cancer cells[222–225]. However, the effect of these cytokines on immunosurveillance of senescent cells has not been elucidated and their efficacy as senolytics in disease models needs to be tested.

While boosting natural immune mechanisms of senescent cell surveillance may have therapeutic potential in years to come, it is worth considering the rapidly growing variety of tools that are becoming available in the immuno-oncology field. Directed cellular approaches such as use of chimeric antigen receptor T (CAR T) cells and NK cells, as well as blocking of immunoinhibitory interactions by blocking interactions with PD1, cytotoxic T lymphocyte-associated protein 4 (CTLA4) and other inhibitory molecules might provide powerful strategies for increasing immunosurveillance. Such approaches depend on the recognition of specific markers on the cell surface of senescent cells. Exciting recent work has demonstrated that senolytic CAR T cell therapy can attenuate senescence-associated diseases[226]. Several studies used unbiased approaches to identify such markers[227–229]. However, the overlap between the extracellular markers identified by the aforementioned strategies is low, when each experiment identified distinct markers, suggesting that such markers might be specific to the cell of origin and/or to the mechanism of senescence induction. One possible way to address this problem is to use the surface molecules on senescent cells that signal to NK cells for their elimination, namely the NKG2D receptor ligands[157]. However, the large repertoire of such ligands, including MICA, MICB and ULBP1–ULBP6, and the different levels of their expression on senescent cells of different origins[157] might provide a challenge in using them for enhancing immune clearance of senescent cells. When different markers are present on cells of different origins, it is possible to envision multiple approaches specific to distinct pathological conditions.

Chimeric antigen receptor T (CAR T) cells

T cells that have been genetically engineered to express T cell receptor developed to bind a defined target in order to eliminate the cells that have the target on their membrane.

PD1

A protein expressed on the cell surface that inhibits the ability of the immune system to target the cells that express the protein. Inhibition of interaction of PD1 with its ligand is a potent immunotherapy approach.

Senomorphics.

An alternative to the complete elimination of senescent cells through senolysis is the use of senomorphic agents. The principle of senomorphics is to disrupt key attributes of senescence, primarily SASP production and secretion, while keeping the cells alive, or to modify their ability to maintain a stable growth arrest (FIG. 5). This approach could interfere with the proinflammatory nature of senescent cells and potentially delay key aspects of ageing and ageing-associated disease.

Novel mechanisms to regulate the SASP, in addition to the transcriptional modulation of SASP factor expression, have been uncovered by using hypothesis-driven strategies and elegant genetic and drug screenings. These include, for example, the mammalian target of rapamycin (mTOR) pathway, which coordinates cell growth and metabolism in response to nutrients, and also promotes SASP production through increased translation of subsets of mRNAs, including the membrane-bound and upstream regulator of NF-κB, IL-1α[230], and the serine/threonine kinase MK2, which indirectly stabilizes many cytokine-encoding transcripts[231]. These mechanistic insights provided a molecular foundation for the use of the mTOR inhibitor rapamycin in pathological settings associated with senescence in vivo. Treatment with rapamycin (and its analogue RAD001)[232] attenuated the protumorigenic SASP[230], prevented senescence[233], impaired SASP-mediated immune recognition of oncogene-expressing cells and ameliorated liver dysfunction in naturally aged mice[231]. Of note, it cannot be excluded that rapamycin may act also via senescence-independent mechanisms. Additionally, rapamycin treatment increased lifespan and delayed certain ageing-related dysfunctions in mice[234].

Compounds that modulate NF-κB signalling, including metformin[137,235–237], apigenin[238], kaempferol[238] and BAY 11–7082 (REF.[118]), have also been shown to decrease SASP production. NAD^+/NADH metabolism was identified as a critical regulator of the magnitude of proinflammatory SASP associated with oncogene activation, and this regulation can be independent of senescence-induced growth arrest[239]. A number of neutralizing antibodies directed against key components of the SASP or their receptors, including IL-6, IL-1α, IL-1β and TNF, also have shown senomorphic properties[104,240–248] (TABLE 1). Additionally, SASP establishment and the secretion of SASP factors can be modulated by inhibiting HSP90 (REFS[214,249]).

Finally, as in many instances cellular senescence is the consequence of the activation of DDR pathways by dysfunctional telomeres, the inhibition of telomeric DDR may prevent or reduce senescence establishment and maintenance. Recently, sequence-specific inhibition of DDR activation by antisense oligonucleotides (ASOs)[250] and their use in cultured cells and in mouse models to specifically inhibit telomeric DDR[38] provided support for this approach.

The use of telomeric ASOs in a mouse model of Hutchinson–Gilford progeria syndrome (an accelerated ageing syndrome) effectively reduced DDR activation, the levels of senescence markers and SASP induction, improved tissue homeostasis and extended lifespan[251]. This or similar approaches that do not depauperate stem or progenitor cell reservoirs but rather promote cell proliferation may provide an alternative or complementary approach to senolysis.

Senolytics may have benefits compared with senomorphics. Firstly, removal of senescent cells has the advantage of their being targeted intermittently and not requiring continuous administration of SASP inhibitors, although repeated treatments are probably necessary. Furthermore, the removal of senescent cells eliminates the possibility of senescence bypassing mutations that can promote tumorigenesis in these damaged cells. Additionally, although there is a strong correlation between the SASP and ageing-associated tissue and organ dysfunction, there is no direct demonstration that the SASP drives these ageing-related defects, as it has not been possible to separate the SASP from senolysis using transgenic mouse models. However, although INK-ATTAC transgenic mouse models in which senescent cells are removed exhibit no apparent detrimental side effects, it remains to be determined whether prolonged or repeated senolysis could become eventually toxic to an organism. Furthermore, it is not known whether senolysis is detrimental or beneficial when the senolysis is induced in advanced age, when the organism has a high senescent cell burden. Pharmacological clearance of a subpopulation of p16/SA-β-gal-positive macrophages has been proposed to contribute to the beneficial effects of senolysis[252,253], but the extent to which this cell type contributes to age-related dysfunctions needs to be further investigated. Lastly, emerging evidence suggests that targeted senolysis in mice may be profoundly toxic in the liver and perivascular tissue, because of the eradication of p16-expressing endothelial cells, adipocytes and macrophages in aged organs, which all have structural functions[254].

Interplay between ‘rejuvenating’ treatments and cellular senescence.

Caloric restriction has been demonstrated to be the most effective strategy to lengthen healthspan and lifespan, and is efficacious in a range of species from yeast to primates[255]. Whether it impacts the number or activity of senescent cells has been surprisingly understudied. Nevertheless, it was reported that caloric restriction reduces p16 levels and the transcriptional expression of genes associated with cellular senescence, including SASP genes, in mice and in the colon of healthy human individuals[256,257]. Caloric restriction in mice reduced the DDR and improved telomere maintenance[258]. Caloric restriction has also been found to reduce the levels of DDR markers and SASP regulators in postmitotic neurons[134]. The link between caloric restriction and reduced DDR signalling and reduced senescence burden may be related to the observation in culture that serum boosts DDR signalling in senescent cells[259].

Inhibiting telomere shortening to prevent and reduce cellular senescence in the context of ageing and so-called telomere syndromes is being considered as a therapeutic approach[260]. In addition to potentially using telomeric ASOs to modulate DDR activation and its consequences[251], other options are being explored, such as the reactivation of an

endogenous telomerase gene using a natural compound[261], but with limited efficacy, and by sex hormones[262], which however has some significant clinical drawbacks. Viral delivery of the telomerase-encoding gene (*Tert*) has been tested with more success in several settings. Systemic delivery of *Tert* reduces several senescence markers and ageing-related conditions and extends the lifespan of wild-type mice, thus demonstrating that telomeric DDR activation plays a role in natural ageing[263]. Idiopathic pulmonary fibrosis is associated with telomere shortening and markers of cellular senescence in humans[264]. In mouse models recapitulating these features, adeno-associated virus particles delivering *Tert* have been shown to reduce DDR and the level of senescence markers and improve lung function[265]. Importantly, concerns regarding the safety of telomerase forced expression in damaged tissues, in particular by fuelling cancer progression, have been mitigated by the recent demonstration that oncogene-expressing mice do not show accelerated tumorigenesis on such treatment[266].

Over the past few decades, heterochronic parabiosis, a process by which young and old small animals are surgically connected by establishing a joined circulation, identified systemic factors that are present in young blood and can ameliorate the function of several aged organs, including liver, muscle, heart and brain[267]. In the brain, similar rejuvenating effects were observed by a simpler procedure of young human plasma transferred into old animals[268]. Exposure to a younger systemic environment was also shown to relieve age-associated tissue dysfunctions in ageing telomerase-deficient mice[269]. Recently, it was reported that blood exchange between young and old mice led to a significant reduction in cellular senescence and SASP marker expression in multiple aged tissues, while at the same time the levels of senescence markers were increased in the young animals exposed to old blood[270]. These observations indicated that systemic factors reverse some features associated with ageing, including defective stem and progenitor cell function, chronic inflammation and senescence burden, and support the hypothesis that intermittent blood exchange in humans may be used as a therapeutic modality for age-related diseases. In line with this, therapeutic plasma exchange is currently being tested in patients with acute sepsis[271] or liver damage[272].

Challenges and future directions

As our understanding of senescent cell characteristics in vitro and in vivo continues to increase, many challenges remain. For example, it is unclear how many ‘senescent phenotypes’ exist. It is possible that there is a very high degree of heterogeneity in the senescent state, at the single-cell level, as well as between cell types and depending on the stimuli that induce senescence. The emerging notion that cellular senescence is a dynamic process that evolves over time further augments its complexity. The identification of a truly universal marker of senescence would be a boon to isolate and characterize senescent cells. The identification of more specific markers to distinguish unequivocally different types of senescent cells would be most useful to characterize them and shed light on their origin in vivo. Presently, single-cell transcriptomic approaches, including spatial transcriptomics, are the only option to fully appreciate senescent cell complexity and to determine the similarities and differences between regulated processes such as cell differentiation and senescence and the impact that senescence has on already differentiated, non-proliferating,

cells. Distinguishing between senescent cell subtypes and identifying what triggers senescence for each sub-type would enable us to identify the particular subsets of senescent cells that may be most deleterious to tissue function, and their targeting would optimize the benefits of senolytic and senomorphic approaches, while minimizing deleterious effects.

The physiological triggers of senescence in vivo are poorly understood. Telomere dysfunction is probably an important one, and only the use of specific telomeric DDR inhibitors will prove its involvement in various physio-pathological conditions. Telomeric ASOs could be both a research tool and a potential treatment, selective for those forms of cellular senescence caused by telomere damage.

The triggers for senescence in seemingly DDR-independent conditions remain elusive. SASP is emerging as the most consequential of all senescent cell phenotypes. Yet, the appreciation of the complexity underlying the mechanisms that control the SASP evolves as more components of the SASP are identified, in different cell types and in different contexts. Also to be considered is the powerful paracrine impact of cellular senescence on non-senescent cells; controlling it will clarify its expected but undemonstrated role in organismal ageing and in a number of conditions associated with the spread of cellular senescence.

Much of our understanding of the contribution of senescent cells to disease comes from animal models for human conditions. However, it remains to be shown that senolysis in humans is safe or effective, which is a prerequisite for devising treatments for patients. The study of long-term effects in rodents is limited to 2–3 years after senolysis, which is a much shorter time frame than their potential use in humans. Therefore, possible long-term toxic effects or negative consequences of senescent cell elimination that require longer observation times simply cannot be assessed with our current models and tools. It is clear that the immune system has the capacity to eliminate senescent cells in certain contexts; however, the clearance process seems to become dysfunctional with age and in disease, possibly explaining the accumulation of senescent cells with age[273]. As the cell components of the immune system are also subjected to senescence, it will be important to determine whether senotherapies eliminate these cells and whether the elimination of senescent immune cells contributes to or mediates the effects of senotherapies. In addition, harnessing the intrinsic ability of the immune system to target these cells, perhaps through engineered T cells, may hold promise for novel therapeutics. In summary, cellular senescence is clearly more complex and nuanced than initially thought, making diverse and occasionally contrasting contributions to physiology and ageing. Importantly, the many years of basic research in this field have set the foundations for a now exploding biotech and industrial activity devoted to turning such knowledge into treatments for patients. The next few years will see whether its promise is fulfilled: exciting times lie ahead.

Acknowledgements

R.D.M. is supported by Telethon (TIGET grant E5), a Career Development Award from the Human Frontier Science Program, an Advanced Research Grant from the European Haematology Association, “Pilot and Seed Grant 2015” from San Raffaele Hospital, a Hollis Brownstein Research Grant from the Leukemia Research Foundation, the Interstellar Initiative on Healthy Longevity of the New York Academy of Sciences and the Japan Agency for Medical Research and Development, and the Italian AIRC under MFAG 2019, ID. 23321 project PI Di Micco Raffaella. V.K. is supported by grants from the European Research Council under the European Union’s

Seventh Framework Proframme (309688) and under Horizon 2020 (856487), from the Israel Science Foundation (634-15; 2633-17), from the Israel Ministry of Health, Minerva Center “Ageing, from Physical Materials to Human Tissues”, and from the Sagol Institute for Longevity Research. V.K. is an incumbent of the Georg F. Duckwitz Professorial Chair. D.B. is supported by the Ellison Medical Foundation, the Glenn Foundation for Medical Research, the US National Institutes of Health (R01AG053229 and R01AG068076), the Mayo Clinic Children’s Research Center, the Alzheimer’s Disease Research Center at Mayo Clinic and the Cure Alzheimer’s Fund. F.d’A.d.F. is supported by AIRC (application 12971), Fondazione Telethon (GGP17111), PRIN 2015, the InterOmics Project, the AMANDA project Accordo Quadro Regione Lombardia-CNR, a European Research Council advanced grant (322726), a European Research Council proof-of-concept grant (875139), AriSLA (project “DDRNA and ALS”), the AIRC Special Program 5 per mille metastases (Project-21091) and the European Joint Programme on Rare Diseases (EJP RD).The authors apologize for not being able to cite all the important contributions of their colleagues owing to space limitations.

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Box 1 |

Senescence biomarkers

A major limitation in the senescence field is the lack of single, universal or model-specific biomarkers to identify senescent cells in culture or tissue samples. At present, the identification of senescent cells relies on a combination of multiple markers that, when present simultaneously, can discriminate between stably arrested senescent cells and quiescent or differentiated counterparts.

The first and still the most widely used biomarker to detect senescent cells in cultured cells and in fresh tissue samples is the accumulation of a lysosomal enzyme termed 'senescence-associated-β-galactosidase' (SA-β-gal)[275]. This marker is detectable by histochemical staining in most senescent cells and is generally not found in presenescent, quiescent or immortal and transformed cells, although SA-β-gal can also accumulate in serum-starved or overconfluent cells in tissue culture and may mark a specific subpopulation of macrophages in vivo as part of a reversible response to immune stimuli[276]. Lipofuscin accumulation is another feature of senescent cells. A recently developed method based on biotin-linked Sudan black B analogue is emerging as a reliable detection system to trace senescent cells in a variety of cell and tissue types[277].

Another peculiarity of senescent cells is the abnormally enlarged and flat morphology with disproportionate increase in the cytoplasm-to-nucleus ratio. While this bulky cytoplasm was originally described as a feature accompanying the establishment of cell senescence, a recent study suggests that increased cell size may play a causative role in driving the senescence-associated growth arrest[278]. In addition, in vivo, SA-β-gal-positive senescent cells have increased cell size compared with SA-β-gal-negative cells as identified on a single-cell level[279]. Another obvious marker for senescent cells is the lack of DNA replication, which is typically detected by the incorporation of nucleoside analogous (for example, 5-bromodeoxyuridine or [^{3}H]thymidine) or by immunostaining for proliferation markers, such as PCNA and Ki-67. These markers do not distinguish between senescent cells and quiescent or differentiated postmitotic cells.

p21 and p16 are two cyclin-dependent kinase inhibitors that are components of the tumour suppressor pathways governed by p53 and RB, and often accumulate in senescent cells. Because p21 and p16 expression levels are sufficient to establish and maintain the senescence-associated growth arrest, they are used to identify senescent cells in tissues and cultured cells. p16, in particular, was used as a surrogate senescence marker for the generation of engineered mouse models where selective eradication of senescent cells has been tested. However, not all senescent cell types express p16 as it can be expressed also by some tumour cells, especially those that have lost RB functions[280].

Nuclear senescence-associated heterochromatin foci (SAHF) are also used to identify senescent cells, but they appear to be specific to the senescence programme induced by activated oncogenes and DNA replication stressors[52]. Persistent DNA damage response factors accumulating at sites of damage as cytologically detectable nuclear foci are also used as markers of senescent cells, and when accumulating at telomeric sequences, telomere-associated foci represent a robust marker of the senescent state[24,35,281].

Lastly, components of the senescence-associated secretory phenotype (SASP), mainly the proinflammatory cytokines interleukin-6 (Il-6) and Il-8, may be used at the transcript and protein levels to evaluate general tissue or cell culture senescence. However, SASP alone cannot be used as a reliable senescence biomarker: indeed, senescence triggered by p16 overexpression does not entail an altered SASP transcriptional programme[85]. Overall, the search for universal senescence biomarkers is constantly challenged by the evidence that senescence phenotypes are highly heterogeneous and may differ depending on the initial trigger and the cell type under study. Therefore, transcriptomic and proteomic studies up to the single-cell level in relevant cell and tissue types will be of paramount importance to find unique or common markers of the senescence state, including cell surface molecules that will allow the prospective isolation of senescent cells from aged and diseased tissues. More recently, the development of innovative imaging-based tools and fluorescent tracers to monitor in real time senescence burden and monitor the therapeutic activity of senotherapies in clinical samples gained the interest of the scientific community[282–284] and may represent a turning point for senescence-based translational medicine applications.

Box 2 |

Senescence and autophagy

Dysfunctional cellular organelles, such as mitochondria and lysosomes, are usually degraded through the activation of an intracellular degradation system named 'autophagy'[285]. However, whether autophagy promotes senescence induction or is an alternative prosurvival mechanism lost during ageing is a matter of intense scientific investigation. Indeed, it was reported that a selective autophagy pathway, via mammalian target of rapamycin (mTOR) activation, contributes to sustain the protein synthesis of many senescence-associated secretory phenotype factors mainly in oncogene-induced senescent cells and that downregulation of several autophagy regulators delays the establishment of oncogene-induced senescence[286,287]. more recently, LC3B, a ubiquitin-like autophagic protein, was found to be associated with the nuclear envelope protein lamin B1 and to contribute to its degradation in the lysosomes in oncogene-induced senescent cells[59,60]. Importantly, lamin-associated chromatin domains are also transported out from the nucleus to the lysosomes via the same mechanism and contribute to the presence of cytosolic chromatin fragments that accumulate in senescent cells. Inhibition of autophagy prevents lamin B1 degradation and ensures nuclear envelope integrity in senescent cells[59].

In the context of therapy-induced senescence for cancer cells, it was also shown that autophagy was triggered as a consequence of senescence establishment to cope with the increased load of accumulating toxic macromolecules, and its pharmacological targeting led to senescent cell elimination[288]. However, autophagy has also been considered to suppress senescence by promoting the degradation of damaged organelles and other cellular components, and several studies have supported such a view. In adult muscle stem cells, basal autophagy maintains stemness by repressing senescence. During ageing, the autophagic activity in muscle stem cells (satellite cells) declines alongside stem cell regenerative capacity, with consequent accumulation of senescent satellite cells in geriatric mice. autophagy restoration in old satellite cells prevents senescence and rescues their regenerative capacity[289]. Similarly, autophagy protects against oxidative stress-induced senescence. enhancing autophagic activity under excessive oxidative stress by mTOR inhibition delays cellular senescence and functionally restores both mitochondrial and lysosomal functions[290]. Further supporting a role of autophagy in preventing senescence, a recent high-throughput screening aimed at identifying compounds that alleviate replicative senescence revealed that the ataxia telangiectasia mutated (ATM) inhibitor KU-60019 enhances the autophagic flux by restoring functional lysosomal activity by blocking the phosphorylation of the vacuolar protein ATP6V1G1. ATM inhibitor treatment also recovered mitochondrial functions and alleviated senescence phenotypes[274]. Together, these seemingly opposite roles may reflect a complex and reciprocal regulation of autophagy and cell senescence that can be linked to several senescence triggers, distinct cell types and a unique spatio-temporal activation of the autophagic programme acting in the senescence regulatory network.

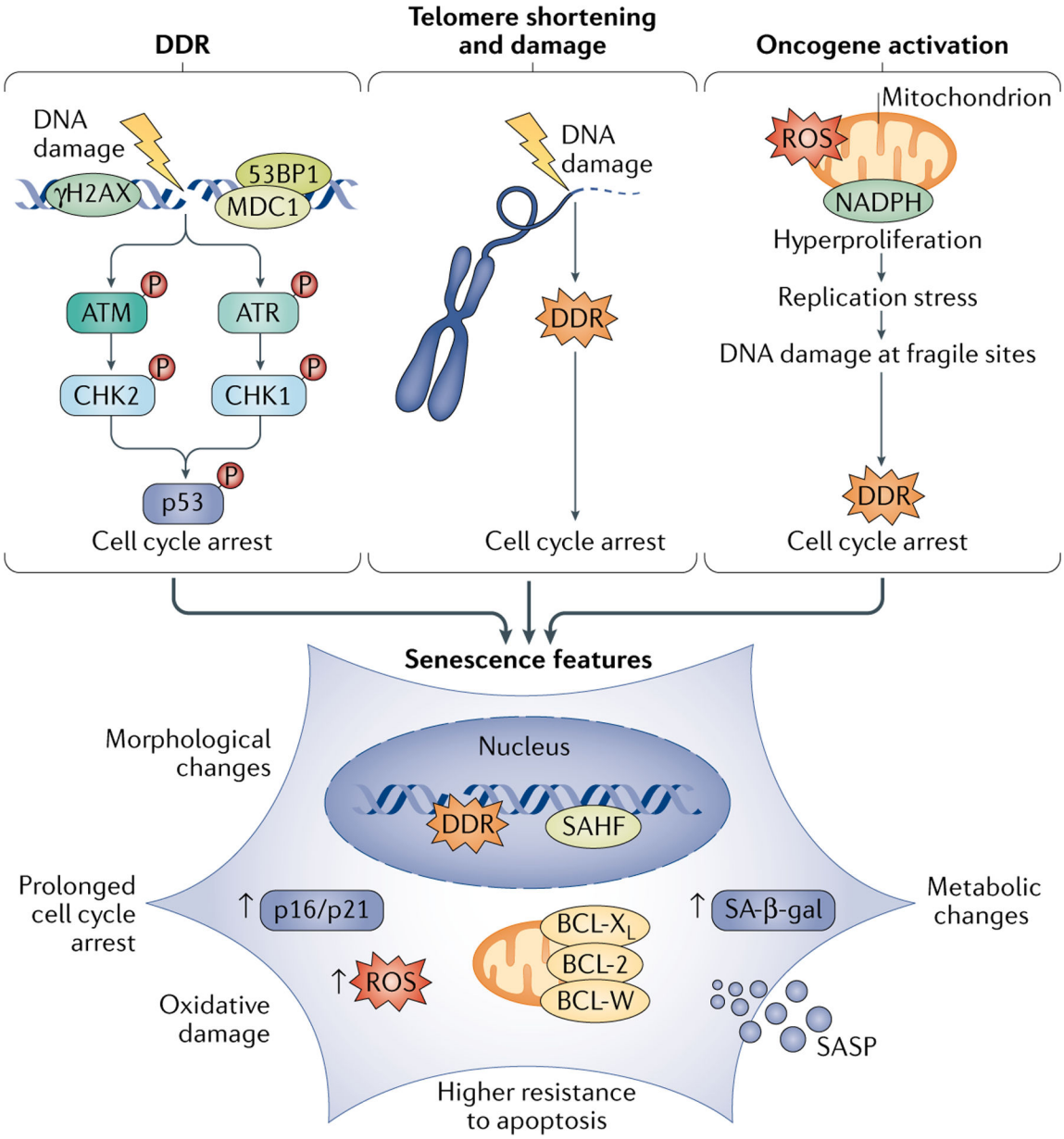

Fig. 1 |. Senescence drivers and phenotypes.

Nuclear DNA damage is often causatively associated with senescence establishment. DNA damage activates a signalling cascade defined as DNA damage response (DDR), characterized by phosphorylated histone H2AX (γH2AX), 53BP1 and MDC1, the apical kinases ataxia telangiectasia mutated (ATM) and ATR and the downstream kinases CHK2 and CHK1. Signals ultimately converge on p53 activation, which in turn elicits cell cycle arrest. Prolonged DDR activation triggers senescence. One or a few DDR signalling telomeres, the ends of chromosomes, are sufficient to trigger replicative cell senescence. Oncogene activation is also a powerful senescence trigger. Specifically, most activated oncogenes, partly via reactive oxygen species (ROS) production, induce hyperproliferation and altered DNA replication patterns that ultimately result in replication stress and DNA

damage accumulation at fragile sites, which include telomeres. Besides prolonged DDR activation, senescence features include cell cycle arrest (by upregulation of p21 and p16 cell cycle inhibitors), oxidative damage (as detected by increased ROS levels), upregulation of the BCL-2 family of antiapoptotic proteins, which induce resistance to apoptosis, metabolic changes (including senescence-associated-β-galactosidase (SA-β-gal) accumulation), senescence-associated heterochromatin foci (SAHF) and a senescence-associated secretory phenotype (SASP).

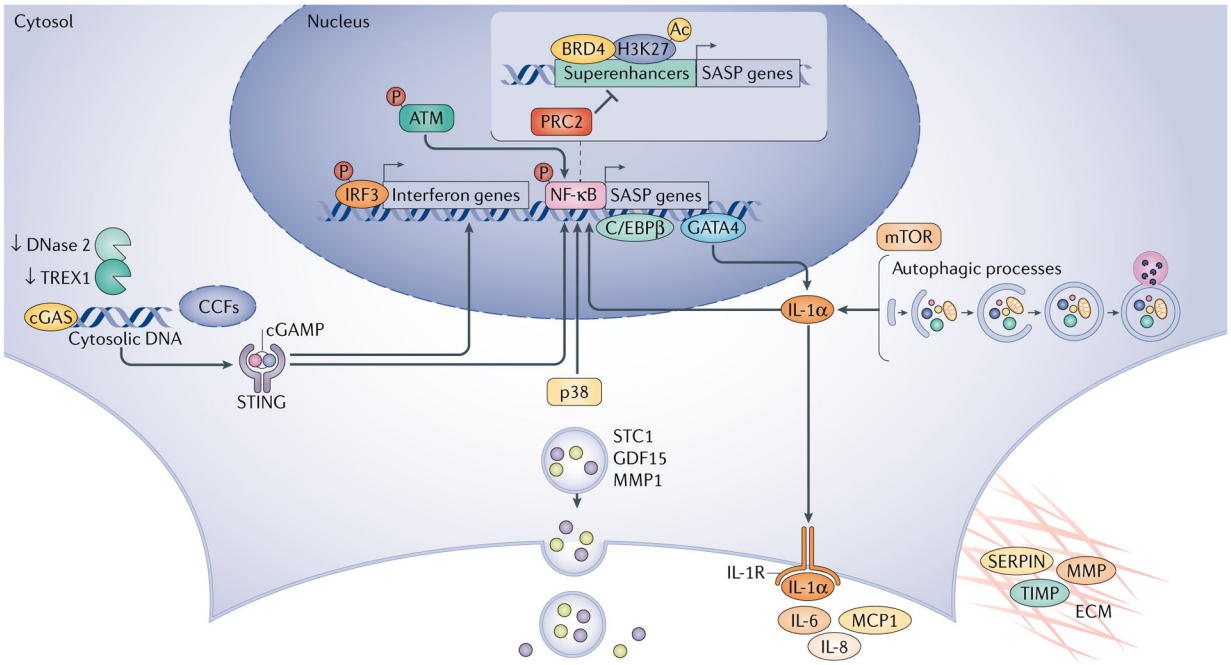

Fig. 2 |. SASP regulation.

Senescence-associated secretory phenotype (SASP) activation is a dynamic process that accompanies cell cycle exit initiated by senescence triggers. A core SASP programme comprises mainly proinflammatory interleukin-6 (IL-6), IL-8 and monocyte chemoattractant protein 1 (MCP1), regulated in an IL-1-dependent manner, and enzymes involved in extracellular matrix (ECM) remodelling, such as matrix metalloproteinases (MMPs), serine/cysteine proteinase inhibitors (SERPINs) and tissue inhibitors of metalloproteinases (TIMPs). More recently, additional core SASP effectors released as soluble molecules or in exosomes were identified, including GDF15, STC1 and MMP1. DNA damage response factors, including the upstream DNA damage response kinase induce SASP genes via nuclear factor-κB (NF-κB). The mitogen-activated protein kinase p38 also induces SASP genes by increasing the activity of NF-κB. Activation of several transcription factors and chromatin regulators has been implicated in SASP activation and regulation. NF-κB the transcription factor CCAAT/enhancer-binding protein-β (C/EBPβ) bind promoters of SASP genes and regulate their activation. GATA4 regulates NF-κB and SASP genes indirectly via IL-1 production. The mammalian target of rapamycin (mTOR) pathway also promotes SASP production through increased translation of subsets of mRNAs, including that encoding for IL-1α. In concert with transcription factors, the epigenetic reader bromodomain-containing protein 4 (BRD4), an acetylated histone-binding protein involved in oncogenesis, is recruited to superenhancers adjacent to SASP genes, thus contributing to the proper execution of cellular senescence. BRD4 binds acetylated histone H3 Lys27 (H3K27), thus competing with Polycomb repressor complex 2 (PRC2), which methylates the same histone residue (to give trimethylated H3K27) for transcriptional repression. Consistent with this, PRC2 inhibits SASP genes in senescent cells. More recently, the DNA sensor cyclic GMP–AMP synthase (cGAS) and the adaptor stimulator of interferon genes (STING) have been reported to be major regulators of the SASP programme across species

and senescence modes, presumably by activating NF-κB and interferon response factor IRF3 on recognition of cytosol DNA and cytosolic chromatin fragments (CCFs). Aberrant activation of the cGAS–STING pathway could be linked to the downregulation of DNases (for example, DNase 2 and TREX1), enzymes normally involved in cytoplasmic DNA degradation. ATM, ataxia telangiectasia mutated; cGAMP, cyclic GMP–AMP; IL-1R, interleukin-1 receptor.

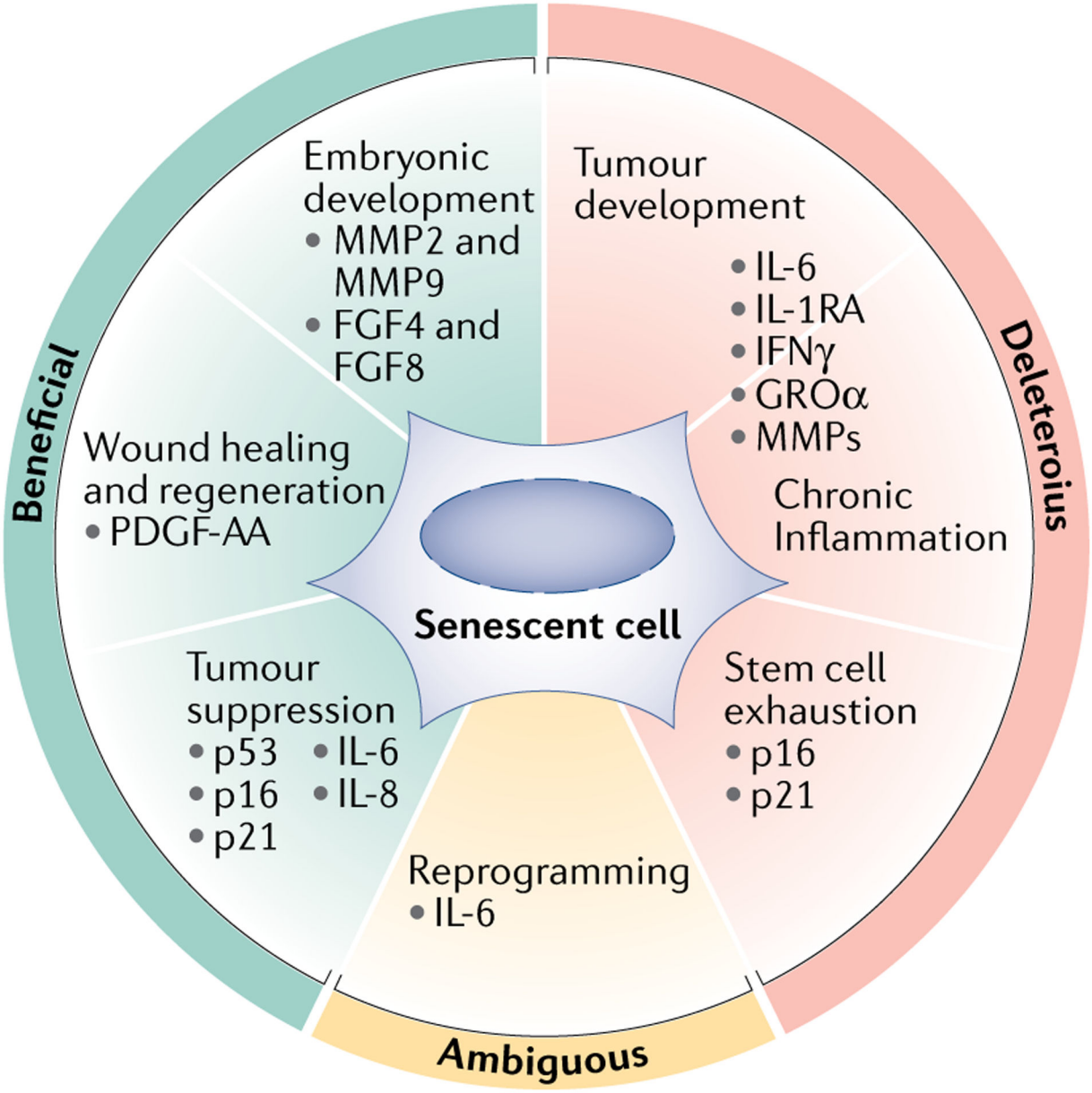

Fig. 3 |. Biological consequences of cell senescence.

Senescent cells execute distinct biological functions, which can have deleterious or beneficial consequences in a context-dependent manner. As beneficial functions, senescent cells guide tissue regeneration and embryonic development in the embryo in transient structures by secretion of FGF4 and FGF8 and shape the placenta structure and function with matrix metalloproteinase 2 and 9 (MMP2 and MMP9). Senescent cells also limit tissue damage by limiting excessive proliferation of cells and promote wound healing in part by secretion of PDGF-AA. One of the most prominent functions of senescence is tumour suppression. Senescent cells limit tumour development by cell-autonomous block of cell cycle progression via upregulation of p53, p16 and p21 and in a cell-non-autonomous manner by promoting senescence in neighbouring cells through secretion of interleukin-6

(IL-6) and IL-8. As deleterious functions, senescent cells can promote a proinflammatory microenvironment and therefore support tumour development in their proximity through multiple senescence-associated secretory phenotype (SASP) components. Similarly, senescent cells promote sterile chronic inflammation during ageing and during multiple age-related diseases. SASP factors, including IL-6, IL-1 receptor antagonist (IL-1RA), GROα and interferon-γ (IFNγ), are the main mediators of this effect. Additional SASP factors, including MMPs, might further damage tissue architecture and promote inflammation and tumorigenesis. When stem or progenitor cells enter senescence due to upregulation of the cell cycle inhibitory proteins, such as p16 and p21, they can no longer perform their function in supporting tissues by providing new cells, thus limiting tissue regenerative potential. Senescent cells also promote reprograming to an embryonic state, at least partially through IL-6. The reprograming, on one hand, can support tissue regeneration and, on the other hand, favours tumour development.

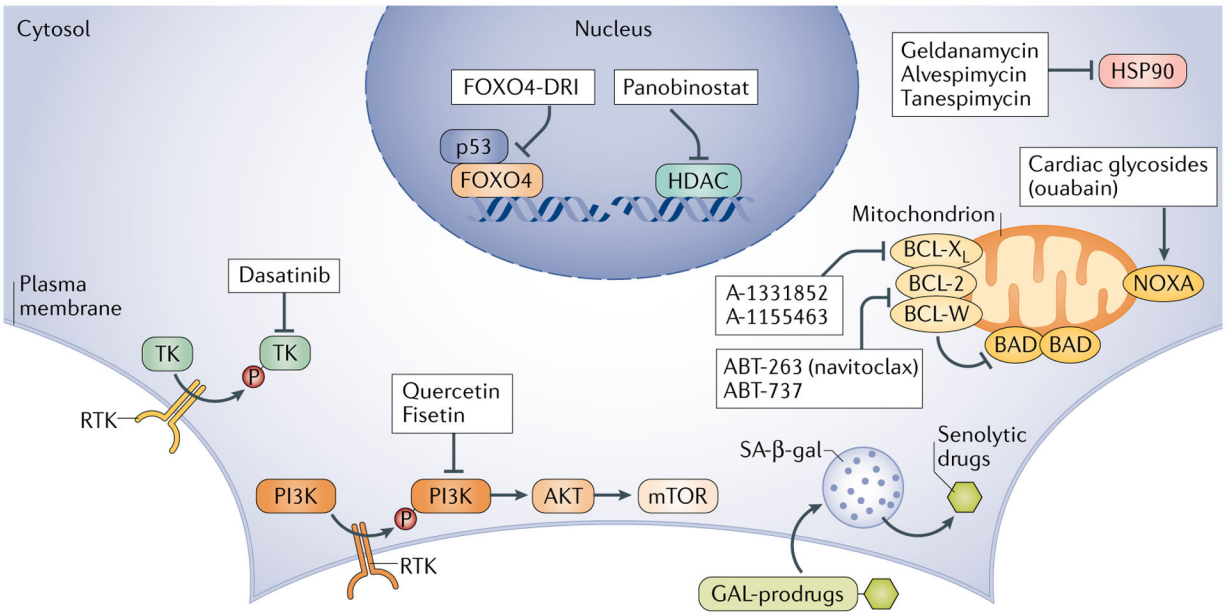

Fig. 4 |. Senolytic therapeutic interventions.

The sensitivities of senescent cells to pharmacological treatments that can promote their death are diverse. A number of known mechanisms of senolytic action are indicated; the various specific compounds that hit these nodes are indicated. Impacting tyrosine kinase (TK) through the use of dasatinib (when used either alone or in combination with the flavonoid quercetin) is capable of initiating death of certain senescent cell types. Quercetin and fisetin are natural flavonoids that impact mammalian target of rapamycin (mTOR) signalling. Inhibitors of the antiapoptotic members of the BCL-2 family are capable of inducing death through mitochondrial-mediated mechanisms, which can also be elicited by the action of cardiac glycosides such as ouabain. Inhibitors of HSP90 or histone deacetylases (HDAC) have also been suggested to promote selective apoptosis of senescent cells. Additionally, disruption of binding of forkhead box protein O4 (FOXO4) to p53, which occurs in senescent cells, through the use of a small peptide liberates p53 to activate apoptosis. Galactose-conjugated senolytic prodrugs (GAL-prodrugs) are processed by senescence-associated-β-galactosidase (SA-β-gal) to exert selective senescence targeting. RTK, receptor tyrosine kinase.

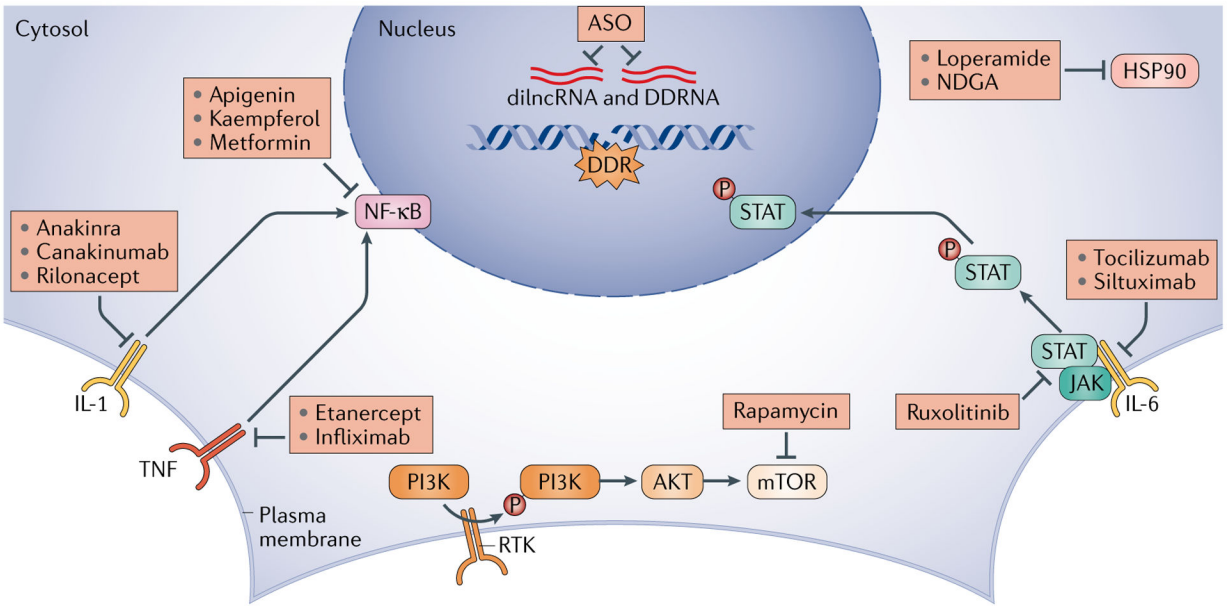

Fig. 5 |. Senomorphic therapeutic interventions.

As an alternative to active killing of senescent cells, senomorphic approaches try to limit the detrimental impacts of these cells, largely through modulation of the senescence-associated secretory phenotype (SASP). As for senolytics, a number of common nodes have been identified that may be unique opportunities for intervention. Rapamycin, a well-characterized inhibitor of mammalian target of rapamycin (mTOR), has been shown to increase lifespan of laboratory mice. Additionally, rapamycin decreases production of the SASP, which may explain the beneficial impacts on life. Nuclear factor-κB (NF-κB) is a critical component for SASP production, and inhibition of NF-κB activity decreases the ability of cells to be proinflammatory. Additionally, inhibition of HSP90 is able to modulate SASP production. Similarly, Janus kinase (JAK)/signal transducer and activator of transcription (STAT) inhibitors and blocking of interleukin-6 (IL-6), IL-1 and tumour necrosis factor (TNF). Taken together, these molecules are beginning to elucidate ways that proinflammatory signalling from senescent cells can be attenuated in the hope of decreasing the consequences of senescent cell accumulation in tissues. ASO, antisense oligonucleotides; DDR, DNA damage response; DDRNA, DNA damage response RNA; dilncRNA, damage-induced long non-coding RNA; NDGA, nordihydroguaiaretic acid; RTK, receptor tyrosine kinase.

Table 1 |

Senolytic and senomorphic compounds

Compound	Targets	Clinical trial status	Refs
Senolytic			
Dasatinib	Pan-receptor tyrosine kinases (including ephrin B1)	Phase II, (NCT02848131) for chronic kidney disease, phase II (NCT04313634) for skeletal health, phase I/II (NCT04063124) for Alzheimer disease	210
Quercetin	Numerous (including PI3K)	Phase II NCT02848131) for chronic kidney disease, phase II (NCT04313634) for skeletal health, phase I/II (NCT04063124) for Alzheimer disease	210
Fisetin	PI3K/AKT/mTOR	Pphase I/II (NCT04210986) for osteoarthritis of the knee, phase II (NCT04313634) for skeletal health	202
ABT-737	BCL-2, BCL-X_L and BCL-W (prosurvival proteins)	Preclinical animal models	14
ABT-263 (navitoclax)	BCL-2, BCL-X_L and BCL-W (prosurvival proteins)	Phase I/II (NCT00445198), phase II (NCT02591095), phase I (NCT02520778), phase II (NCT02079740) for various cancers	15,165,175,197,228
A-1331852	BCL-X_L (prosurvival protein)	Preclinical models of senescence in vitro	202
A-1155463	BCL-X_L (prosurvival protein)	Preclinical models of senescence in vitro	202
EF24	Proteosomal degradation of BCL-2 family proteins (prosurvival proteins)	Preclinical models of senescence in vitro	205
Cardiac glycosides (including oubain and digoxin)	BCL-2, BCL-X_L and BCL-W (prosurvival proteins)	Preclinical animal models	203,204
Azithromycin	Autophagy, metabolic changes	Preclinical models of senescence in vitro	217
Roxithromycin	Autophagy, metabolic changes	Preclinical models of senescence in vitro	217
Proxifim	p53	Preclinical animal models	206
UBX0101	MDM2 and p32	Phase II (NCT04129944) for osteoarthritis of the knee	201
Panobinostat	HDAC	Approved for multiple myeloma	55
Geldanamycin	HSP90	Preclinical models of senescence in vitro	214
Tanespimycin	HSP90	Preclinical models of senescence in vitro	214
Alevspimycin (17-DMAG)	HSP90	Preclinical models of senescence in vitro	214
Piperlongumine (and analogues)	OXR1 (unknown)	Preclinical models of senescence in vitro	215,216
Galactose-conjugated nanoparticles	Lysosomal activity of senescent cells	Preclinical animal models	218
Galactose-modified cytotoxic agents	Lysosomal activity of senescent cells	Preclinical animal models	219,220

Compound	Targets	Clinical trial status	Refs
BET protein degrader	Bromodomain and extraterminal domain family protein	Preclinical animal models	104
Senomorphic			
Metformin	IKK and/or NF-κB	Approved for type 2 diabetes	137,235–237
Apigenin	NF-κB p65 subunit and IκB	Naturally occurring flavonoid	238
Kaempferol	NF-κB p65 subunit and IκB	Naturally occurring flavonoid	238
BAY 11–7082	NF-κB p65 subunit and IκB	Preclinical models of senescence in vitro	118
Rapamycin	mTOR	Approved for immunosuppression	230,231,233,234
RAD001	mTOR	Approved for tuberous sclerosis complex-associated diseases	232
SB203580	p38 MAPK	Preclinical models of senescence in vitro	88
(5*Z*)-7-Oxozeaenol	TAK1	Preclinical models of senescence in vitro	232
Ruxolitinib	JAK	Approved for graft-versus-host disease	99
Senomorphic (cont.)			
KU-60019	ATM	Preclinical animal models	274
NDGA	HSP90	Naturally occurring antioxidant	249
Loperamide	HSP90	Approved for treatment of diarrhoea	214
Simvastatin	IL-6, IL-8, MCP1	Preclinical models of senescence in vitro	240
Cortisol	IL-6 secretion	Steroid hormone	241
Anakinra	IL-1R	Approved for rheumatoid arthritis	242
Canakinumab	IL-1β	Approved for cryopyrin-associated periodic syndromes	243
Rilonacept	IL-1α and IL-1β	Approved for cryopyrin-associated periodic syndromes	244
Etanercept	TNF	Approved for autoimmune diseases	245
Infliximab	TNF	Approved for autoimmune diseases	246
Tocilizumab	IL-6R	Approved for autoimmune diseases	247
Siltuximab	IL-6	Approved for multicentric Castleman disease	248
Telomeric antisense oligonucleotides	Telomeric non-coding RNA fuelling DDR	Preclinical animal models	251

ATM, ataxia telangiectasia mutated; DDR, DNA damage response; HDAC histone deacetylase; IL, interleukin; IL-1R, interleukin-1 receptor; IL-6R, interleukin-6 receptor; IκB, inhibitor of nuclear factor-κB; IKK, inhibitor of nuclear factor-κB kinase; JAK, Janus kinase; MCP1, monocyte chemoattractant protein 1; mTOR, mammalian target of rapamycin; nuclear factor-κB; NF-κB; NGDA, nordihydroguaiaretic acid; TNF, tumour necrosis factor.

Nat Rev Mol Cell Biol. Author manuscript; available in PMC 2021 August 06.