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REVIEW

Context-dependent roles of cellular senescence in normal, aged, and disease states

Victoria Moiseeva[1], Andrés Cisneros[1], Aina Calls Cobos[1], Aida Beà Tarrega[1], Claudia Santos Oñate[1], Eusebio Perdiguero[1], Antonio L. Serrano[1] and Pura Muñoz-Cánoves[1,2,3]

1 Department of Experimental and Health Sciences, Pompeu Fabra University (UPF), CIBER on Neurodegenerative Diseases (CIBERNED), Barcelona, Spain
2 ICREA, Barcelona, Spain
3 Spanish National Center on Cardiovascular Research (CNIC), Madrid, Spain

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Correspondence
P. Muñoz-Cánoves, Department of Experimental and Health Sciences, Pompeu Fabra University (UPF), CIBER on Neurodegenerative Diseases (CIBERNED), 08003 Barcelona, Spain
Tel: +34 93 3160891
E-mail: pura.munoz@upf.edu

Victoria Moiseeva, Andrés Cisneros and Aina Calls Cobos contributed equally to this article.
Eusebio Perdiguero and Antonio L. Serrano contributed equally to this article.

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Cellular senescence is a state of irreversible cell cycle arrest that often emerges after tissue damage and in age-related diseases. Through the production of a multicomponent secretory phenotype (SASP), senescent cells can impact the regeneration and function of tissues. However, the effects of senescent cells and their SASP are very heterogeneous and depend on the tissue environment and type as well as the duration of injury, the degree of persistence of senescent cells and the organism's age. While the transient presence of senescent cells is widely believed to be beneficial, recent data suggest that it is detrimental for tissue regeneration after acute damage. Furthermore, although senescent cell persistence is typically associated with the progression of age-related chronic degenerative diseases, it now appears to be also necessary for correct tissue function in the elderly. Here, we discuss what is currently known about the roles of senescent cells and their SASP in tissue regeneration in ageing and age-related diseases, highlighting their (negative and/or positive) contributions. We provide insight for future research, including the possibility of senolytic-based therapies and cellular reprogramming, with aims ranging from enhancing tissue repair to extending a healthy lifespan.

Introduction

Regeneration to restore the architecture and function of damaged tissue is a dynamic process that requires tight coordination of multiple signals and cell types. The outcome of this process depends on the tissue injured and the extent of the damage. Unresolved regeneration can lead to tissue dysfunction, disease and organ failure, and the capacity of mammalian tissues to regenerate dramatically declines with ageing.

Senescent cells are key players in the regenerative process after injury. Broadly, senescence is defined as a permanent cell proliferation arrest state, induced by the accumulation of cyclin-dependent kinase (CDK)

Abbreviations
ALS, amyotrophic lateral sclerosis; CKD, chronic kidney disease; DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; FAPs, fibro-adipogenic progenitors; GWAS, genome-wide association studies; iNKT, invariant natural killer T cells; IPF, idiopathic pulmonary fibrosis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; SASP, senescence-associated secretory phenotype; SCs, satellite cells; TECs, tubular epithelial cells.

inhibitors (especially $p16^{INK4a}$ and $p21^{CIP1}$) [1,2]. In 1961, Hayflick and Moorehead described the 'degeneration' of cell cultures *in vitro* as 'a phenomenon attributable to intrinsic factors, which are expressed as senescence' [3,4]. Cellular senescence can be triggered or accelerated by excessive stress, including DNA damage, mitochondrial dysfunction, oxidative stress and oncogene activation [5,6]. Senescent cells are highly heterogeneous, and there is no unique and universal senescence marker [7–9]. Commonly used senescence markers are the so-called senescence-associated β-galactosidase (SA-β-gal) activity and increased lysosomal β-galactosidase activity [10,11]—which however can also be detected in non-senescent cells [12,13]. Additional traits include DNA damage markers (such as 53BP1 and γH2AX), loss of nuclear lamin B1 (Lmnb1), senescence-associated heterochromatic foci (SAHF), telomeric dysfunction, cytoskeleton modifications, stronger substrate adhesion, accumulation of lipofuscin, increased lysosomes and granularity, enlarged cell size and high reactive oxygen species (ROS) production (Fig. 1) [14–17]. Senescent cells also have altered mitochondria and aberrant metabolism, and secrete high levels of SASP proteins [18–20]. Importantly, senescent cells are apoptosis resistant and can upregulate members of the anti-apoptotic Bcl-2 family of proteins, including Bcl-2, Bcl-xL and Bcl-w [21].

SASP largely varies depending on the types of cells, senescence triggers and kinetics [8,22,23] but generally comprises cytokines, growth factors, extracellular matrix (ECM) components and ECM-remodelling proteins. SASP can have significant effects on neighbouring cells and even convert them into new senescent cells (so-called secondary senescence or the bystander effect) [20]. Furthermore, the time of persistence of the senescent cells affects the tissue [20]: the transient presence of senescent cells can have a beneficial role on tissue regeneration after injury, while long-term (chronic) accumulation of senescent cells can harm tissue [24]. Of note, the progressive immune system dysfunction that occurs with age, a process defined as immunosenescence [25], altogether with increased levels of cellular damage over time, contributes to the accumulation of senescent cells.

The specific elimination of senescent cells, named senolysis, has made it possible to identify their role in many different contexts either by the use of genetic models inducing specific ablation of senescent cells or by small molecule drugs known as senolytics. These strategies will be further detailed in this review, which will focus on the role of cellular senescence in two distinct contexts: during tissue regeneration after acute damage, and in tissues under chronic stress (such as in age-related degenerative diseases). We will also discuss the link between senescence and cellular

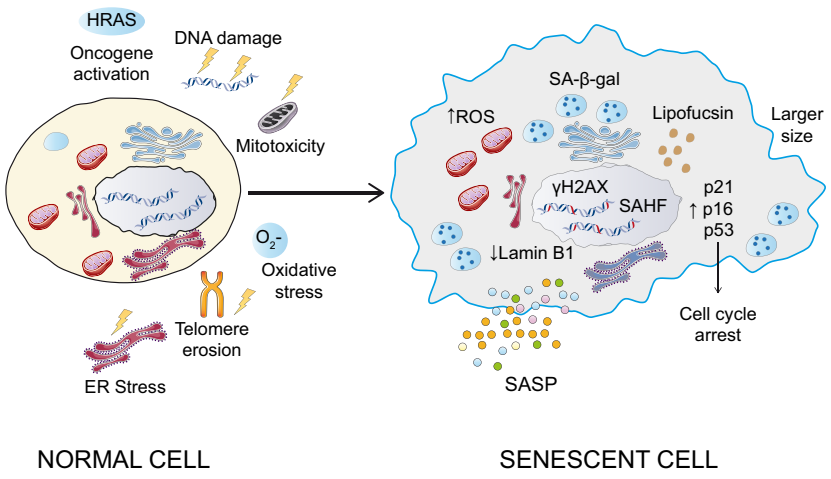

Fig. 1. Hallmarks of cellular senescence. After different types of damage, cells can enter into a senescent state, characterized by permanent cell cycle arrest due to upregulation of p53 and the cell cycle inhibitors $p16^{INK4a}$ and $p21^{CIP1}$. Senescent cells present morphological alterations, being enlarged and with an irregular shape. Their nuclear integrity is compromised due to the loss of Lamin B1, which is accompanied by the accumulation of heterochromatin foci (SAHF) and increased expression of DNA damage response markers in the nuclei, such as γH2AX. Senescent cells have dysfunctional mitochondria that produce high levels of reactive oxygen species (ROS), higher lysosomal content that induces the increased activity of the senescence-associated β-galactosidase (SA-β-gal) enzyme, and accumulation of lipofuscin. Finally, senescent cells show a secretory phenotype known as SASP comprised of cytokines, chemokines, interleukins and extracellular matrix components. Artwork created with BioRender.com.

reprogramming in the context of chronic diseases and ageing.

Cellular senescence in tissue regeneration after acute damage

Senescent cells appear at the tissue injury site after damage. Multiple stressors, such as damage-associated molecular patterns (DAMPs), oxidative stress and mitochondrial dysfunction, are present in high quantities in damage sites and also trigger cellular senescence [26,27]. Senescence has an important role in tissue remodelling in skin wound healing, damaged kidney and liver, and infarcted heart (see below). Moreover, senescent cells accumulate transiently in animal models with a high regenerative response after acute injuries, such as zebrafish hearts and fins or salamander limbs [28,29]. Although present in low proportions, senescent cells can influence the outcome of tissue repair, likely by modulating the regenerative niche through their SASP. In this context, several studies report that senescent cells can limit fibrosis during regeneration in various tissues [30,31], suggesting a positive role in tissue repair. However, SASP can be either beneficial or detrimental for tissue repair depending on their composition as well as on the cell types affected and on the type of injury [26]. Here, we discuss the role of cellular senescence in the context of skin wound healing, cardiac, liver and kidney regeneration, bone fracture healing and spinal cord recovery (Fig. 2).

Skin: Senescent fibroblasts and endothelial cells appear after skin wounding, secrete platelet-derived growth factor-AA (PDGF-AA) and transforming growth factor beta 1 (TGFβ1), and promote differentiation of fibroblasts into myofibroblast [31,32]. Genetic ablation of senescent cells via ganciclovir (GCV) in p16-3MR mice, or by knocking down both $p16^{INK4a}$ and $p21^{CIP1}$, delays wound closure. As compared to normal injuries, senescence-free wounds present larger wound gaps that have: (a) longer persistence of necrotic cells and cellular debris, (b) slower angiogenesis, (c) poor formation of granulation tissue, and (d) more abundant fibrosis. Indeed, inhibition of PDGF-AA delays wound closure in normal injuries, whereas its topical administration to senescence-free wounds rescues the delayed wound closure and lack of myofibroblast differentiation. Of note, administration of PDGF-AA does not rescue collagen accumulation (fibrosis) in senescence-free wounds [31], suggesting that fibrosis prevention by senescent cells during wound healing depends on other factors, most likely on the acquisition of an anti-fibrotic SASP.

After skin wounding, cellular communication network factor 1 (CCN1) is upregulated and induces fibroblast senescence upon binding $\alpha_6\beta_1$ integrin and heparan sulphate proteoglycan (HSPG), which results

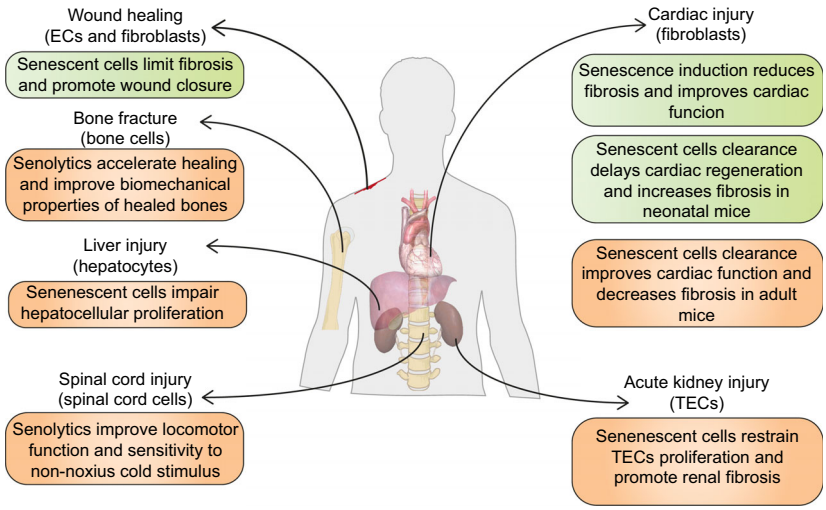

Fig. 2. Contribution of senescent cells to acute injury. Distinct populations of senescent cells appear and modulate regeneration in many tissues of the organism after acute damage. Depending on the factors, such as the tissue or the age of the animal, senescent cells have been reported to be beneficial (boxes in green) or detrimental (boxes in orange). The specific contribution of senescent cells in different regeneration contexts, the mechanisms by which senescent cells act in these contexts, and how different factors, such as age, affect the role of senescent cells during regeneration needs to be further evaluated. ECs, endothelial cells; TECs, tubular epithelial cells. Artwork created with BioRender.com.

in ROS generation and p53 and $p16^{INK4a}$ activation. CCN1-induced senescent cells express antifibrotic genes; consistent with this, skin injury in mice with knockout (KO) for *Ccn1* has reduced levels of senescent cells and exacerbated fibrosis, which can be rescued by topical administration of CCN1 [33]. In human skin, administration of CCN2 (which is highly homologous to CCN1) promotes senescence in fibroblasts and reduces fibrosis in cutaneous wound healing. However, CCN2 inhibition does not affect senescence induction during wound repair or the outcome of injury. Unlike the constant expression in wounds of CCN1, CCN2 is highly expressed during the initial inflammatory phase but not during the proliferative or remodelling stages of wound healing [34]. Finally, overactivation of the nuclear factor erythroid 2-related factor 2 (Nrf2) induces fibroblast senescence, leading to improved re-epithelization during wound repair; however, it also promotes skin tumorigenesis [35]. The ability of senescent cells to limit fibrosis and to promote wound closure and repair during skin wound healing is maintained in ageing, as shown *in vivo* by genetic elimination of $p16^{INK4a}$+ cells, which delays skin wound closure in both young and old mice [36].

Heart: Senescent cells (and particularly senescent fibroblasts) appear during the cardiac repair. At both neonatal and adult heart stages, downregulation of p53 and $p16^{INK4a}$ reduces the number of senescent fibroblasts, while that of non-senescent fibroblasts increases. This is accompanied by higher collagen deposition, leading to fibrosis, and impaired cardiac function [37–41]. In accordance with this, haplodeficiency of the gene for ataxia-telangiectasia mutated (ATM), which acts upstream of p53 and $p16^{INK4a}$, also reduces the number of senescent cells during heart regeneration and increases myofibroblast accumulation, fibrosis, cardiac dilatation, and deterioration of the left ventricle, thus leading to impaired cardiac function [42]. In contrast, overexpression of CCN1 after heart injury promotes senescence of fibroblasts, which restrains their proliferation and in turn reduces fibrosis and improves cardiac function in both neonatal and adult mice [37,43]; thus, induction of senescence during heart regeneration seems to be beneficial. In contrast, clearance of senescent cells can have either positive or negative effects. In neonatal mice, the clearance of senescent fibroblasts with the senolytic compound navitoclax (also called ABT-263), a specific inhibitor of the anti-apoptotic BCL-2, and BCL-xL pathways, delays cardiac regeneration and increases fibrosis [37]. In line with this, senescent cells exhibit a positive role during cardiac development when they contribute to organo- and morphogenesis [44]. In contrast, navitoclax treatment of adult mice reduces the expression of proinflammatory, profibrotic and anti-angiogenic cytokines after cardiac injury, which leads to decreased fibrosis and improved cardiac function [45]. Therefore, clearance of senescent cells in the heart has a negative impact in neonatal stages but a positive outcome later in life after an injury.

Liver: Senescent hepatocytes emerge after liver damage. In human acute liver disease and murine liver injury models, p21-dependent hepatocellular senescence is proportionate to disease severity and is associated with impaired regeneration [46]. In these conditions, there is a spread of senescence to local uninjured hepatocytes (secondary senescence) that depends on macrophage-derived TGFβ1. Downregulation of $p21^{CIP1}$ or inhibition of TGFβR1 reduces the number of senescent cells after liver injury, increases hepatocellular proliferation, and improves regeneration [46]. Of note, senescent cells can aggravate biliary injuries through the same mechanisms. In this context, inhibition of TGFβ signalling can also interrupt the deleterious effects of senescent cells and restore liver function [47]. Furthermore, senescence has been related to declining liver regeneration with ageing. Downregulation of $p21^{CIP1}$ partially rescues the regenerative capacity of old animals after liver resection, whereas pre-treatment with the senolytic compound ABT-737 (in the same class as navitoclax) before liver resection decreases $p21^{CIP1}$ expression, enhances liver regeneration and function, normalizes serum liver enzymes and minimizes lipidosis [48]. In contrast, accumulation of senescent hepatic stellate cells (HSCs) leads to enhanced fibrosis upon prolonged liver damage with CCl_4 treatment, thus limiting regeneration. Senescent HSCs limit the secretion of ECM components and enhance immunosurveillance, which results in a faster resolution of fibrosis and reduced scar formation [30]. These findings indicate that different cell populations in the same tissue might behave in completely distinct ways upon their entry into senescence, clarifying how senescence can have both beneficial and deleterious roles in liver regeneration.

Kidney: During acute kidney injury (AKI), several cell types undergo senescence, with tubular epithelial cells (TECs) being the most significant ones. Maladaptive repair after AKI can lead to chronic kidney disease (CKD), and senescence influences the outcome of regeneration in AKI and the transition from AKI to CKD. The presence of senescent TECs restrains the overall TEC proliferation and activates the fibroblast-to-myofibroblast transition, which in turn promotes renal fibrosis. Inactivation of TLR/IL-1R signalling (which has been related to secondary senescence) by

inhibition of Myd88 reduces the number of senescent cells and the level of fibrosis [49]. Senescent TECs, inflammation and fibrosis after AKI can be reduced by depleting senescent cells by treating mice with a combination of senolytic drugs known as 'D+Q'. This cocktail of drugs consists of the tyrosine kinase inhibitor dasatinib, which inhibits SRC, c-KIT, ephrin receptors' and other kinases, and the flavonoid quercetin which targets multiple kinases and kinase receptors and inhibits the PI3K-AKT pathway [50]. In addition to D+Q, improved outcomes after AKI can be also achieved by treating p16-3MR mice with GCV [49]. Of note, the senolytic peptide FOXO4-DRI, which was designed to block the interactions of forkhead box O 4 (FOXO4) and p53 [51], is as effective as GCV in reducing senescent cells but does not ameliorate either inflammation or fibrosis [49].

Senescence has also complex and diverse roles during renal regeneration. Aged mice have reduced regeneration upon AKI, but that can be improved by pre-treatment with the senolytic navitoclax before AKI [52]. Senolytic pre-treatment of aged mice promotes tubular cell proliferation, increases organ weight and reduces the markers of kidney damage and fibrosis. These effects are in part mediated by TFGβ1 production by senescent cells [53]. Furthermore, renal regeneration is improved in mice by downregulation of $p21^{CIP1}$ and $p16^{INK4a}$, inhibition of p53 before AKI, or treatment with CDK4/6 inhibitors before AKI, while $p21^{CIP1}$ KO mice have a reduced ability to recover after AKI [54–56]. Thus, senescence has also complex and diverse roles during renal regeneration.

Bone: Senescent cells also accumulate after a bone fracture, reaching their peak between one and 2 weeks later. Reducing the number of senescent cells using the senolytics D+Q accelerates bone regeneration, as observed by a larger relative callus area 2 weeks after fracture. Furthermore, healed bones of treated mice display better biomechanical properties, sustain higher maximal torque and show a tendency towards greater bone stiffness after 5 weeks [57].

Spinal cord: Senescent neurons accumulate at the lesion periphery of injured spinal cords both in mice and zebrafish [58]. In the zebrafish model, senescent cells accumulate 1 month after injury but are eventually cleared and returned to basal levels. In contrast, in mice, the induced accumulation of senescent cells persists at the lesion periphery, with no improvement in the time frame followed. Reducing the number of senescent cells in the mouse injury model using the senolytics navitoclax or D+Q improves locomotor performance and bladder function (a common complication of such injury), and mice also recover normal sensitivity to a non-noxious cold stimulus [58]. Thus, senescent cells have a detrimental impact after a spinal cord injury.

Senescence surveillance in tissue regeneration

In non-pathological situations, senescent cells are efficiently removed from injured tissues by the immune system, a process known as senescence surveillance. Different cells of the immune system are likely to participate in this process, including macrophages,

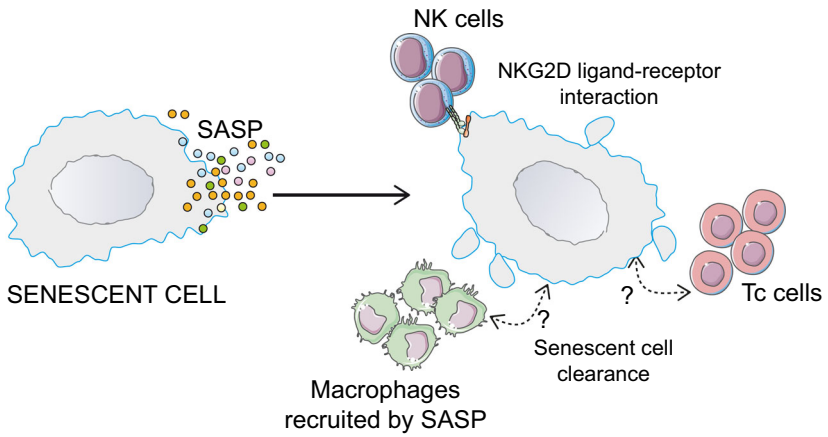

Fig. 3. Clearance of the senescent cells by the immune system. Senescent cells upregulate ligands in their cell surface that are recognized by the NKG2D receptor of NK cells, initiating a cytotoxic response and subsequent clearance. Although also involved, the exact role of other immune cell types like macrophages or lymphocytes in the clearance of senescent cells is not fully understood. The immune system's ability to clear senescent cells is impaired with ageing, leading to senescent cells accumulation in the organism and contributing to the appearance and progression of age-related diseases. Artwork created with BioRender.com.

dendritic cells, neutrophils, T lymphocytes and natural killer (NK) cells (Fig. 3), although their exact roles may vary depending on the pathophysiological context [29,59–63]. Although not well understood, senescent cells induced by different stimuli appear to become immunoreactive due to the expression of several ligands of the NK cell receptor (NKG2D), including the MHC class I-chain-related protein A (MICA) and the UL16-binding proteins 1/2 (ULBP1 and ULBP2) [30,64,65]. The NKG2D ligand-receptor interaction is essential for the NK-mediated cytotoxicity towards senescent cells *in vitro* [65]. The expression of NKG2D ligands is regulated by the DNA damage response and ERK signalling pathway, both of which are highly active in senescent cells [66]. NK lymphocytes kill senescent cells mainly via the perforin-based system through the granule exocytosis mechanism. Indeed, mice in which granule-exocytosis-mediated apoptosis (and therefore immune surveillance) is disabled have an overall lower survival rate and exhibit higher senescent cell tissue burden and chronic inflammation, as well as multiple age-related disorders [67]. In addition to NK cells, macrophages are also important for clearing senescent cells [29,60,62,68]; however, it is not known whether senescent cells are phagocytized by macrophages in a similar way to apoptotic cells, which are recognized by so-called 'find-me' (chemoattractants released by cells to guide phagocytes to their location) and 'eat-me' (a signal on a cell surface inducing a phagocyte to phagocytose the cell) signals [69]. A recent study showed that inducing $p21^{CIP1}$ in senescent cells places them under immunosurveillance by recruiting macrophages and cytotoxic T cells, with the former being responsible for eliminating target cells [63].

In advanced age, senescent cells tend to accumulate in tissues, suggesting that their clearance is compromised (see below). A recent study showed that the specific induction of DNA damage in the hematopoietic lineage (resembling an aged immune system) leads to increased levels of senescent cells in non-lymphoid tissues [70]. Similarly, the specific decline in mitochondrial function in T cells leads to premature ageing of adult mice, and this correlates with higher levels of the senescence hallmarks p53, $p21^{CIP1}$ and SA-β-gal activity in many peripheral organs (e.g. liver, heart and pancreas) [71]. Altogether, these data suggest that immunosenescence promotes the systemic accumulation of senescent cells in mice and causes premature ageing. Indeed, activation of invariant natural killer T (iNKT) cells, a subset of natural killer T (NKT) cells that declines in frequency and function in humans with age, leads to a removal of senescent cells in white adipose tissue of mice fed a high-fat diet [72].

The permanent accumulation of senescent cells in peripheral tissues with ageing may also be related to age-related gene expression changes in these cells that modify their interactions with the immune system. Senescent human dermal fibroblasts can evade immune clearance by expressing the non-classical MHC-class Ib molecule HLA-E, a ligand of the inhibitory receptor NKG2A [73]. Furthermore, blocking the interaction between HLA-E and NKG2A boosts the immune response against senescent cells *in vitro* [73]. Interestingly, the production of IL-6 by senescent cells (as part of the SASP) induces the expression of HLA-E in non-senescent cells in a paracrine fashion [73], and IL-6 levels are chronically increased in the serum of elderly people [74].

Cellular senescence in ageing and chronic pathologies

Senescence is considered a hallmark of ageing, as higher levels of senescent cells are observed in different tissues over time [75,76]. The expression of key cell cycle arrest and senescence regulators $p15^{INK4b}$, $p16^{INK4a}$ and $p19^{ARF}$ (encoded by the *INK4/ARF* gene locus) is considered an ageing-related biomarker [77]. Moreover, genome-wide association studies (GWAS) have shown that the *INK4/ARF* locus is one of the highest disease susceptibility-associated hotspots, correlating with age-related diseases, such as cancer, type 2 diabetes (T2D), atherosclerosis and glaucoma [78], and is significantly related to physical dysfunction in the elderly [79]. Furthermore, key proinflammatory SASP factors (such as IL-6, CCL4 and TNFα) contribute to age-related inflammation ('inflammaging') and correlate with frailty and multimorbidity at an advanced age [22,80,81].

Senescent cells accumulate in tissues of normally ageing mice and in accelerated ageing models, such as the progeroid BubR1 mice, which have a shortened lifespan and age-related phenotypes (e.g. sarcopenia, infertility, cardiac arrhythmias and impaired wound healing) [36,82,83]. Inactivation of $p16^{INK4a}$ or inducible elimination of $p16^{INK4a}$-expressing cells increases lifespan and delays the onset of sarcopenia and cataracts (among other ageing-related features) in BubR1 mice (and in INK-ATTAC BubR1 mice, a transgenic mouse model that expresses a death cassette and green fluorescent protein under the control of a minimal INK4a/ARF promoter fragment) [82,83]. Elimination of $p16^{NK4a}$-expressing cells also extends lifespan and healthspan in naturally aged INK-ATTAC mice and prevents kidney dysfunction, cardiomyocyte hypertrophy and lipodystrophy [36]. Treating normally aged

mice with the senolytic compound navitoclax rejuvenates hematopoietic and muscle stem cells [84]. Similarly, selective elimination of senescent cells with the FOXO4-DRI peptide improves fitness, hair density and renal function in both fast-ageing and naturally aged mice [51]. Strikingly, transplantation of a small number of senescent cells is sufficient to induce physical dysfunction in young mice and to further exacerbate it in old ones, while treatment with the senolytic drugs D+Q abrogates the negative effects of senescent cell transplantation and ameliorates physical dysfunction in naturally aged (24 months old) mice [85]. Of note, the elimination of $p16^{INK4a}$-expressing cells in mice disrupts blood–tissue barriers and leads to liver fibrosis, due to the elimination of vascular endothelial cells in liver sinusoids [86], highlighting the nuanced, tissue-dependent roles of senescent cells. In the following sections, we discuss the role of cellular senescence in the context of different age-related pathologies (Fig. 4).

Sarcopenia is the state of progressive loss of skeletal muscle mass and function with ageing, which is associated with fall-related injuries, frailty and mortality in the elderly. Various senescence markers have been detected in muscles of aged rats, including increased mRNA expression of $p16^{INK4a}$ and $p21^{CIP1}$, coinciding with the presence of atrophic myofibers [87]. With ageing, skeletal muscle also loses its regenerative capacity. Several studies have attributed this loss to the entrance of a fraction of muscle satellite cells (SCs) in senescence after injury [88–90]. Naturally aged very old (geriatric) mice exhibit increased SA-β-gal activity in SCs, high expression levels of $p15^{INK4b}$, $p16^{INK4a}$ and *Igfbp5*, and increased p38α/p38β signalling, resulting in a delayed regenerative process upon injury and a reduced ability to engraft and form new myofibers even after transplantation into young mice [91,92]. The causes of senescence entry in aged SCs have been identified as impaired autophagy (and mitophagy), which leads to the accumulation of dysfunctional mitochondria, and excessive ROS production [93]. Additionally, excessive upregulation of TGFβ in injured aged muscle leads to imbalanced pSmad3 and Notch signalling and overexpression of CDK inhibitors, $p15^{INK4b}$, $p16^{INK4a}$, $p21^{CIP1}$ and $p27^{KIP1}$ in aged SCs [94]. Besides SCs, other cell types undergo senescence in aged muscle; for instance, fibro-adipogenic progenitors (FAPs) express high levels of $p15^{INK4b}$, $p16^{INK4a}$, $p21^{CIP1}$ and other markers of senescence in BubR1 progeroid mice, suggesting the involvement of FAPs in defective muscle regeneration in these mice [95]. In agreement with previous results [85], treatment with the senolytics D+Q over 4 months improved muscle function of old mice, indicating the negative impact of accumulated senescent cells in old muscle tissue. Interestingly, senolytic treatment before an acute injury did not affect muscle regeneration either in young or old mice [96]. This result highlights the difference between induction of senescence after an acute injury (see Section 1) and accumulation of senescence due to ageing and sarcopenia, as well as the importance of timely use of senolytic drugs to influence either process.

Duchenne muscular dystrophy (DMD) is a fatal, X-linked muscle degenerative disorder characterized by a lack of functional dystrophin protein, and it remains incurable. Dystrophin is required for proper linkage between the myofiber cytoskeleton and the ECM and the mechanical stability of the myofibers. Loss of dystrophin leads to a high susceptibility to damage upon muscle contraction, resulting in functional loss and progressive wasting. Because of the continuous degenerative–regenerative cycles, SCs in DMD proliferate

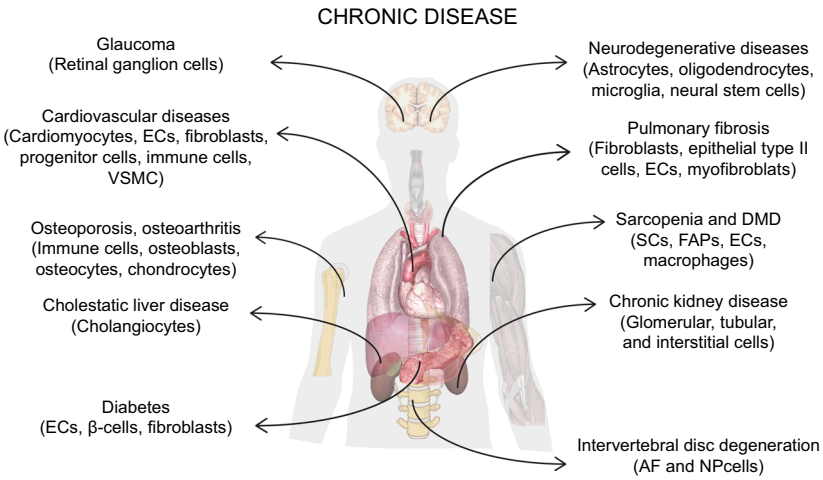

Fig. 4. Contribution of senescent cells to chronic diseases. Distinct populations of senescent cells have been observed in many tissues of the organism under different pathological conditions. The exact contribution of the distinct senescent cell types to each chronic disease needs to be further elucidated. AF, annulus fibrosus; ECs, endothelial cells; FAPs, fibroadipogenic progenitors; NP, nucleus pulposus; SCs, satellite cells; VSMC, vascular smooth muscle cells. Artwork created with BioRender.com.

in an attempt to restore the architecture of muscle tissue; however, they eventually lose their regenerative potential [97]. Several studies have shown that the DMD-like mdx mouse model has increased levels of senescent cells in muscles as compared to age-matched normal counterparts, correlating with the chronic muscle degeneration, although mdx mice show normal lifespan and milder clinical symptoms than DMD patients [98,99]. The mdx/mTR^{KO} mouse model (mdx mice with no telomerase activity) shows a more severe disease phenotype, better resembling human DMD [100]. Interestingly, the shortened telomeres of SC-derived myoblasts from mdx/mTR^{KO} mice lead to their compromised proliferative capacity, muscle loss and fibrosis, which account for severe muscle degeneration [100]. Senescence of SCs was later confirmed by several studies performed in different mouse models of DMD and myotonic dystrophy type I, correlating with impaired proliferation rate and myogenic program development [99,101–103]. However, FAPs, endothelial cells and macrophages also present signs of cellular senescence in a rat model of DMD and mdx mice, suggesting that different cell populations undergo senescence in skeletal muscle [104,105]. Senolytics and genetic interventions to ablate senescent cells improve muscular function and regeneration and reduce fibrotic and adipose infiltrates in the skeletal muscle of dystrophic rats, which in turn prevents dystrophy-associated loss of muscle strength and body weight [104]. Most importantly, markers of senescence have also been detected in biopsy samples from DMD patients, suggesting that interventions with senolytics might be a therapeutic option to attenuate disease progression [104].

Idiopathic pulmonary fibrosis (IPF) is a chronic fatal disease, affecting people around 70 years old, and is characterized by progressive inflammation and deposition of ECM in the lung interstitium, with the destruction of lung structure and function [106]. Different cell populations, such as epithelial and endothelial cells, and fibroblasts undergo senescence in the lungs of patients with IPF (reviewed in [107]). Senescent fibroblasts produce pro-fibrotic SASP, including TGFβ, MMP2 and MMP12, and induce fibrogenesis in a paracrine manner, further exacerbating pulmonary fibrosis [108]. Interestingly, lung myofibroblasts of aged mice acquire a senescent, apoptosis-resistant phenotype, correlating with impaired fibrosis resolution observed with ageing [109]. The mechanism behind it relies on the overexpression of Nox4, a ROS-generating enzyme, which leads to alterations in redox homeostasis and impaired Nrf2 antioxidant response. Targeting Nox4 is sufficient to reduce senescent cells burden and diminish myofibroblasts accumulation, fibrosis and mortality [109]. However, induction of p53-dependent senescence in alveolar type II epithelial cells suffices for initiation and development of progressive lung fibrosis in mice, which is abrogated by p53 inhibition or treatment with senolytic drugs [110]. Elimination of senescent cells in bleomycin-treated mice (an experimental lung fibrosis model) using the two different strategies—genetically in INK-ATTAC mice, and pharmacologically with D+Q both *in vivo* and *ex vivo*—results in decreased fibrotic markers, improved pulmonary functions and attenuated weight loss and lethality [108,111,112]. Furthermore, a pilot clinical study conducted on patients with IPF showed improved physical function after treatment with D+Q senolytics [113].

Chronic kidney disease (CKD) comprises different renal conditions, mainly characterized by progressive glomerulosclerosis, interstitial fibrosis, tubular atrophy and loss of kidney functions [114]. Both senescent cell accumulation in the kidney and the risk of developing CKD increase with age [115–118]. Systemic elimination of senescent cells in naturally aged INK-ATTAC mice reduces glomerulosclerosis and blood urea levels, suggesting that it improves kidney function [36]. Comparably, targeting senescent cells either with a modified FOXO4-DRI interfering peptide or genetically using a p16-3MR system in mice leads to reduced levels of urea in plasma, recovering renal function in naturally aged and in Xpd$^{TTD/TTD}$ fast-ageing mice [51]. In kidney biopsies from patients with CKD, senescent cells are detected within glomerular, tubular and interstitial populations, based on p16^{INK4A} staining [116,118–120]. Moreover, the presence of the SASP components TNF-α and IL-6 correlates with more rapid CKD progression [121]. However, targeting senescent cells in CKD has both detrimental and beneficial outcomes. Mouse models of diabetic nephropathy in p21^{CIP1} and p27^{KIP1} KO mice are protected from proteinuria and glomerular expansion [122,123]. However, p16^{INK4a} KO models have exacerbated fibrosis in unilateral ureteric obstruction, but enhanced functional recovery after ischaemia-reperfusion injury, and limited atrophy and fibrosis in renal transplant models [54,124,125]. Thus, the role of senescent cells in kidney disease highly depends on the type of injury, which needs further investigation. Importantly, a pilot study in patients with chronic diabetic kidney disease showed that administration of D+Q was efficiently reducing senescent cells, assessed by p16^{INK4a}, p21^{CIP1} and SASP levels. Furthermore, D+Q treatment decreased macrophage recruitment and the formation of crown-like structures, particularly of adipose tissue in diabetes and obesity [126].

Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are liver diseases that present chronic inflammation, biliary damage, liver fibrosis and ultimately organ failure [127]. Currently, a liver transplant is the only effective treatment for PBC (whereby the age at diagnosis is usually 40–60 year olds) and PSC (30–40 year olds) [127–129]. Senescent cells have been detected in chronic liver diseases [130–132], and senescence markers correlate with fibrosis levels and are associated with disease severity in patients with PSC and PBC [133,134]. Senescence of cholangiocytes was confirmed with $p16^{INK4a}$, $p21^{CIP1}$ and SA-β-gal markers (among others) in both PSC and PBC [47,132,135,136]. The SASP components of senescent cholangiocytes increase fibrosis and inflammation *in vitro* [137] and *in vivo* [47] and induce bystander senescence in hepatic parenchyma in PSC [47,132]. Similarly, the SASP components CCL2 and CX3CL1 promote infiltration of T cells and macrophages in PBC [133,138]. Targeting of senescent cells with fisetin in $Mdr2^{-/-}$ mice, an experimental model of PSC, has beneficial effects on disease progression [139]. Similarly, downregulation of $p16^{INK4a}$ expression in $Mdr2^{-/-}$ mice using morpholino technology *in vivo* or by crossing them with $p16^{INK4a}$ KO or INK-ATTAC mice, restores fibrosis, intrahepatic bile duct mass and SASP levels [139,140]. The anti-apoptotic BCL-xL pathways are upregulated by senescent cholangiocytes, and their Inhibition via a BH3 mimetic or a Bcl-xL-specific siRNA reduces senescence and fibrosis [141]. Importantly, induction of senescence, and in particular in cholangiocytes of the $K19Cre^{ERT2}Mdm2^{-/-}$ mouse model, recapitulates a phenotype observed in PSC and PBC, suggesting that senescent cholangiocytes might be a driver rather than a consequence of the biliary disease [47].

Neurodegeneration: Old age is the most important determinant for the development and progression of neurodegenerative diseases [142], including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and multiple sclerosis. Despite differences in pathology, neurodegenerative diseases are typically characterized by a chronic and progressive loss of neurons and their synaptic connections, resulting in gradual functional decline [143,144]. In addition to their tight link with age, most neurodegenerative diseases also share similar etiopathogenic mechanisms, such as neuroinflammation, DNA damage, altered proteostasis, oxidative stress and mitochondrial dysfunction [145], all of which are mechanisms that have long been associated with senescence [146]. Many studies now show that different cellular populations residing in the nervous system acquire a senescence-like phenotype with the expression of senescence hallmarks in both *in vitro* and *in vivo* models of neurodegenerative disease. These populations include astrocytes, microglia, oligodendrocytes, neuronal stem cells and (rather surprisingly) the post-mitotic neuronal population (for extended reviews on this topic, see [145,147,148]).

Removing senescent cells or restoring senescent cells to their normal phenotype may be a potential therapeutic strategy for neurodegenerative diseases. Senescent cells clearance is sufficient to prevent pathology and cognitive decline in mouse models of Alzheimer's disease [149–151]. Similarly, senescent cells depletion in a Parkinson's disease mouse model induced by paraquat administration ameliorates disease progression [152]. Although clearing senescent cells has not been linked experimentally to ALS progression, some studies point out that targeting SASP improves disease phenotype in the ALS mouse model $SOD1^{G93A}$ [153,154]. These beneficial effects have also been shown in other neurodegenerative diseases and age-related conditions of the nervous system. For example, clearance of senescent cells ameliorates chemotherapy-related peripheral neuropathy [155]. Similarly, removing senescent cells from ageing mice decreases activation of microglia and SASP levels, resulting in alleviation of both age-related brain inflammation and cognitive impairment [156].

Cardiovascular diseases (CVDs) are a leading cause of death worldwide, particularly in the aged populations [157]. Senescent cells accumulation in the cardiovascular system can impair heart function and promote age-related CVDs, including atherosclerosis, myocardial infarction, valvular heart disease and heart failure (HF) [158]. All cardiac cell types can undergo senescence in old age or cardiac disease [36,159]. Senescence of old human and murine cardiomyocytes (which rarely divide) may be induced by persistent DNA damage at telomeres, which is driven by mitochondrial dysfunction and occurs independently of cell division and telomere length. Length-independent telomere damage in cardiomyocytes activates senescence pathways, such as $p16^{INK4a}$ and $p21^{CIP1}$, and secretion of an atypical SASP, including growth differentiation factor 15 (GDF15), TGFβ2 and endothelin 3 (EDN3). In contrast, senescence of dividing neonatal cardiac cells is characterized by cell cycle arrest, DNA damage, upregulation of $p16^{INK4a}$, $p21^{CIP1}$ and p53 and secretion of typical SASP components (e.g. IL-6, CXC-chemokine ligand 1 and 2 (CXCL1, CXCL2), TNFα, vascular endothelial growth factor (VEGF), MMP1, MMP3 and plasminogen activator inhibitor 1 (PAI1)). SASP components have paracrine effects on surrounding cardiac cells, promoting fibroblast

activation and differentiation, diminished angiogenic potential, endothelial cell dysfunction and cardiomyocyte hypertrophy [159]. Atherosclerotic plaques contain senescent endothelial cells, VMSC and immune cells [160,161], and targeting senescent cells in advanced cases suffice to reduce plaque size [162]. Global clearance of senescent cells in hearts with cardiac hypertrophy using INK-ATTAC mice or the senolytic navitoclax reduces fibrosis and cardiac hypertrophy; however, the associated mechanisms are still poorly understood [38,159]. Senescence may also play a direct role in chemotherapy-induced and radiation-induced cardiomyopathy [163]. A correlation between cellular senescence and the pathophysiological pathways of HF has been shown, suggesting that senescence suppression has therapeutic potential for treating and even preventing HF [164,165]. After myocardial infarction, a beneficial role for cellular senescence has been reported [41]; however, negative effects have been also shown [45]. In summary, although targeting senescent cardiovascular cells is a potential strategy to prevent cardiac failure in ageing and CVDs (reviewed in detail in [166,167]), a better understanding of the involved mechanisms is needed.

Glaucoma designates progressive optic neuropathies characterized by degeneration of retinal ganglion cells (RGCs) and changes in the optic nerve, which may progress to blindness. Multiple genetic and environmental risk factors have been related to the development and progression of glaucoma, including increased intraocular pressure (IOP) (reviewed in [168]) and ageing [169]. An age-related increase in resistance to trabecular outflow of aqueous humor is largely responsible for high IOP in elderly people [168]. Senescence can contribute to glaucoma establishment, with increased SA-β-gal activity in the cornea, sclera, and outflow of patients with primary open-angle glaucoma (POAG) [170] and increased expression of the senescence-related gene *Cdkn2a* in the retina of a rat model of glaucoma [171]. In line with this, the increased expression of $p16^{INK4a}$ in a mouse model of increased IOP results in enhanced senescence of RGCs and their later degeneration, while a lack of the $p16^{INK4a}$ gene in $p16^{INK4a}$ KO mice protects RGCs from cell death caused by elevated IOP [172]. RGC senescence plays a detrimental role in a phase before RGC death; for instance, overexpression of the Tank binding kinase 1 (TBK1) leads to $p16^{INK4a}$ upregulation and senescence acquisition of RGCs in a preclinical model of acute IOP [173]. Early selective removal of senescent cells in the p16-3MR mice improves survival and function of RGCs after increased IOP [174], suggesting senolysis as a potential tool for treating glaucoma.

Diabetes mellitus is an autoimmune disease. Type 1 diabetes (T1D) is characterized by a loss of pancreatic β-cells, leading to insulin deficiency and disruption of glucose metabolism, with a typical onset in the early decades of life. In contrast, T2D is characterized by normal plasma insulin levels and resistance to insulin in peripheral organs, and its incidence increases with age [175]. Senescent cells accumulation in ageing is involved in the pathogenesis of T2D through direct impact on pancreatic β-cell function, SASP-mediated tissue damage and adipose tissue dysfunction. Moreover, cellular senescence can be promoted by the diabetic microenvironment, such as by high circulating glucose levels, perturbations of the growth hormone axis and upregulated ceramide synthesis [176–178]. High glucose levels induce senescence in endothelial cells, renal mesangial cells, adipose-derived stem cells and fibroblasts; however, the mechanisms behind this are not clear [179–181]. High glucose-induced senescence contributes to diabetic complications, including nephropathy, retinopathy, vasculopathy and cardiovascular disease, suggesting that senescent cells may initiate and exacerbate diabetes [182]. In non-obese diabetic (NOD) mice, senescent β-cells elimination with senolytics interrupts immune-mediated β-cell destruction and prevents T1D by preserving β-cell mass [183], while senolysis assays in mice have also shown beneficial effects on T2D, for instance by improving glucose metabolism and β-cell function in an aged model [178]. However, even though senolytics are promising tools in preclinical models, further studies are required to test their benefits in translational trials [184].

Intervertebral disc degeneration (IDD): Age-related degeneration of the intervertebral discs is the most common cause of joint-related disability in the elderly [185,186]. The hallmark of IDD is the loss of proteoglycan (PG) in ECM, which leads to reduced capacity to resist compressive forces [187]. Senescence markers, such as $p16^{INK4a}$ and SA-β-gal, are increased in discs of older persons and correlate with degeneration grades of disc tissue [188–190]. Senescent cells can interfere with matrix homeostasis and promote loss of PG, thereby fostering disc degeneration [191]. Senescence in this condition can be triggered by various causes, including DNA damage as well as inflammatory and oxidative mechanical and nutritional stresses (reviewed in [186]). Targeting senescent cells with the senolytics D+Q or the HSP90 inhibitor 17-DMAG improves PG content and extends the healthspan in a progeroid $Ercc1^{-/\Delta}$ mouse model [21,192]. Beneficial effects of senescent cell elimination have also been observed in physiologically aged p16-3MR or wild-

type mice: long-term treatment with GCV or the senolytics D+Q, respectively, increases PG content, reduces matrix protease MMP13 levels, reduces inflammation and improves histological disc features [193,194]. Similarly, $p16^{INK4a}$ KO mice maintain disc heights and have higher water content and glycosaminoglycan levels and reduced levels of MMPs upon tail suspension (a procedure used to induce IDD in mice), as compared to wild-type mice [188]. Moreover, elimination of $p16^{INK4a}$-expressing cells in the intervertebral disc, using Acan-CreERT2-$p16^{INK4a}$ KO mice, leads to lower SASP levels, improved matrix homeostasis, and reduced apoptosis [195]. These studies suggest that senescence is a driver of age-related IDD. Targeting senescent cells is, therefore, an attractive approach to treating IDD in a rapidly growing aged population.

Osteoporosis is a bone disorder characterized by fragility and deteriorated bone tissue [196]. Bone formation and resorption are dynamic processes carried out by osteoblasts and osteoclasts, respectively, and their imbalance in favour of resorption leads to osteoporosis. Under normal conditions, bone marrow stromal cells (BMSCs) efficiently differentiate into osteoblasts, chondrocytes and adipocytes. However, this ability is impaired in old age, in part due to their senescence entry and aberrant signalling, affecting bone formation with a risk of developing osteoporosis [197–199]. Markers of senescence are also significantly higher in B cells, T cells, myeloid cells, osteoblast progenitors, osteoblasts and osteocytes in the bone microenvironment with ageing [200]. Elimination of senescent cells in old INK-ATTAC mice (either genetically or with senolytics) leads to increased bone mass and bone strength. Interestingly, the SASP increases osteoclastogenesis by promoting the survival of monocyte osteoclast progenitors. JAK1/2 inhibition, which can suppress the SASP, prevents age-related bone loss, suggesting that the SASP disrupts the proper control between bone resorption and formation in ageing [201,202]. Likewise, inhibiting oxidative stress by antioxidant strategies (e.g. using pyrroloquinoline quinone or *N*-acetylcysteine) can reduce senescence and SASP levels, promote bone formation, limit bone resorption and prevent bone loss [203,204]. The compound tetramethylpyrazine (TMP) can be used to treat and prevent glucocorticoid-induced osteoporosis in mice, and its administration locally into bone marrow reduces the senescent phenotype observed in this niche, attenuating bone loss, and improving metabolic microenvironments in aged mice [205]. Similarly, targeting senescent cells with senolytics or genetically in INK-ATTAC mice efficiently alleviates radiation-induced bone loss [206,207]. However, genetic elimination of senescent cells does not improve bone mass or strength in old p16-3MR mice. Although GCV treatment in this mouse model abrogates the age-associated increase in osteoclastogenesis, it does not reduce $p16^{INK4a}$ levels in osteocytes, the major senescent population in the bone microenvironment [208]. Albeit significant research has been done in the field, it is not clear which specific populations are targeted by the genetic or pharmacological approaches presented here, or which populations (and which SASP components) are responsible for any observed beneficial effects.

Osteoarthritis is a disease of the synovial joints, characterized by cartilage degradation, thickening of the subchondral bone, osteophyte formation, inflammation, degeneration of ligaments and hypertrophy of the joint capsule. Ageing is one of the most common risk factors for developing osteoarthritis, leading to pain and disability in the elderly [209]. The number of senescent chondrocytes and synovial fibroblasts increases with age, as shown by $p16^{INK4a}$ expression and SA-β-gal activity [210,211]. Cellular senescence, arising from ageing as well as from trauma, has been implicated in osteoarthritis (reviewed in [212]). Patients with osteoarthritis present high levels of pro-inflammatory cytokine IL-6 and extracellular vesicles (EVs) in synovial fluids [213,214]. Strikingly, parabiosis experiments showed that old mice that shared circulation with young mice present less severe osteoarthritis, suggesting that circulating factors might protect from or contribute to disease progression [215]. Clearance of naturally occurring $p16^{INK4a}$ senescent cells ameliorates age-related cartilage degeneration, as shown by cartilage thickness, increase proteoglycans levels, and a normalized Osteoarthritis Research Society International score [216]. Transplantation of senescent fibroblasts into knee joints induces cartilage erosion, formation of osteophyte, pain and mobility loss in mice, suggesting that accumulation of senescent cells with ageing might not just predispose but drive osteoarthritis [217]. Similarly, senescent chondrocytes inhibit proliferation and chondrogenesis, while promoting apoptosis and senescence, in BMSCs *in vitro* and *in vivo*. A pro-senescent environment induces overall delayed cartilage regeneration, and this can be partially rescued by senolytic navitoclax treatment in a cartilage-defective rat model [218]. Likewise, Evs derived from patients with osteoarthritis induce paracrine senescence in chondrocytes, leading to decreased proteoglycan production [214]. Targeting senescent cells with the senolytic UBX0101, which inhibits the interaction between p53 and MDM2 and prevents p53 degradation, results in reduced EV numbers in

chondrocytes from patients with osteoarthritis *in vitro* [214]. Furthermore, elimination of senescent cells with local injection of the GCV or UBX0101 senolytic results in ameliorated post-traumatic osteoarthritis in p16-3MR mice, as observed by reduced proteoglycan loss and pro-chondrogenic environment [216]. Other senolytic and senomorphic (i.e. targeting the SASP) strategies have been described as potential tools to induce a pro-regenerative niche in cartilage, including fenofibrate (a PPARa antagonist), metformin, and rapamycin [219–222]. Indeed, the senolytic UBX0101 has been recently tested in phase I clinical trial, where dose-dependent improvements in pain and function were observed [223]. However, other studies are yet to be evaluated to determine the potential use of senolytics for osteoarthritis in patients.

Cellular senescence in reprogramming

Differentiated cells (including aged cells) can be reprogrammed to a pluripotent cell state by ectopic expression of the transcription factors Oct3/4, Sox2, Klf4 and c-Myc ('OSKM'), which then allows subsequent differentiation to the desired cell type [224]. Induction of a pluripotent state *in vitro* reverts age-related cellular changes, as shown by extended telomeres, and rejuvenated mitochondrial metabolism transcriptomic profiles. Importantly, these improvements are maintained after the re-differentiation of these cells [225]. In naturally aged mice or mouse models of premature ageing, transient OSKM expression prolongs longevity and restores tissue regeneration in the pancreas and skeletal muscle after injury [226,227]. Similarly, OSKM induction leads to de-differentiation of cardiomyocytes in heart of adult mice, while partial reprogramming in early infarcted myocardial tissue ameliorates damage and improves cardiac function [228]. In OSKM-inducible transgenic mice, partial reprogramming reduces fibrosis and improves healing in cutaneous wounds, while avoiding tumour formation [229].

Reprogramming by OSKM is very inefficient because of cell-intrinsic barriers, which are activated by cell damage and mediated by the tumour suppressors p53, $p16^{INK4a}$, and $p19^{ARF}$, leading to a stable cell cycle arrest [230,231]. These stress response mediators can also induce cell senescence. The effects of senescence on reprogramming are still ambiguous, as cell-intrinsic and non-cell–extrinsic senescence can have a distinct impact on the process of reprogramming [232]. In the initial reprogramming steps, senescence acts as a barrier by preventing proliferation through upregulation of the *Ink4*/*Arf* locus and activation of p53 [230,232]. However, senescent cells are needed for the efficient *in vivo* reprogramming of nearby cells [233]. Reprogramming is blunted in *Ink4a*-null mice that do not activate an efficient senescent response upon OSKM induction, showing that *Ink4a* is critical for senescence-induced paracrine stimulation of reprogramming [233]. Furthermore, *in vivo* reprogramming is more efficient when combined with tissue injury and in ageing, as both conditions enhance cellular plasticity while inducing senescence, thus favouring reprogramming [99,234]. The mechanism behind this relies on the inflammatory cytokine IL-6, which provides crucial paracrine signals to enhance reprogramming through the *Ink4*/*Arf* locus [231,234]. When IL-6 or its downstream kinase effector is blocked, the efficiency of *in vivo* reprogramming is significantly reduced. Thus, cellular senescence alters the tissue microenvironment and promotes de-differentiation and plasticity of surrounding cells through the SASP, which is needed for efficient tissue repair through a non-cell autonomous mechanism. This cellular plasticity favours OSKM reprogramming in neighbouring cells [235].

Cellular reprogramming needs to be further dissected before it can be used in rejuvenating and regenerative therapies, as there are potential risks of the *in vivo* expression of OSKM factors, such as cellular transformation. Additionally, it is still not clear whether the ameliorated age-related effects are temporary or persist over time [227]. The relationship between senescence and reprogramming requires additional insight, as senescence is a hallmark of ageing and its effects on cellular reprogramming are ambivalent. Nevertheless, reprogramming technology is a powerful tool with a high potential to develop therapeutic strategies for tissue regeneration and amelioration of ageing hallmarks, with possible uses in regenerative medicine [236].

Concluding remarks and future directions

Senescence is an extremely heterogeneous cell state. Not only do the molecular and transcriptomic phenotypes and the SASP vary among the cell populations, triggers and contexts but also the role itself of senescent cells is heterogeneous. Paradoxically, the field of cellular senescence is reigned by a generalized rule on the role of cellular senescence: transient senescence is considered beneficial for repair after an acute injury, while chronic senescence is linked to its detrimental role in ageing and age-related diseases. However, as we show in this review, this is not always the case. Senescent cells can induce fibrosis and delay regeneration, for instance for bone, liver and kidney repair

after acute injury; conversely, elimination of senescent cells in old age and disease can also be deleterious, for instance in the aged liver and CKD [46,50,57,86,124]. These studies indicate that the complex state of cellular senescence cannot be reduced to a few simple rules and highlight the difficulty of timely senescence ablation interventions. Rather, the contributions of senescent cells should be studied in an in-depth way for each context.

Because ageing and age-related diseases share some features, including the accumulation of senescent cells, it is difficult to uncouple whether senescence is a trigger or a consequence of disease and/or ageing. Some studies suggest that senescent cells can drive disease onset and not merely artifacts of its progression [195]. Moreover, amelioration of disease may be related to both systemic and tissue-specific elimination of senescent cells. A few studies have demonstrated that senescent cells aggravate the disease state at strict locations. Thus, different strategies with a more specific, directed focus are needed to uncouple the roles of senescent cells in ageing and disease when they converge, as is the case of almost all chronic diseases.

An unsolved issue of general targeting senescent cells (which has mainly been performed with senolytics and genetic approaches in a very broad way) is that it may underestimate or camouflage diverging roles of distinct senescent populations. For instance, the SASP produced by different senescent cells during liver regeneration has been reported to have both beneficial and detrimental roles [30,46]. Additionally, distinct targeting strategies do not reproduce the same outcome, such as the FOXO4-DRI peptide vs GCV in p16-3MR mice for AKI, and D+Q vs GCV treatment for osteoporosis. These studies suggest that different senescent populations with distinct roles in AKI and osteoporosis might be targeted by these treatments [49,50,201,208]. Thus, the contribution of distinct cell types must be established in each scenario. Moreover, we should keep in mind that many of the so-called SASP factors can also be pro-inflammatory, anti-inflammatory or tissue remodelling signals secreted by non-senescent cells. Therefore, without a transcriptional analysis at the single-cell level, it will be hard to distinguish between different senescent cell types and non-senescent cells with the overlapping transcriptional program. New mouse models to induce genetic elimination of particular senescent cell types, or cell type-specific senolytics, should be generated and tested to elucidate these points.

Finally, systemic administration of senolytics could have adverse effects on the organism. Although natural senolytics (e.g. curcumin analogues, quercetin and fisetin) seem to have lower toxicity than more specific targeting ones (e.g. inhibitors of the Bcl-2 family proteins, inhibitors of Hsp90 and deacetylase and UBX101 or FOXO4-DRI interfering peptide), they are generally less potent. However, almost all the targeting senolytics have on-target and/or off-target toxicities. For instance, navitoclax is one of the most potent and broad-spectrum senolytic agents identified to date, but studies show that it (and other small molecular inhibitors of the Bcl-2 family proteins) can induce platelet apoptosis, resulting in thrombocytopaenia [237–239]. This may substantially limit the clinical use of some senolytics as anti-ageing agents, as older people are more susceptible to adverse drug effects than younger ones. Indeed, in patients, there is a paucity of data on the efficacy, safety and side effects of senolytic therapy [240]. Studies aiming to reduce the toxicity of known targeting senolytics are needed [241,242]. However, recent studies propose novel molecules and strategies to induce senolysis. Suda and colleagues showed that vaccination against glycoprotein nonmetastatic melanoma protein B (GPNMB), which appeared upregulated in senescent vascular endothelial cells, attenuated senescence in adipose tissue, and improved systemic metabolic abnormalities in mice fed on a high-fat diet [243]. Similarly, the urokinase-type plasminogen activator receptor (uPAR) is broadly induced in cell surface during senescence, and uPAR-specific CAR T cells efficiently ablate senescent cells *in vivo* [244]. Another novel and targeted strategy against senescent cells were described by Johmura et al. [245]. In their study, the authors showed that due to the lowered pH in lysosomes of senescent cells, the kidney-type glutaminase (KGA) enzyme expression was upregulated, resulting in enhanced glutaminolysis. By inhibiting KGA-dependent glutaminolysis in aged mice, senescent cells were specifically eliminated and age-associated organs dysfunction ameliorated. Finally, it has been recently shown that bromodomain and extra-terminal domain (BET) family protein inhibitors (BETi) have a senolytic effect by attenuating non-homologous end joining (NHEJ) and upregulating autophagic gene expression [246]. Treatment of obese mice with the BETi compound ARV825 eliminates senescent hepatic stellate cells and reduces liver cancer development [246].

Another limitation of some senolytics is that although they extend life and healthspan in mice, they do not prevent important features of ageing. Although reprogramming could be (as a concept) a more efficient solution than rejuvenating and restoring impaired tissue regeneration, safety reasons blunt its feasibility for humans at the present.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

All authors contributed to the writing, editing, discussion and review of the manuscript.

Data availability statement

Data used in this review article are derived from public domain resources. Data sharing not applicable.

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