Cellular senescence: from physiology to pathology

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Abstract | Recent discoveries are redefining our view of cellular senescence as a trigger of tissue remodelling that acts during normal embryonic development and upon tissue damage. To achieve this, senescent cells arrest their own proliferation, recruit phagocytic immune cells and promote tissue renewal. This sequence of events — senescence, followed by clearance and then regeneration — may not be efficiently completed in aged tissues or in pathological contexts, thereby resulting in the accumulation of senescent cells. Increasing evidence indicates that both pro-senescent therapies and antisenescent therapies can be beneficial. In cancer and during active tissue repair, pro-senescent therapies contribute to minimize the damage by limiting proliferation and fibrosis, respectively. Conversely, antisenescent therapies may help to eliminate accumulated senescent cells and to recover tissue function.

More than five decades ago Leonard Hayflick and Paul Moorhead discovered that normal human fibroblasts have a finite proliferative capacity in culture, a phenomenon that they named 'cellular senescence', and speculated that it could be an underlying cause of ageing[1]. This property of normal cells is in contrast to the behaviour of cancer cells, which possess an indefinite capacity to proliferate. Today, we know that the phenomenon observed by Hayflick and Moorhead in fibroblasts reflects one particular type of cellular senescence produced by the loss of telomeres after extensive proliferation in the absence of endogenous telomerase activity. The various stimuli and cellular contexts that induce senescence in multiple physiological and pathological processes are becoming increasingly appreciated, and this is the main focus of this Review.

Senescent cells differ from other non-dividing cells (such as quiescent or terminally differentiated cells) by several markers and morphological changes (BOX 1). These features include the absence of proliferative markers, senescence-associated β-galactosidase (SAβGAL) activity, expression of tumour suppressors and cell cycle inhibitors, and often also of DNA damage markers, nuclear foci of constitutive heterochromatin and prominent secretion of signalling molecules. Although none of these markers is on its own completely specific or universal for all senescence types, there is ample consensus that senescent cells express most of them.

Work during the past decade has convincingly demonstrated that senescence has beneficial and detrimental roles (as detailed in this Review). In general, transient induction of senescence followed by tissue remodelling is beneficial, because it contributes to the elimination of damaged cells. Conversely, persistent senescence or the inability to eliminate senescent cells is detrimental. In our view, the general biological purpose of senescence is to eliminate unwanted cells, which is conceptually similar to apoptosis. Senescence and apoptosis are the most important mechanisms to eliminate damaged cells. This is particularly relevant in cancer and ageing, which are both characterized by the accumulation of severe cellular damage. In agreement with this, senescence is a crucial barrier against cancer progression (see below), and senescent cells accumulate with ageing (BOX 2).

We begin by describing the most important molecular mechanisms underlying cellular senescence. Then, we focus on the most recent findings that implicate senescence both in normal physiology and in a remarkably wide range of pathological disorders. This is followed by the proposal of a unified model for senescence as a tissue remodelling mechanism. Finally, we review current emerging pro-senescent and antisenescent therapies, and their therapeutic potential for cancer, chronic disorders and ageing.

Mechanisms of senescence

The number of stimuli that induce senescence is constantly increasing and the mechanisms involved have been extensively reviewed[2–8]. These stimuli are signalled through various pathways, many of which activate p53 (encoded by *TP53* in humans and by *Trp53* in mice), and essentially all of them converge in the activation

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Box 1 | **Morphology of senescent cells and biomarkers**

Cellular senescence in *in vitro* culture is usually accompanied by morphological changes; in general, cells become large, flat, vacuolized and, occasionally, multinucleated. However, *in vivo* senescent cells retain the normal morphology dictated by tissue architecture. A collection of markers, when used in combination, are generally accepted to define senescence both in cultured cells and in tissues[167]. The most widely used assay for senescence is the histochemical detection of β-galactosidase activity at pH 6.0, which is known as senescence-associated β-galactosidase (SAβGAL)[168]. This activity is based on the increased lysosomal content of senescent cells, which enables the detection of lysosomal βGAL at a suboptimal pH (pH 6.0)[169], and this probably reflects the increased autophagy occurring in senescent cells together with an enlargement of the lysosomal compartment[170]. A limitation of the SAβGAL assay is the need to use fresh or frozen samples. However, it has been recently reported that senescent cells are also positively stained with Sudan Black B, which detects the complex lysosomal aggregate known as lipofuscin, and it has the advantage of being applicable to formalin-fixed and paraffin-embedded tissue samples[171]. As cellular senescence is based on a stable cell cycle arrest, the absence of proliferative markers, such as Ki67 protein or 5-bromodeoxyuridine (BrdU) incorporation, is an essential condition to document senescence. Other canonical senescence markers comprise the most common mediators of senesence, including p16, ARF, p53, p21, p15, p27 and hypophosphorylated RB. Foci of heterochromatin are also a feature of some senescent cells and are known as senescence-associated heterochromatic foci (SAHF)[172,173]. These foci contain hallmarks of heterochromatin, such as trimethylation at Lys9 of histone 3 (H3K9me3), heterochromatin protein 1 homologue-γ (HP1γ) and macroH2A. Of note, SAHF are preferentially formed during oncogene-induced senescence but not during replicative senescence or upon ageing[174,175]. In addition, senescent cells secrete a number of extracellular factors, including transforming growth factor-β (TGFβ), insulin-like growth factor 1 (IGF1)-binding proteins, plasminogen activator inhibitor 1 (PAI1), and inflammatory cytokines and chemokines that can reinforce and propagate senescence in an autocrine and paracrine manner[39,42,45]. Other markers that are frequently present in senescent cells are decoy receptor 2 (DCR2; also known as TNFRSF10D) and DEC1 (also known as TNFRSF10C)[176]. A decrease in lamin B1 (LMNB1) levels has also been found to be a common feature of many types of senescence[177,178]. Finally, it is important to highlight that most of the above senescence markers have been validated *in vivo*, both in association with pre-malignant tumours[4] and in association with developmental, physiological and pathological processes (as discussed in this Review).

of the cyclin-dependent kinase (CDK) inhibitors p16 (also known as INK4A; encoded by *CDKN2A*), p15 (also known as INK4B; encoded by *CDKN2B*), p21 (also known as WAF1; encoded by *CDKN1A*) and p27 (encoded by *CDKN1B*) (FIG. 1). The inhibition of CDK–cyclin complexes results in proliferative arrest, and the crucial component responsible for the implementation of senescence is the hypo-phosphorylated form of RB[9]. In addition to multiple senescence triggers and senescence-activating pathways, it is conceivable that the mechanisms that ultimately lead to senescence may also vary depending on the cell type and conditions. Similar to apoptosis, for which several subtypes of apoptotic cell death have been recently defined[10], future studies may define senescence subtypes. In this section, we briefly summarize the main mechanisms involved in 'damage-induced senescence', which includes various subtypes, such as 'replicative senescence', 'DNA-damage-induced senescence', 'stress-induced senescence' and 'oncogene-induced senescence'.

Telomere shortening and DNA-damage response. Telomeres function as molecular clocks that keep a record of the replicative history of primary cells[11]. In particular, telomere 'erosion' through consecutive cell divisions that fail to maintain telomere length can result in critically short telomeres and a type of senescence known as 'replicative senescence' (REF. 2). The loss of telomeres is sensed by cells as a type of DNA damage and therefore triggers a DNA-damage response (DDR), which is similar to that produced by external DNA-damaging agents, such as ionizing radiation and chemotherapeutic drugs. In addition to telomere shortening, telomeres are particularly sensitive to external DNA damage[12,13] owing in part to the fact that telomeres, from yeast to humans, are poorly accessible to the DNA damage repair machineries[14]. The main mediators of the DDR are the DNA damage kinases ATM, ATR, CHK1 and CHK2, which phosphorylate and activate several cell cycle proteins, including p53 (REF. 2) (FIG. 1). In turn, phosphorylated p53 protein activates the expression of p21, which binds to and inhibits some CDK–cyclin complexes, particularly those involving CDK2.

CDKN2A *locus derepression.* Replicative senescence is also linked to the *CDKN2A* locus (also known as *INK4A* and *ARF*), which encodes two crucial tumour suppressors, p16 and ARF (FIG. 1). Whereas p16 is an inhibitor of CDK4 and CDK6, ARF regulates p53 stability through inactivation of the p53-degrading E3 ubiquitin protein ligase MDM2 (REFS 15,16). The *CDKN2A* locus is normally expressed at very low levels in young tissues but becomes derepressed with ageing[17]. Although the molecular mechanisms responsible for *CDKN2A* derepression are not completely understood, it is well established that it depends to a large extent on the loss of Polycomb repressive complexes[18,19]. Of note, DNA damage may reduce ARF protein levels by inducing its degradation[20,21].

Stress-induced senescence and reactive oxygen species. Levels of reactive oxygen species (ROS) increase after many different types of stresses, including chemotherapeutic drugs, loss of telomeric protective functions, DNA damage and oncogene activation[22,23]. The relevant role of oxidative stress to senescence is demonstrated by the fact that treatment with antioxidants delays or prevents senescence[24–26]. Mechanistically, high intracellular levels of ROS induced by the RAS–RAF–MEK–ERK cascade activate the p38 MAPK, which leads to increased transcriptional activity of p53 and upregulation of p21 (REF. 27) (FIG. 1).

Oncogene-induced senescence. Normal cells respond to the activation of many oncogenes by undergoing cellular senescence. Oncogene-induced senescence was originally observed when an oncogenic form of RAS was expressed in human fibroblasts[28]. The list of oncogenes able to induce senescence has since increased to about 50 oncogenes[5]. Similarly, loss of tumour suppressors can trigger senescence as exemplified by the losses of PTEN[29], NF1 (also known as neurofibromin)[30] or von Hippel-Lindau disease tumour suppressor (VHL)[31]. Importantly, it is well demonstrated that oncogene-induced senescence occurs *in vivo*, functioning as a brake during the early stages of tumorigenesis[4]. A general feature of oncogene-induced senescence is the derepression of the *CDKN2A* locus[15,16].

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Box 2 | **Senescence and ageing**

The idea that cellular senescence contributes to ageing stems from the initial report by Hayflick and Moorhead[1]. Indeed, senescent cells accumulate in some, but not in all, tissues in aged humans[168], monkeys[179] and mice[180]; the most prominent increases are observed in the skin, liver, lung and spleen[168,179,180]. The primary triggers of ageing-associated senescence probably consists of DNA damage, loss of telomere protective functions and derepression of the *CDKN2A* locus encoding p16 and ARF[3,181]. We propose that the central purpose of senescence is to initiate a sequence of processes that eliminate damaged cells and culminate in tissue regeneration (we refer to this sequence of events as senescence–clearance–regeneration). However, this beneficial process can be corrupted, particularly in aged tissues, by a combination of factors: on the one hand, clearance of senescent cells by the immune system may become impaired leading to a net accumulation of senescent cells, which may further aggravate tissue dysfunction through the senescence-associated secretory phenotype (SASP)[39,182]; on the other hand, senescence may not only affect differentiated cells but also stem and progenitor cells, thus limiting the regenerative capacity of tissues. The combination of inefficient clearance, excessive SASP and ineffective regeneration may explain the accumulation of senescence during ageing and its active contribution to some ageing phenotypes (FIG. 3). Importantly, the elimination of senescent cells in mice with high levels of constitutive damage (due to a hypomorphic mutation in *Bub1b* (budding uninhibited by benzimidazoles 1 homologue beta)) can rejuvenate some damaged tissues, such as muscle and fat, and protects from cataracts[126]. Therefore, the elimination of senescent cells is a promising strategy to reduce chronic systemic inflammation and to rejuvenate tissues[6,183]. In summary, senescence is a response that is primarily designed to eliminate damaged cells; however, with advancing age, the full sequence of senescence–clearance–regeneration is not entirely accomplished and senescence may become part of the problem rather than its solution. As a result of this duality, senescence is considered an example of 'antagonistic pleiotropy' (REF. 184) and it has been categorized as an 'antagonistic' hallmark of ageing[181].

In addition, this type of senescence may also induce a robust DDR owing to the DNA damage that is caused by aberrant DNA replication[32,33] and/or ROS[22,23] (FIG. 1). The relative importance of these mechanisms (p16, ARF or DDR-induced p53) varies across cell types. For example, in the case of PTEN loss, DNA damage does not seem to have an important role in senescence[29]. Also, in mice the ARF–p53 pathway is a crucial activator of oncogene-induced senescence[34], whereas in humans the DDR–p53 pathway seems to have a more important role than the ARF–p53 pathway[35]. Finally, p16 plays a modest part in promoting senescence in mice but is prominent in human cells[36].

Senescence-associated secretory phenotype. Senescent cells implement a complex pro-inflammatory response known as senescence-associated secretory phenotype (SASP)[36–39] (FIG. 1). The SASP is mediated by the transcription factors nuclear factor-κB (NF-κB) and CCAAT/enhancer-binding protein-β (CEBPβ), and includes the secretion of pro-inflammatory cytokines (interleukin-6 (IL-6) and IL-8), chemokines (monocyte chemoattractant proteins (MCPs) and macrophage inflammatory proteins (MIPs)), growth factors (transforming growth factor-β (TGFβ) and granulocyte–macrophage colony-stimulating factor (GM-CSF)) and proteases[40–42]. The secretion of these and similar proteins by senescent cells causes inflammation and, at least in some cases, it may be pivotal for the clearance of senescent cells by phagocytosis[43,44]. SASP components, most notably TGFβ, can also trigger senescence in neighbouring cells in a paracrine manner, through a mechanism that generates ROS and DNA damage[45–47]. Therefore, the SASP has powerful autocrine and paracrine activities, which suggests that senescence creates an inflammatory microenvironment that may lead to the elimination of senescent cells.

Paracrine
Refers to a mode of signalling in which the cell responding to a signalling molecule is near the cell secreting the molecule.

Autocrine
Activation of cellular receptors by ligands produced by the same cell.

Senescence in development and physiology

The role of senescence has been mostly restricted to contexts of cellular damage or stress. This view, however, has been recently expanded by the identification of senescence in a large number of embryonic structures and in some specialized normal adult cells (FIG. 2; TABLE 1).

Senescence in the embryo. The surprising finding that senescence occurs during development is mostly based on the analysis of mouse embryos[48,49]. However, developmental senescence has also been observed in human[48], chicken[49] and quail[50] embryos, which suggests that this is a conserved feature of embryonic development across vertebrates (FIG. 2; TABLE 1). Examples of senescence-positive structures include the mesonephric tubules during mesonephros involution, the endolymphatic sac of the inner ear, the apical ectodermal ridge (AER) of the limbs, the regressing interdigital webs and the closing neural tube[48,49]. Indeed, senescence was detected at multiple additional embryonic structures, and we anticipate that more comprehensive analyses will reveal even more developmental processes associated with senescence.

In the particular cases of the mesonephros, the endolymphatic sac, the AER and the neural tube, the occurrence of senescence was substantiated not only by SAβGAL staining but also by other features of senescence (BOX 1), such as absence of proliferation (negative Ki67 staining and 5-bromodeoxyuridine (BrdU) incorporation), increased heterochromatin markers (histone 3 Lys9 trimethylation (H3K9me3) and heterochromatin protein 1 homologue-γ (HP1γ; also known as CBX3)) and increased expression of cell cycle inhibitors (p15, p21 and p27)[48,49]. Of note, DNA damage markers were absent in the structures undergoing developmental senescence. It should also be mentioned that SAβGAL-positive staining has been reported in the visceral endoderm of the early mouse embryo (embryonic day 5.5 (E5.5)–E7.5) and in the visceral endoderm layer of the yolk sac[51]. However, this SAβGAL signal was not associated with proliferative arrest or with other markers of senescence, and it was concluded that, in this particular case, SAβGAL is not indicative of senescence[51]. Altogether, cellular senescence seems to be common throughout the developing embryo, but it has distinctive features compared to damage-induced senescence.

Mechanisms of developmental senescence. Three particular developmental structures have been analysed in detail regarding senescence: the mesonephros (the transitory embryonic kidney), the endolymphatic sac (a filtering tube that regulates the pressure and composition of the endolymph of the inner ear) and the AER (a transient structure of specialized ectoderm marking the dorsoventral boundary of the limb bud)[48,49]. Remarkably, genetic analysis of senescence in these structures revealed an essential role of the cell cycle

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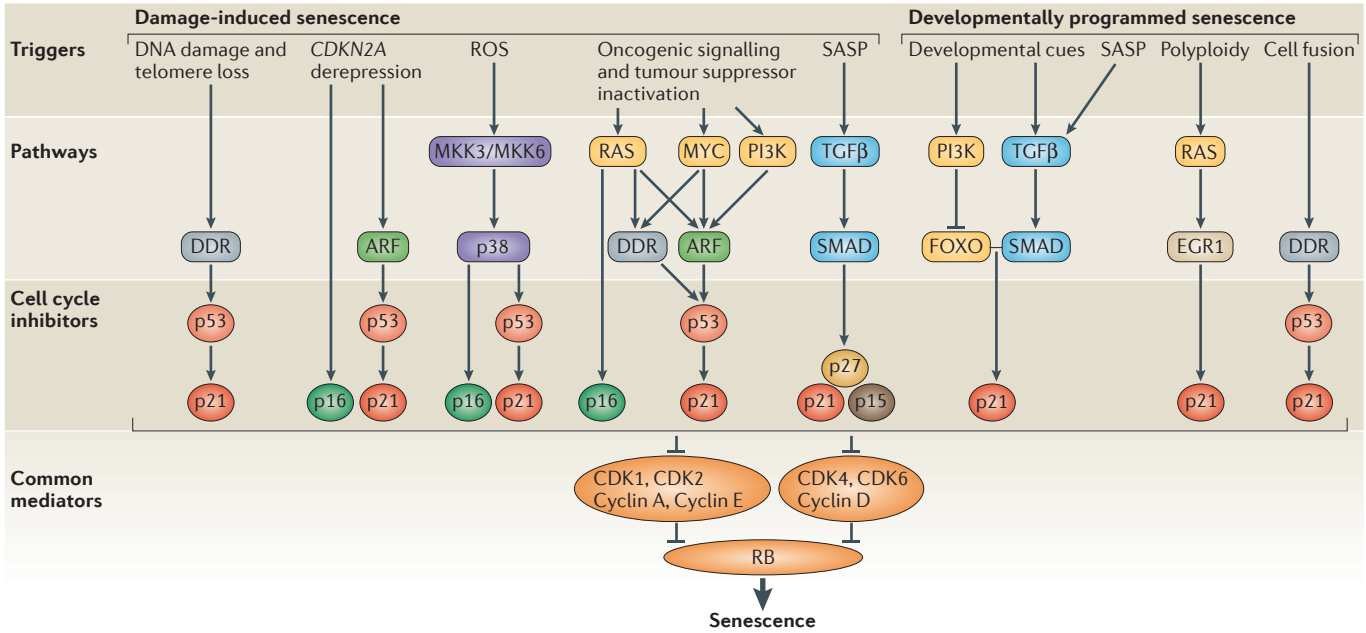

Figure 1 | **Molecular pathways of senescence.** Multiple stressors and damaging agents (triggers) activate signalling cascades (pathways) that converge on the activation of cell cycle inhibitors and the tumour suppressor RB. DNA damage agents and telomere loss activate the DNA-damage response (DDR), which directly activates p53 and its downstream transcriptional target p21. Many types of senescence are associated with the epigenetic derepression of the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) locus (encoding the cell cycle inhibitor p16 and the p53 activator ARF). Reactive oxygen species (ROS) activate p16 and p53 through the kinases MKK3 (also known as MAPKK3) and MKK6 (also known as MAPKK6), and their downstream kinase effector p38. Oncogenic signalling or loss of tumour suppressors activate p16 and p53 with the participation of the DDR and ARF. Transforming growth factor-β (TGFβ) is a notable component of the senescence-associated secretory phenotype (SASP) pathway, which upregulates the cell cycle inhibitors p21, p27 and p15 through the SMAD complex. Developmental cues induce senescence through p21 by inducing the PI3K and TGFβ pathways (see above). Polyploidization and cell fusion also upregulate p21 through the DDR and p53, and also by RAS-induced activation of the transcription factor early growth response protein 1 (EGR1). FOXO, forkhead box protein O.

inhibitor p21, as shown by the absence of senescence hallmarks (including SAβGAL, proliferative arrest and senescence-associated heterochromatin foci) in *Cdkn1a*-null embryos[48,49]. This dramatic effect was not observed in embryos lacking other cell cycle inhibitors, including *Cdkn2a*-null or even *Trp53*-null embryos.

The gene expression profiles of the senescent mesonephros and AER demonstrated gene expression changes that are characteristic of developmental pathways, particularly TGFβ, WNT and Hedgehog, and a secretory phenotype similar to the SASP, notably including fibroblast growth factor 4 (FGF4) and FGF8 (REFS 48,49). Mechanistic and genetic analyses of the mesonephros and endolymphatic sac have revealed that p21 upregulation and senescence is controlled by the TGFβ–SMAD and PI3K–forkhead box protein O (FOXO) pathways[48] (FIG. 1). Of note, these pathways also participate in damage-induced senescence in adult somatic cells (FIG. 1). In addition, the secretion of growth factors, such as FGF4 and FGF8, activates the ERK pathway in mesenchymal cells that are proximal to the AER and, in turn, mesenchymal cells signal back to the AER to maintain senescence[49].

Altogether, these studies suggest that developmentally programmed senescence is characterized by developmental cues that converge on p21.

Clearance, compensatory processes and morphological defects. There is compelling evidence showing that cells that undergo damage-induced senescence are often removed by immune-mediated clearance[43,44,52]. In the case of the regressing mesonephros and the AER, macrophages surround senescent cells at days E13.5–E14.5 (REFS 48,49). Interestingly, in the absence of *Cdkn1a*, the mesonephros is free of macrophages at day E14.5, thereby suggesting a causal connection between senescence and macrophage recruitment. Remarkably, however, both the mesonephros and the AER are eventually eliminated in *Cdkn1a*-null embryos in a process that involves compensatory apoptosis and late infiltration of macrophages[48,49].

The importance of apoptosis during embryonic development is well established[53]. However, inhibition of the apoptotic programme during development produces modest morphological defects restricted to imperforated vaginas and partial persistence of interdigital webs[54,55], which indicates the existence of compensatory mechanisms. Similarly, failure to undergo senescence activates a compensatory apoptotic programme[48,49], which again illustrates the robustness of embryonic development. It is therefore conceivable that senescence and apoptosis are interconnected, which enables their mutual compensation during development.

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The endolymphatic sac exemplifies another role of developmental senescence that is different from the elimination of transient structures. The epithelium of the endolymphatic sac is composed of different cell populations, with one of them identified by the expression of protein pendrin (an anion exchanger that is important for the function of the endolymphatic sac). Interestingly, senescence only affects a proportion of the epithelial cells and this occurs concomitantly with an expansion of pendrin-positive cells[48]. However, in *Cdkn1a*-null embryos, the increase of pendrin-positive cells is notably reduced, which indicates that senescence in this case regulates the relative ratios of different cell populations within the same structure. The absence of *Cdkn1a* results in numerous aberrant infoldings of the endolymphatic sac epithelium into the lumen, but these aberrant infoldings are eliminated at birth by macrophages independently of senescence.

Despite compensation by apoptosis, absence of senescence produces some morphological defects affecting a proportion of adult animals[48]. The Wolffian duct is a longitudinal canal that connects the mesonephric tubules with the kidney and the cloaca. In females, the Wolffian duct undergoes *Cdkn1a*-dependent senescence[48], and, during this process, it participates in the formation of the vagina (by guiding the Müllerian duct). Interestingly, a small proportion of *Cdkn1a*-null females present with vaginal septa, which is consistent with an abnormal function of the Wolffian duct in vaginal morphogenesis[48]. Consequently, at least in the case of the mesonephros and its derived structures, an impaired senescent programme results in morphological defects in the adult.

Physiological senescence in adult cells. Apart from embryonic development, senescence also occurs in a physiologically programmed manner in adult organisms. In particular, normal megakaryocytes[56] and placental syncytiotrophoblasts[57] undergo senescence as part of their natural maturation programmes. In the case of mouse and human megakaryocytes, senescence is characterized by SAβGAL activity, proliferative arrest and accumulation of HP1γ[56]. Interestingly, megakaryocyte senescence, similar to developmentally programmed senescence, is dependent on p21 but is independent of p16, p53 or p27 (REF. 56) (FIG. 1). Remarkably, megakaryocytes from myeloproliferative disorders, such as primary myelofibrosis, do not express p21 and do not undergo senescence, which suggests that senescence is physiologically relevant[56].

The human placenta shows marked SAβGAL activity at the syncytiotrophoblast in association with DNA damage markers, p16, p21 and p53 (REF. 57) (FIG. 1). This specialized structure consists of a large syncytium that covers the vascular embryonic villi and that is formed by the fusion of underlying cytotrophoblasts, which become themselves polyploid through a process of endoreduplication.

It is remarkable that these two cell types, megakaryocytes and syncytiotrophoblasts, are among the few mammalian cell types that undergo endoreduplication, which

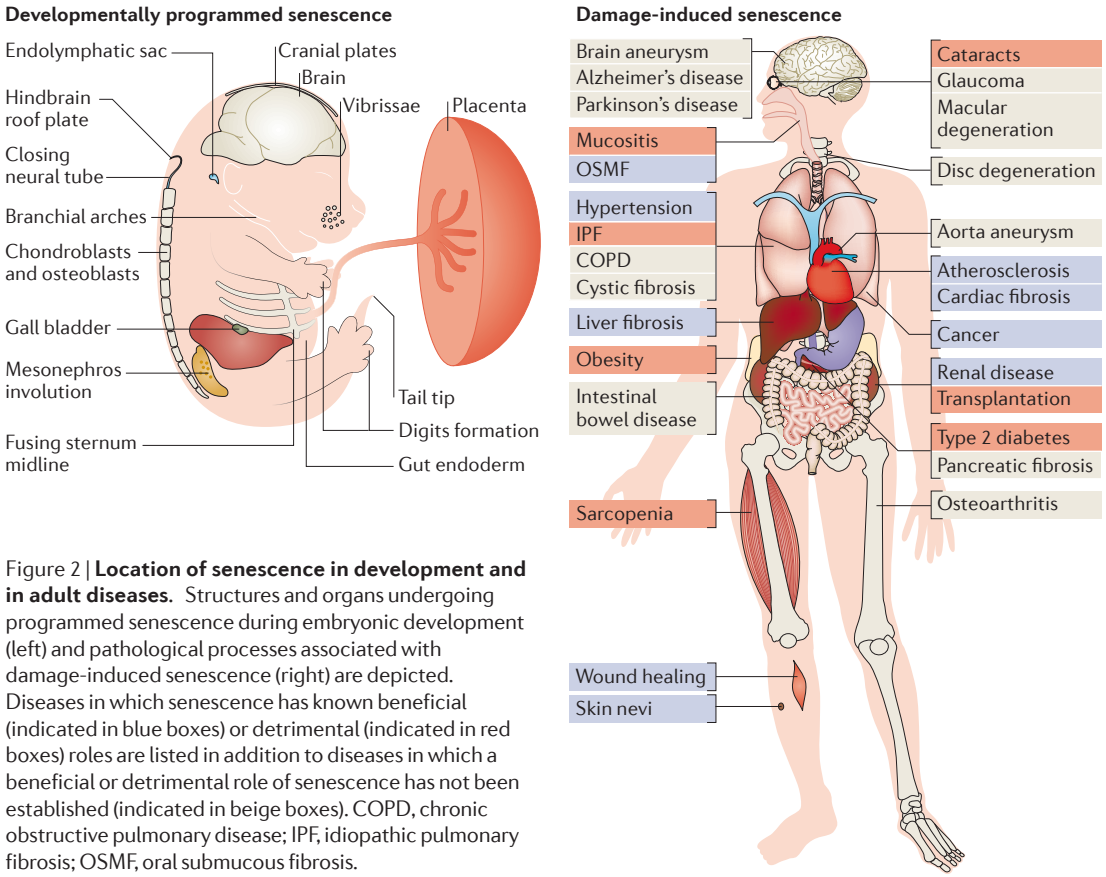

Figure 2 | **Location of senescence in development and in adult diseases.** Structures and organs undergoing programmed senescence during embryonic development (left) and pathological processes associated with damage-induced senescence (right) are depicted. Diseases in which senescence has known beneficial (indicated in blue boxes) or detrimental (indicated in red boxes) roles are listed in addition to diseases in which a beneficial or detrimental role of senescence has not been established (indicated in beige boxes). COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; OSMF, oral submucous fibrosis.

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Table 1 | **Developmental and physiological senescence**

Structures	Developmental stage*	Refs
Embryonic structures		
Branchial arches	Embryonic day 9.5 (E9.5) Chicken Hamburger–Hamilton stage 28 (HH28)	49
Eye	Chicken HH28	49
Gut endoderm	E9.5–E10.5	49
Apical ectodermal ridge (AER) of limbs	E9.5–E13.5	48,49
Tip of tail	E10.5–E17.5	48,49
Fusion of cranial plates	E10.5–E12.5	49
Pallium ventricular zone	E11.5	49
Cystic primordium (gall bladder)	E11.5	49
Neural tube	E11.5–E14.5 Chicken HH28	48,49
Endolymphatic sac of the inner ear	E12.5–E14.5 Human 9 weeks	48,49
Mesonephric tubules	E12.5–E14.5 Quail HH40 Human 9 weeks	48,50
Interdigital webs of limbs	E13.5–E14.5	48,49
Ventral and fusing sternum midline	E14.5	48,49
Vibrissae follicles	E14.5–E15.5	48
Vertebral osteoblasts (cervical region)	E17.5–E18.5	48
Vertebral chondroblasts (cervical region)	E17.5–E18.5	48
Adult cells		
Placental syncytiotrophoblasts	Adult	57
Megakaryocytes	Adult	56
Decidual natural killer cells	Adult	185

*Unless otherwise specified, stage refers to mouse embryonic development.

leads to poliploidy[58]. The occurrence of physiologically programmed senescence in two cases of natural polyploidy suggests that this could be the underlying cause. In support of this, fusion of various unrelated cell types, including cancer cells, also induces senescence[57]. These findings may also have a pathological correlate during infectious processes with fusogenic viruses, such as measles virus[57]. It is worth mentioning that mature osteoclasts, which are multinucleated cells formed by a process of cell fusion, also have SAβGAL activity[59]. However, further studies are necessary before concluding that osteoclasts constitute another case of physiological senescence.

Finally, natural killer (NK) cells are abundant at the maternal–fetal interface, where they contribute to a successful pregnancy by remodelling the maternal vasculature. Interestingly, decidual NK cells, as well as NK cells stimulated with fetal antigens, manifest hallmarks of senescence, including SAβGAL, DDR markers, HP1γ, p21 and SASP[185].

Beneficial effects of senescence in diseases

In addition to normal development and physiology, senescent cells have been associated with multiple pathological processes (FIG. 2; TABLE 2), in which senescence can have both beneficial and detrimental effects. The discussed pathologies are subdivided according to the current understanding of the role of senescence in each disease.

Senescence counteracts cancer progression. It is well established that the intensity of the oncogenic signalling flux progressively increases during the early stages of tumorigenesis until it reaches a threshold that activates the key tumour suppression pathways p16 and p53 (FIG. 1). When this happens, cell cycle inhibitors offset the oncogenic signalling and cells enter into senescence, which prevents the expansion of pre-cancerous cells. Accordingly, at the pathological level, senescence is detectable in the benign stage of tumorigenesis, which depending on the tissue type is known as adenoma, tumour *in situ*, intraepithelial neoplasia and others[4].

Compelling evidence exists that cells that undergo damage-induced senescence can be removed by immune-mediated clearance[43,44,52]. This is due in part to direct immune recognition of the senescent cells by T helper cells[52] and is also due to the recruitment of inflammatory phagocytic cells[43], which are probably attracted by SASP factors[37,39].

In addition, there is evidence for the therapeutic effect of pro-senescent therapies in already established malignant tumours. In particular, acute activation of p53 in hepatocellular carcinomas and sarcomas induces senescence, which is followed by tumour elimination[43,60]. Also, as discussed below in further detail, pharmacologic CDK4 inhibitors induce senescence in many cancer cells[61–63] and are showing promising activity in human clinical trials[64–66]. As a word of caution, however, DNA-damage-induced senescence that is associated with cyclical chemotherapeutic regimes may promote disease progression through SASP components, such as WNT16B[67].

Attenuation of liver fibrosis. Liver fibrosis, being a main precursor of cirrhosis, is characterized by the accumulation of fibrotic tissue and the concomitant loss of liver function. It is triggered by chronic liver damage associated with hepatitis virus infection, alcohol abuse or liver steatosis (fatty liver disease). During chronic damage, hepatic stellate cells (HSCs) become activated and abnormally proliferate as myofibroblasts (damage-activated fibroblasts). Eventually, these myofibroblasts become senescent and produce a stable fibrotic scar with abundant collagen and other extracellular matrix components. In human patients, SAβGAL-positive cells accumulate in the periphery of the fibrotic scar[68]. In rodents, chronic treatment with carbon tetrachloride (a liver-damaging agent) or bile duct ligation produces liver fibrosis, which is characterized by positive SAβGAL staining[69–71]. Histological analyses indicated that these SAβGAL-positive cells derive from activated HSCs that upregulate p53, p21 and p16 (REF. 69). The acquisition of senescence by the HSCs is associated with the SASP, attraction of immune cells, clearance of the senescent HSCs by NK cells and partial elimination of the fibrotic scars.

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Table 2 | **List of senescence-related diseases**

Diseases	Overall effect of senescence	Disease description and therapeutic strategies	Refs
Cancer			
Multiple pre-malignant tumour types	Beneficial	• Senescence is associated with the pathology and restricts tumour progression • Cyclin-dependent kinase 4 (CDK4) inhibitors are pro-senescent and can induce tumour regression	4, 61–66, 166
Fibrosis			
Idiopathic pulmonary fibrosis	Detrimental	• Senescence is associated with the pathology and favours fibrosis • NADPH oxidase 4 (NOX4) inhibitors and anti-inflammatory agents are antisenescent and revert fibrosis	112–120
Cystic fibrosis	Not determined	• Senescence is associated with the pathology	153
Liver fibrosis	Beneficial	• Senescence is associated with the pathology and restricts fibrosis • Interleukin-22 (IL-22), CCN family member 1 (CCN1) and statins are pro-senescent and revert fibrosis	68–71, 73–75
Skin wound healing and oral submucous fibrosis	Beneficial	• Senescence is associated with the pathology and restricts fibrosis • CCN1 is pro-senescent and limits fibrosis	76–78
Renal fibrosis	Beneficial	• Senescence is associated with the pathology and restricts fibrosis upon urethral obstruction • A CDK4 inhibitor is pro-senescent and favours kidney repair upon ischaemic injury	72, 79–87
Myocardial infarction and cardiac fibrosis	Beneficial	Senescence is associated with the pathology and restricts fibrosis	89
Pancreatic fibrosis	Not determined	Senescence is associated with the pathology	164
Vascular diseases			
Atherosclerosis	Beneficial	Senescence is associated with the pathology and restricts atherosclerotic plaque formation	90–101, 106,107
Brain aneurysm and aortic aneurysm	Not determined	Senescence is associated with the pathology	142,143
Metabolic disorders			
Obesity	Detrimental	Senescence is associated with obesity and contributes to its pathological effects (systemic inflammation and insulin resistance)	122–126
Type 2 diabetes	Detrimental	Senescence is associated with the pathology and contributes to the disease	128–133
Neurological disorders			
Alzheimer's disease	Not determined	Senescence is associated with the pathology	149
Parkinson's disease	Not determined	Senescence is associated with the pathology	150
Muscle disorders			
Sarcopenia	Detrimental	• Senescence is associated with sarcopenia and aggravates the pathology • Inhibitors of MAPK p38α (also known as MAPK14) and MAPK p38β (also known as MAPK11) are antisenescent and restore the regenerative potential	125,126, 137–140
Bone and cartilage disorders			
Osteoarthritis	Not determined	Senescence is associated with the pathology	155,156
Intervertebral disc degeneration	Not determined	Senescence is associated with the pathology	157,158
Ocular diseases			
Macular degeneration	Not determined	Senescence is associated with the pathology	159,160
Glaucoma	Not determined	Senescence is associated with the pathology	146
Cataracts	Detrimental	Senescence is associated with cataracts and aggravates the pathology	125,126
Other diseases			
Pulmonary hypertension	Beneficial	• Senescence is associated with hypertension and restricts the pathology • Nutlin 3a is pro-senescent and reverts hypertension	108–110
Chronic obstructive pulmonary disease	Not determined	Senescence is associated with the pathology	161–163
Renal transplantation	Detrimental	Senescence is associated with mucositis and decreases transplantation success	79,88
Radiation-induced oral mucositis	Detrimental	• Senescence aggravates the pathology • Rapamycin is antisenescent and protects from radiation-induced mucositis	141
Intestinal bowel disease	Not determined	Senescence is associated with the pathology	154

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The beneficial role of senescence in restricting liver fibrosis is demonstrated by the analysis of senescence-deficient mice. In particular, upon liver damage, mice lacking *Trp53* and/or *Cdkn2a* present senescence-negative fibrotic areas that are larger than those in senescence-competent mice[69,73]. Similarly, the extracellular matrix protein CCN1 (also known as CYR61; a member of the CCN family (comprising CYR61, connective tissue growth factor (CTGF) and protein NOV homologue (NOV))) produced by damaged hepatocytes is a key mediator for the induction of senescence in HSCs. Accordingly, mice with *Cyr61*-null hepatocytes do not execute HSC senescence and this results in a lower secretion of matrix metalloproteinases (MMPs) and an exacerbated fibrotic response[71]. Also, production of IL-22 by activated HSCs induces HSC senescence in association with p53 activation through STAT3 (signal transducer and activator of transcription 3)–SOCS3 (suppressor of cytokine signalling 3) and increased production of MMPs[74]. In agreement with this, transgenic mice overexpressing IL-22 in the liver show a more efficient and faster resolution of fibrosis[74].

Together, the above studies suggest that the induction of HSC senescence could be a possible therapeutic strategy to limit liver fibrosis. There are promising results in mouse models indicating that treatments with recombinant protein CCN1, or with adenoviruses expressing CCN1 or IL-22 are able to revert already established hepatic fibrosis by promoting HSC senescence[70,71,74]. Moreover, statins also induce senescence in HSCs and thereby attenuate hepatic fibrosis upon bile duct ligation in rats[75].

Reduction of skin scarring and oral fibrosis. Skin fibrosis is part of the process of wound healing and it shows remarkable similarities to liver fibrosis. In particular, the above-mentioned matricellular protein CCN1 is crucial for the induction of senescence in dermal fibroblasts, the associated expression of pro-inflammatory cytokines and antifibrotic MMPs[76]. As in the case of liver fibrosis, *Cyr61*-deficient mice do not activate senescence in the dermal fibroblasts that participate in cutaneous healing, which leads to an exacerbated fibrosis[76]. Moreover, topical treatment with recombinant purified CCN1 protein restricts fibrosis during wound healing[76]. Mechanistically, CCN1 activates NADPH oxidase 1 (NOX1) through RAC1 and this, in turn, generates ROS that induce the DDR, ERK and p38 MAPK pathways, which results in the upregulation of p53 and p16 (REFS 76,77). These studies have led to a model in which wound-activated fibroblasts (myofibroblasts), characterized by the abundant production of extracellular matrix, are converted by CCN1 into senescent fibroblasts, which are then characterized by their capacity to degrade the extracellular matrix. Therefore, senescence participates in restricting the initial fibrotic wound response[77].

Senescence of activated fibroblasts has also been proposed to ameliorate the effect of oral submucous fibrosis, a disease associated with tobacco and areca nut chewing[78]. In particular, senescent fibroblasts accumulate during oral submucous fibrosis progression by a telomere-independent mechanism involving DDR, ROS and increased p16 expression, and senescence may reduce fibrosis by the increased expression of MMPs[78].

Mitigation of renal fibrosis. In the case of the kidney, increased activity of senescence pathways has been observed in multiple renal diseases (reviewed in REF. 79). In particular, reports in humans, rats and mice have documented high levels of p16 and p21 expression, and SAβGAL activity in association with renal damage and ageing[80–86]. Interestingly, renal injury by urethral obstruction results in renal fibrosis associated with SAβGAL activity, whereas, in *Cdkn2a*-null mice, SAβGAL is absent and renal fibrosis is aggravated[72]. Conversely, in a model of renal injury by ischaemia, treatment of mice with a CDK4 inhibitor (which can be considered a 'p16 mimetic'; see below) reduces the extent of renal damage[87]. However, it should be mentioned that in a similar model of ischaemia renal repair was improved in the absence of *Cdkn2a* encoding p16 and ARF[88].

Limitation of cardiac fibrosis upon infarction. Cellular senescence has additionally been shown to play a part in regulating cardiac fibrosis after myocardial infarction[89]. Myocardial infarction promotes the accumulation of senescent myofibroblasts in the heart and the expression of key senescence regulators, especially p53 but also p16, p21 and ARF, which decrease collagen production and cardiac fibrosis. Importantly, ablation of *Trp53* significantly attenuates cardiac fibroblast senescence, inflammation, macrophage infiltration and MMP production, and increases collagen deposition at the fibrotic scar, thereby aggravating cardiac fibrosis[89]. Based on the above-mentioned findings regarding the reversion of liver and skin fibrosis by pro-senescent treatments[71,74,76], it is possible that similar treatments could also revert cardiac fibrosis and improve heart function recovery.

Protection against atherosclerosis. Cellular senescence has been increasingly linked to the development of vascular pathologies, in particular to atherosclerosis, which has been the topic of excellent recent reviews[90–93]. Advanced atherogenic plaques consist of a cap, which is formed by vascular smooth muscle cells (VSMCs) and collagen, that surrounds a necrotic core comprising lipids, lipid-laden macrophages (foam cells) and debris. Plaque formation involves a series of events initiated by endothelial cell dysfunction, vascular permeability, and the monocyte- and macrophage-mediated release of pro-inflammatory cytokines. These events induce an initial phase of VSMC proliferation that is subsequently followed by the onset of senescence[92]. The SASP associated with senescent VSMCs amplifies the pro-atherogenic inflammatory environment and spreads senescence in a paracrine manner to other VSMCs, as well as to endothelial cells[92]. This is in line with the observation that atherosclerotic plaques from human coronary arteries contain endothelial cells and VSMCs that are positive for SAβGAL and express high levels of p15, p16, p21, p53 and ARF (REFS 93–95). In addition to

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hypercholesterolaemia, multiple additional factors contribute to trigger vascular senescence, including mitogens, inflammatory molecules, angiotensin II, oxidants and antioxidants, nitric oxide, high glucose, advanced glycation end products, mitochondrial damage and oxidized low-density lipoprotein[90,92].

The beneficial effect of senescence on atherosclerosis has been inferred genetically. In particular, mouse models with deficiencies in ARF[96], p53 (REFS 97,98), p21 (REF. 99) or p27 (REF. 100) all show increased susceptibility to developing atherosclerosis. Conversely, mice with increased *Trp53* gene expression are protected from atherosclerosis[101]. Together, these results suggest that pro-senescent pathways restrict the formation of atherosclerotic plaques, most probably by limiting the proliferation of the associated VSMCs and macrophages. However, it should also be mentioned that agonists of the nuclear receptor oxysterols receptor LXRβ decrease senescence and protect from atherosclerosis[102].

Remarkably, unbiased genetic screens in humans and in mice have also pointed to p16 and ARF as protectors of atherosclerotic diseases. In particular, multiple independent genome-wide association studies (GWASs) have consistently identified polymorphisms in a genomic region near the *CDKN2A–CDKN2B* locus[103,104]. Furthermore, in the case of one particular polymorphism, it has been possible to establish that the pro-atherosclerotic allele is associated with reduced expression of the three genes encoded by the *CDKN2A–CDKN2B* locus[105]. In mice, deletion of the genomic region that is homologous to the human interval containing atherosclerosis-associated polymorphisms results in decreased *Cdkn2a–Cdknb* expression and increased mortality upon high cholesterol diets[106]. Moreover, genetic mapping of an atherosclerosis-prone mouse strain identified a low-expression allele of the *Cdkn2a* locus (but not *Cdkn2b*) as responsible for the atherosclerosis susceptibility[107]. These genetic evidences in human and mice strongly suggests that lower levels of *CDKN2A* expression result in a higher risk of atherosclerosis, which further supports the concept that pro-senescent pathways protect from atherosclerosis.

Protection against pulmonary hypertension. Pulmonary hypertension has been also proposed to induce senescence in pulmonary artery smooth muscle cells from patients with chronic obstructive pulmonary disease (COPD)[108]. Experimental models of mouse pulmonary hypertension have been used to study the effect of pro-senescent pathways. In these models, the absence of *Trp53* or *Cdkn1a* aggravates pulmonary hypertension[109,110], whereas p53 stimulation with nutlin 3a ameliorates the disease[110]. Therefore, pro-senescent therapies, such as nutlin 3a, could provide therapeutic benefit to patients with pulmonary hypertension.

Detrimental effects of senescence in diseases

In this section, we review the pathologies for which there is evidence that senescence has a detrimental role (FIG. 2; TABLE 2). Studies on haematopoietic stem cells are intentionally excluded from this Review, because stereotypic senescence has not yet been described in these cells; however, ageing has a clear negative impact on haematopoietic stem function and is associated with telomere shortening, increased DNA damage and upregulation of cell cycle inhibitors[111].

Senescence aggravates pulmonary fibrosis. Cellular senescence has been implicated in idiopathic pulmonary fibrosis (IPF), a chronic and ultimately fatal disorder that is characterized by a progressive loss of lung function. A small percentage of human patients with IPF (<10%) have abnormally short telomeres, which in some cases are due to mutations in genes encoding telomerase components[112,113]. In mice, treatment with the DNA-damaging agent bleomycin recapitulates many features of human IPF. The cellular bases of IPF are complex and involve loss of alveolar cells, expansion of interstitial fibroblasts and epithelization of the fibrotic masses with bronchial cells[114]. Interestingly, SAβGAL activity is present in the three cell types involved in IPF — alveolar, bronchial and mesenchymal — in both human and mouse lungs[115–118]. The cell cycle inhibitor p16 is highly upregulated in fibroblasts of the fibrotic lesions and in the overlying epithelial cells[118]. Similarly, p21 is greatly expressed in human bronchial cells covering the fibrotic masses, and *in vitro* analyses indicated TGFβ as a key factor in the p53-independent induction of p21 (REF. 117), a mechanism that is reminiscent of developmental senescence (see above). Senescent bronchial cells also secrete IL-1β amounts that are proposed to induce fibroblast-to-myofibroblast differentiation and increased extracellular matrix deposition[117].

The detrimental role of senescence in lung fibrosis has been revealed in the context of caveolin (CAV). In particular, CAV1 has an important role in this process, as revealed by the fact that *Cav1*-null mice are partially protected from bleomycin-induced IPF, and this is accompanied by an impaired senescence and SASP response[119]. Also, the NADPH-dependent oxidase NOX4 contributes to the pathogenesis of lung fibrosis by generating high levels of ROS. Importantly, treatment of mice with a chemical inhibitor of NOX4 decreased p16 and p21 levels and reverted bleomycin-induced lung fibrosis in mice[118]. Finally, the anti-inflammatory agent rupatadine also impairs senescence upon bleomycin-induced fibrosis and diminishes the extent of lung fibrotic damage[120].

Adipocyte senescence is associated with obesity. Caloric overload, due to nutritional excess and/or low energy expenditure, leads to the storage of energy in adipose tissue. The storage capacity of adipocytes eventually reaches a threshold that triggers a stress response and the recruitment of macrophages. This inflammatory response of the adipose tissue initiates a cascade of events with systemic pathological consequences, including liver steatosis and insulin resistance, which constitute the hallmarks of metabolic syndrome[121]. Interestingly, the adipose tissue of obese subjects presents evidence of senescence, which is characterized by SAβGAL activity, presence of the SASP, and upregulation of p53 and p21, both in mouse models and in human patients[122–124].

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Mouse genetics has demonstrated a key role of p53 and p16 in adipose tissue senescence. In particular, whole-body deletion of the *Trp53* gene prevents adipose tissue senescence, and selective deletion of *Trp53* in adipose tissue protects mice from insulin resistance induced by chronic high-fat diet[122]. Also, a mouse model of accelerated ageing, caused by a hypomorphic allele of the *Bub1b* (budding uninhibited by benzimidazoles 1 homologue beta) gene, presents high levels of SAβGAL activity in the adipose tissue[125]. This senescent phenotype is absent in *Cdkn2a*-null mice, and, more impressively, it can be reverted by ablation of *Cdkn2a*-positive cells[125,126]. Therefore, adipose tissue senescence is associated with obesity and it contributes to its pathological effects.

Senescence contributes to type 2 diabetes. Insulin resistance due to obesity and ageing is initially compensated through an overproduction of insulin by pancreatic β-cells and an expansion of these cells[127], but this chronic challenge eventually leads to proliferative exhaustion and loss of β-cell mass[103]. In agreement with this, the number of β-cells and their proliferation rate are increased in mice after 4 months on a high-fat diet; however, after 12 months β-cell mass is reduced and proliferation rates are even lower than in mice on a standard diet[128]. Intriguingly, after 12 months on a high-fat diet the atrophic mouse β-cell islets are strongly positive for SAβGAL activity[128]. Moreover, type 2 diabetes induced by specific genetic manipulations also correlates with SAβGAL activity in the islets, as it does in the case of *Pttg1* (pituitary tumour-transforming gene 1; encoding securin)-null mice[129,130], *Cdk2*-null mice overexpressing *Myc* in β-cells[131], *Lig4* (ligase 4, DNA, ATP-dependent)-null mice and *Trp53*-hypomorphic mice[132]. In addition, studies using multiple genetic manipulations uncovered that, compared to other tissues, β-cells islets are particularly sensitive to reduced levels of CDK activity or increased levels of DNA damage (reviewed in REF. 133). Therefore, there is a robust association between type 2 diabetes and senescence.

Interestingly, a remarkable number of GWASs have found that polymorphisms that are in close proximity to the *CDKN2A–CDKN2B* locus are linked to type 2 diabetes [103,104,134]. It remains to be determined whether the type 2 diabetes risk alleles increase or decrease the expression of *CDKN2A–CDKN2B*. On the one hand, it is well established that p16 is a negative regulator of β-cell proliferation[135]. On the other hand, however, mice overexpressing *Cdkn2a* do not have defects in glucose homeostasis[135], and mice overexpressing the entire *Cdkn2a–Cdkn2b* locus are even protected from ageing-associated glucose intolerance and insulin resistance[136]. It is therefore conceivable that moderate levels of p16 could contribute to maintain the quiescence of β-cell progenitors and, thereby, their long-term functionality.

Senescence aggravates sarcopenia. Loss of muscle function is one of the most prevalent ageing-associated pathologies. Recent studies have implicated senescence of the muscle stem cells (also known as satellite cells) as the underlying cause of ageing-associated sarcopenia and loss of muscle regenerative potential. Aged muscles from humans and mice accumulate p16 and are positive for SAβGAL[137–139]. The upregulation of p16 and the loss of regenerative potential occurs through a mechanism that involves p38 MAPK[138,140] and CCN1 (REF. 139). Interestingly, genetic inactivation of p16 (REF. 137) or chemical inhibition of p38 (REFS 138,140) rejuvenates aged satellite cells and promotes muscle regeneration upon injury. Moreover, genetic elimination of p16-expressing satellite cells also ameliorates sarcopenia in a progeroid mouse model, which indicates that the presence of senescent satellite cells is detrimental by itself beyond their lack of regenerative capacity[126].

Other diseases worsened by senescence. Other pathological processes in which senescence has a detrimental role are cataracts[125,126] and radiation-induced oral mucositis[141]. In particular, elimination of senescent cells in a mouse model of progeria ameliorates cataracts[125,126] indicating that cellular senescence directly contributes to this pathology. In the case of oral mucositis, radiation induces senescence in a manner that requires the activity of mTOR and treatment with rapamycin protects mice from developing senescence and, thereby, from mucosal ulcerations[141]. Finally, the presence of senescent cells in pre-transplantation kidneys correlates with a poor outcome in renal transplantation and failed post-transplantation kidneys also have high levels of p16 (REF. 79). Furthermore, the kidneys of *Cdkn2a*-deficient mice have reduced senescence and transplantation of these kidneys results in increased mouse survival compared to mice transplanted with wild-type kidneys[88].

Emerging diseases associated with senescence

In this section, we review diseases that have been associated with senescence but that remain to be characterized in further detail, including whether senescence contributes to the pathology or, on the contrary, whether it serves to limit the extent of the pathological process (FIG. 2; TABLE 2). Senescence has been associated with human aneurysms in the brain[142] and the heart[143], including SAβGAL activity[143]. Interestingly, various GWASs have linked the *CDKN2A–CDKN2B* locus to both brain[144] and aortic[145] aneurysms. Glaucoma is also associated with senescence, as evidenced by an increase in SAβGAL activity in the trabecular meshwork cells involved in regulating the intraocular pressure[146]. Again, independent GWASs have found *CDKN2A–CDKN2B* linked to glaucoma and increased intraocular pressure[147,148]. Astrocyte senescence has been proposed as a component of Alzheimer's disease[149] and Parkinson's disease[150]. It is of note that the human brain cortex presents an increase in the number of astrocytes expressing p16 and MMP1 (also known as interstitial collagenase); this is exacerbated in age-matched patients with Alzheimer's disease[149]. This is in line with two independent GWASs that found significant linkage between Alzheimer's disease and polymorphic variants in the vicinity of the *CDKN2A–CDKN2B* locus[151,152].

Other human diseases associated with senescence and SAβGAL activity, are cystic fibrosis[153], intestinal bowel disease[154], osteoarthritis[155,156] and intervertebral disc degeneration[157,158]. The number of senescent cells

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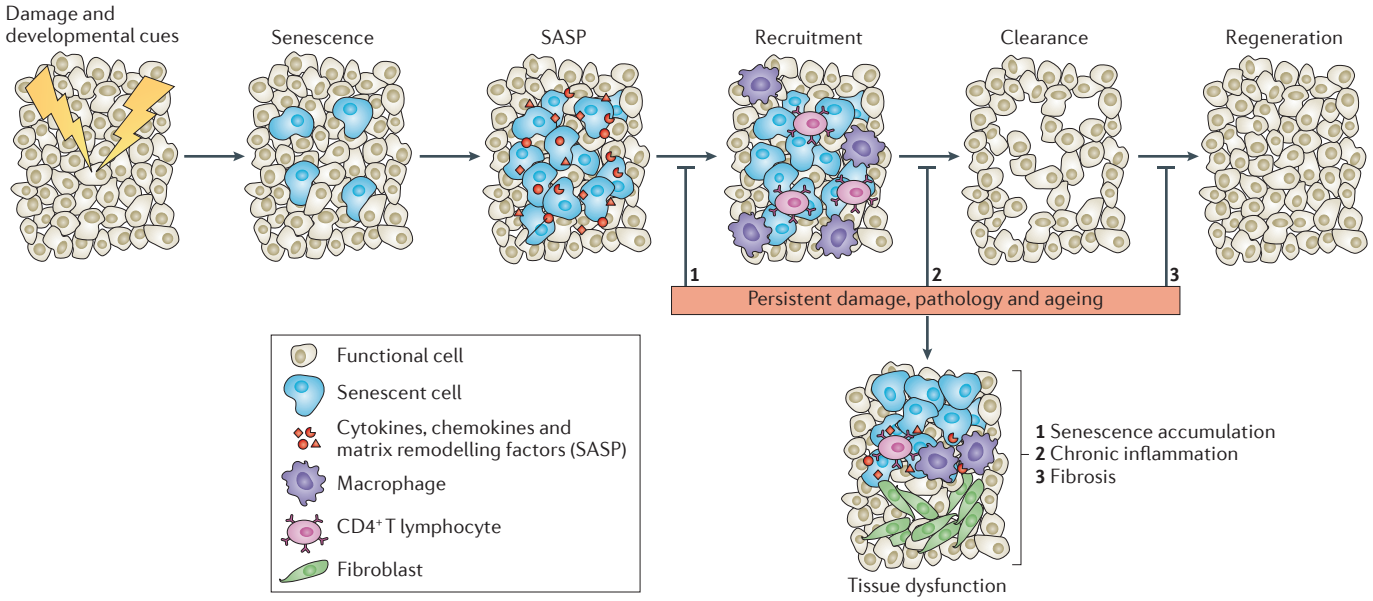

Figure 3 | **Unified model of senescence.** Senescence initiates a tissue remodelling process by recruiting immune cells through the senescence-associated secretory phenotype (SASP). Macrophages clear the senescent cells, and progenitor cells repopulate and regenerate the damaged tissue. This sequence of senescence–clearance–regeneration may be impaired upon persistent damage, pathological states or ageing. In these cases, senescent cells are not efficiently cleared and the tissue is not fully regenerated. Resolution of the damage in these cases involves a fibrotic scar with senescent cells, inflammatory cells and fibrotic tissue.

increases with ageing in the retinal pigmented epithelium of rhesus monkeys[159], and this could contribute to macular degeneration[160]. COPD is another severe lung disease linked to ageing and cigarette smoking and is characterized by disruption of the alveolar epithelium and often associated with fibrosis[161]. Interestingly, exposure of mice to cigarette smoke induces SAβGAL activity in alveolar cells[162], and the alveolar epithelium of patients with COPD contains high levels of p16 and nuclear NF-κB[163]. Finally, chemically-induced damage to the pancreas in rats results in fibrosis with areas of abundant senescent pancreatic stellate cells[164].

Therefore, the list of diseases associated with senescence keeps growing and we anticipate that senescence will be relevant for even more pathologies.

A unified model

Based on the above-discussed evidence, we propose that senescence is a key component of tissue remodelling both in normal development and physiology, and in multiple pathologies. We also propose that, in general, cellular senescence coordinates tissue remodelling through three sequential processes: first, a stable proliferative arrest; second, a secretory phenotype (SASP) that recruits immune cells, notably including T helper lymphocytes and macrophages; and, third, the mobilization of nearby progenitor cells that repopulate the tissue (FIG. 3). This model can be applied, with some adjustments, to developmental senescence, which in some cases achieves the elimination of transitory embryonic structures (such as the mesonephric tubules and the interdigital webs) and in others the elimination of one cell population in favour of another (as in the endolymphatic sac of the inner ear)[48,49]. In the case of pre-malignant tumours, oncogenically stressed cells become senescent and may also trigger clearance and tumour elimination. Benign melanocytic lesions (or nevi) are a notable exception. These lesions contain oncogenic *BRAF* (v-raf murine sarcoma viral oncogene homologue B) mutations and are senescent[165]; however, for reasons that remain to be elucidated, they are not cleared and indeed are stable for years. The senescence–clearance–regeneration model probably functions in cases of occasional adult somatic damage, which leads to a complete restoration of the damaged tissue. In support of this, mice that are genetically deficient in senescence pathways are more sensitive to various tissue injuries, and they present with exacerbated fibrosis and more severe tissue damage (see above). However, after persistent damage or in aged tissues, clearance and regeneration may be compromised owing to poor macrophage recruitment or a deficient regenerative response (FIG. 3). In these circumstances, senescent cells accumulate and create a stable senescent lesion that may aggravate the pathology. Therefore, the balance between the beneficial and detrimental effects of senescence probably depends on whether senescent cells are only transitory or whether they accumulate over time.

Senescence as a therapeutic target

Current available evidence indicates that both pro-senescent and antisenescent approaches can be desirable depending on the therapeutic context. During the course of ongoing tissue damage, the promotion of senescence can be beneficial by limiting the fibrotic response. In the case of cancer, pro-senescent therapies are emerging and have been the topic of a recent review[166]. CDK4 inhibitors

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can be considered as p16 mimetics; indeed, they induce senescence (and not apoptosis) in many human cancer cell lines[61–63]. A special mention must be made to the CDK4 inhibitor palbociclib (PD-0332991; Pfizer), which shows promising results for mantle cell lymphoma[64], breast cancer[65] and liposarcomas[66]. Moreover, the CDK4 inhibitor LEE011 (Novartis) has also shown interesting preclinical activity in neuroblastoma[63]. Phase III clinical trials will unequivocally show the real clinical potential of these inhibitors. A desirable feature of pro-senescent therapies against cancer is the possibility of a subsequent complementary treatment to completely eliminate the senescent cells, thereby impairing a possible tumorigenic reversion and the pro-tumorigenic effects of the SASP on neighbouring cells[67]. In this regard, it has been found that senescent cells sustain high levels of proteotoxicity, which require a high lysosomal activity[186]. This is actually an Achilles heel of senescent cells, because they are particularly sensitive to chemical inhibitors of lysosomal ATPases, as shown in an *in vivo* model of chemotherapy-induced senescence[186].

In addition to cancer, proof of principle for the therapeutic benefit of pro-senescent agents has been provided in the cases of renal fibrosis (by using palbociclib)[87], liver fibrosis (by treatment with the senescence mediators IL-22 (REF. 74) and CCN1 (REFS 70,71)) and cutaneous fibrosis (by treatment with CCN1 (REF. 76)).

Pro-senescent therapies, as discussed above, may facilitate the resolution of the damage during repair.However, in the long term, the resulting senescent wounds or scars may impose a marked tissue dysfunction. In this case, their elimination by antisenescent therapies may be beneficial. This strategy has been elegantly demonstrated in mice with persistent DNA damage (due to a hypomorphic mutation in *Bub1b*), in which senescent cells greatly accumulate and their genetic elimination partially ameliorates some phenotypes[126]. Several potential pharmacological agents may function through inhibition of senescence. In particular, lung fibrosis can be improved by decreasing senescence through chemical inhibition of NOX4 with GKT137831 (Genkyotex)[118] or with the anti-inflammatory agent rupatadine[120]. Similarly, oral fibrosis can be improved by inhibiting senescence with the mTOR inhibitor rapamycin[141]. Finally, pharmacological inhibition of the p38 MAPK pathway by SB203580 (Tocris) or SB202190 (EMD Chemicals) ameliorates age-associated sarcopenia and reveals a potential therapy for the treatment of progressive muscle wasting[138,140].

Therefore, pro-senescent therapies can be useful for the treatment of cancer and for ongoing tissue repair processes, whereas antisenescent therapies can be beneficial to eliminate senescence and fibrosis in 'resolved' injuries or to rejuvenate the aged muscle.

Conclusions

Here, we review the main mechanisms involved in the induction of senescence and the role of senescence in a large variety of physiological and pathological processes. We conclude that senescence is mainly designed to coordinate processes of tissue remodelling. In the case of embryonic development, senescence is important to eliminate unwanted cells and, thereby, contributes to morphogenesis, elimination of transient structures and the establishment of correct cell numbers. It remains to be determined how developmentally programmed senescence is initiated and how it is functionally connected with apoptosis.

Senescence also participates in tissue remodelling processes upon damage, but its final effect in human diseases depends on the type of pathology. Pro-senescent therapeutic strategies have shown benefits for the treatment of cancer and there are promising results in preclinical mouse models for their use to improve tissue repair after injury. At the same time, future antisenescent therapies may help to eliminate the burden of senescent cells associated with stabilized fibrotic scars that accumulate during ageing or chronic damage.

A challenge for the immediate future is to understand the interplay between senescence and regeneration. Understanding this will provide a clearer picture of the roles of senescence in different pathologies. This is crucial to design pro-senescent or antisenescent therapies.

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Competing interests statement
The authors declare no competing interests.

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