Cellular senescence: when bad things happen to good cells

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Abstract | Cells continually experience stress and damage from exogenous and endogenous sources, and their responses range from complete recovery to cell death. Proliferating cells can initiate an additional response by adopting a state of permanent cell-cycle arrest that is termed cellular senescence. Understanding the causes and consequences of cellular senescence has provided novel insights into how cells react to stress, especially genotoxic stress, and how this cellular response can affect complex organismal processes such as the development of cancer and ageing.

Renewable tissue
A tissue in which cell proliferation is important for tissue repair or regeneration. Renewable tissues typically contain, but sometimes recruit, mitotic cells upon injury or cell loss.

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Cellular senescence was formally described more than four decades ago when Hayflick and colleagues showed that normal cells had a limited ability to proliferate in culture[1] (see BOX 1 for descriptions of different types of senescence). These classic experiments showed that human fibroblasts initially underwent robust cell division in culture. However, gradually — over many cell doublings — cell proliferation (used here interchangeably with cell growth) declined. Eventually, all cells in the culture lost the ability to divide. The non-dividing cells remained viable for many weeks, but failed to grow despite the presence of ample space, nutrients and growth factors in the medium.

Soon after this discovery, the finding that normal cells do not indefinitely proliferate spawned two important hypotheses. At the time, both were highly speculative and seemingly contradictory. The first hypothesis stemmed from the fact that many cancer cells proliferate indefinitely in culture. Cellular senescence was proposed to be an anti-cancer or tumour-suppressive mechanism. In this context, the senescence response was considered beneficial because it protected organisms from cancer, a major life-threatening disease. The second hypothesis stemmed from the fact that tissue regeneration and repair deteriorate with age. Cellular senescence was proposed to recapitulate the ageing, or loss of regenerative capacity, of cells *in vivo*. In this context, cellular senescence was considered deleterious because it contributed to decrements in tissue renewal and function. For many years, these hypotheses were pursued more or less independently. However, as an understanding of the senescence response grew, these hypotheses coalesced, bringing new insights to the fields of cancer and ageing. Here, we review recent progress in understanding the causes

of cellular senescence, and the evidence that it is important for suppressing cancer and a possible contributor to ageing.

Senescence in an evolutionary context
To understand how cellular senescence can be both beneficial and detrimental, and the origins of its regulation, it is important to understand the nature of cancer and the evolutionary theory of ageing. Cancer is often fatal and therefore poses a major challenge to the longevity of organisms with renewable tissues. Tissue renewal is essential for the viability of complex organisms such as mammals. However, cell proliferation is essential for tumorigenesis, and renewable tissues are at risk of developing cancer[2]. Moreover, cancer initiates and, to a large extent, progresses owing to somatic mutations[3], and proliferating cells acquire mutations more readily than non-dividing cells[4]. The danger that cancer posed to longevity was mitigated by the evolution of tumour-suppressor mechanisms. One such mechanism was cellular senescence, which stops incipient cancer cells from proliferating[5–7].

The environment in which cellular senescence evolved was replete with extrinsic hazards such as infection, predation and starvation. Hence, organismal lifespans were relatively short owing to death from these hazards. Therefore, tumour-suppressor mechanisms needed to be effective for only a relatively short interval (a few decades for humans, several months for mice). Should such mechanisms be deleterious later (for example, if the regenerative capacity were to decline or if dysfunctional senescent cells were to accumulate), there would be little selective pressure to eliminate the harmful effects. Therefore, some tumour-suppressor mechanisms can be both beneficial

- Senescence derives from *senex*, a Latin word meaning old man or old age. In organismal biology, senescence describes deteriorative processes that follow development and maturation, and the term is used interchangeably with ageing.

- The term senescence was applied to cells that ceased to divide in culture[1], based on the speculation that their behaviour recapitulated organismal ageing. Consequently, cellular senescence is sometimes termed cellular ageing or replicative senescence.

- Cells that are not senescent are termed pre-senescent, early passage, proliferating or, sometimes, young.

- Telomere shortening provided the first molecular explanation for why many cells cease to divide in culture[61,68]. Dysfunctional telomeres trigger senescence through the p53 pathway. This response is often termed telomere-initiated cellular senescence.

- Some cells undergo replicative senescence independently of telomere shortening[16,53,101]. This senescence is due to stress, the nature of which is poorly understood. It increases p16 expression and engages the p16–retinoblastoma protein (pRB) pathway. This response is termed stress-induced or premature senescence, stasis or M0 (mortality phase 0).

- Certain mitogenic oncogenes or the loss of anti-mitogenic tumour-suppressor genes induce senescence in normal cells[83,92,93,95]. This is known as oncogene-induced senescence.

- Cells that do not divide indefinitely are said to have a finite or limited replicative (or proliferative) lifespan and are (replicatively) mortal. Cells that proliferate indefinitely are termed (replicatively) immortal.

- Immortal cells are not necessarily transformed (tumorigenic) cells. Although historically the terms immortalization and transformation have been used interchangeably, the replicative lifespan of cells can be expanded indefinitely by the expression of telomerase without the phenotypic changes that are associated with malignant transformation[138].

- Telomere-initiated senescence is sometimes termed M1 (mortality phase 1)[153]. Some cells (for example, fibroblasts) undergo telomere-initiated senescence with few signs of genomic instability. Other cells (for example, some epithelial cells) arrest with obvious signs of genomic instability and are termed agonescent[154].

- Human cells that escape telomere-initiated senescence (M1) or agonescence owing to the loss of p53 function can proliferate until they enter a state that is termed crisis, mitotic catastrophe or M2 (mortality phase 2)[153]. This state is characterized by extensive genomic instability and cell death.

Antagonistic pleiotropy
The hypothesis that genes or processes that were selected to benefit the health and fitness of young organisms can have unselected deleterious effects that manifest in older organisms and thereby contribute to ageing.

Mitotic cell
A cell that has the ability to proliferate. *In vivo*, mitotic cells often exist in a reversible growth-arrested state that is termed quiescence or G0 phase, but such cells can be stimulated to proliferate in response to appropriate physiological signals.

Post-mitotic cell
A cell that has permanently lost the ability to proliferate, usually due to differentiation.

and deleterious, depending on the age of the organism[8]. This concept — that a process can be beneficial to young organisms but harmful to old organisms — is the essence of antagonistic pleiotropy, an important evolutionary theory of ageing[9]. There is now substantial evidence that cellular senescence is indeed a potent tumour-suppressor mechanism[6,7,10,11], and there is also mounting — but still largely circumstantial — evidence that cellular senescence promotes ageing[12–14].

Characteristics of senescent cells

Complex organisms such as mammals contain both mitotic cells and post-mitotic cells (BOX 2). Cellular senescence is confined to mitotic cells, from which cancer can arise. Although mitotic cells can proliferate, they can also spend long intervals in a reversibly arrested state termed quiescence or G0. Quiescent cells resume proliferation in response to appropriate signals, including the need for tissue repair or regeneration. By contrast, post-mitotic cells permanently lose the ability to divide owing to differentiation.

Mitotic cells can senesce when they encounter potentially oncogenic events (discussed below). When this occurs, the cells cease proliferation (known as growth arrest), in essence irreversibly. They often become resistant to cell-death signals (apoptosis resistance) and they acquire widespread changes in gene expression (altered gene expression). Together, these features comprise the senescent phenotype (FIG. 1).

Growth arrest. The hallmark of cellular senescence is an inability to progress through the cell cycle. Senescent cells arrest growth, usually with a DNA content that is typical of G1 phase, yet they remain metabolically active[15–18]. Once arrested, they fail to initiate DNA replication despite adequate growth conditions. This replication failure is primarily caused by the expression of dominant cell-cycle inhibitors (see below). In contrast to quiescence, the senescence growth arrest is essentially permanent (in the absence of experimental manipulation) because senescent cells cannot be stimulated to proliferate by known physiological stimuli.

The features and stringency of the senescence growth arrest vary depending on the species and the genetic background of the cell. For example, most mouse fibroblasts senesce with a G1 DNA content, although a defect in the stress-signalling kinase MKK7 primarily induces a G2–M arrest[19]. Likewise, some oncogenes (see below) cause a fraction of cells to senesce with a DNA content that is typical of G2 phase[20–22]. Furthermore, tumour cells can senesce with G2- or S-phase DNA contents. Although tumour cells usually proliferate indefinitely in culture, some of them retain the ability to undergo a senescence-like arrest, especially in response to certain anti-cancer therapies[23]. Finally, human and rodent cells differ strikingly in the stringency of the senescence growth arrest[24]. Like human cells, many mouse and rat cells have a finite proliferative capacity in culture, although, as discussed below, the mechanisms that limit this proliferation probably differ. However, rodent cell cultures frequently acquire spontaneous variants that can divide indefinitely. Such variants are exceedingly rare in human cultures.

Apoptosis resistance. Apoptosis entails the controlled destruction of cellular constituents and their ultimate engulfment by other cells[25]. Like senescence, apoptosis is an extreme response to cellular stress and is an important tumour-suppressive mechanism[26]. But, whereas senescence prevents the growth of damaged or stressed cells, apoptosis quickly eliminates them.

Many (but not all) cell types acquire resistance to certain apoptotic signals when they become senescent. For example, senescent human fibroblasts resist ceramide-induced apoptosis but endothelial cells do not[27]. Senescent human fibroblasts also resist apoptosis caused by growth-factor deprivation and oxidative stress, but do not resist apoptosis caused by engagement of the Fas death receptor[28,29]. Resistance to apoptosis might partly explain why senescent cells are so stable in culture. This attribute might also explain why the number of senescent cells increases with age, although, as discussed below, several factors probably contribute to this phenomenon.

Box 2 | Mitotic and post-mitotic cells

Mitotic cells are capable of proliferation, and they include the epithelial, stromal (fibroblastic) and vascular (endothelial) cells that comprise the major renewable tissues and organs such as the skin, intestines, liver, kidney and so on. They also comprise major components of the haematopoietic system, and cells such as the glia, which support the survival and function of non-dividing neurons. Mitotic cells also include the undifferentiated stem and progenitor cells that provide many of these tissues with the differentiated cells that are required for their function. Mitotic cells are susceptible to malignant transformation (that is, transformation into a cancer cell). They are also susceptible to undergoing cellular senescence when challenged by stimuli that have the potential to cause cancer.

Post-mitotic cells are incapable of proliferation. They include the differentiated neurons and muscle cells that comprise the brain, heart and skeletal muscle. Recent findings suggest that tissues that are composed mainly of post-mitotic cells can undergo limited repair and regeneration; however, this regeneration is not due to the proliferation of post-mitotic cells, but rather to the recruitment of mitotic stem cells or their progeny (progenitor cells)[155–157]. Because they have already lost the ability to proliferate, post-mitotic cells do not undergo cellular senescence as currently defined.

Post-mitotic and senescent cells are irreversibly blocked from re-entering the cell cycle. The mechanisms that prevent these cells from undergoing cell division are incompletely understood, but probably share some common effectors.

Quiescence
A reversible non-dividing state from which cells can be stimulated to proliferate in response to physiological signals.

Senescent phenotype
The combination of changes in cell behaviour, structure and function that occur upon cellular senescence. For most cell types, these changes include an essentially irreversible growth arrest, resistance to apoptosis and many alterations in gene expression.

Oncogene
A gene that contributes to the malignant transformation of cells. Oncogenes can be cellular or viral in origin. Cellular oncogenes are usually mutant or overexpressed forms of normal cellular genes. Viral oncogenes can also originate from cellular genes, acquiring mutations during viral capture, but they can also be distinctly viral in origin.

Chromatin
The DNA and complex of associated proteins that determine the accessibility of large DNA regions to the transcription machinery and other large protein complexes.
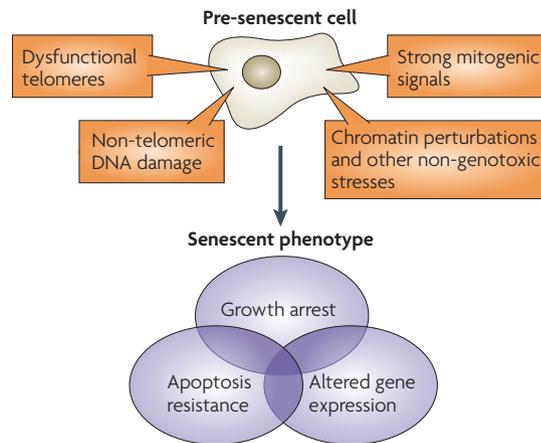
It is not clear what determines whether cells undergo senescence or apoptosis. One determinant is cell type; for example, damaged fibroblasts and epithelial cells tend to senesce, whereas damaged lymphocytes tend to undergo apoptosis. The nature and intensity of the damage or stress may also be important[30,31]. Most cells are capable of both responses. Moreover, manipulation of pro- and anti-apoptotic proteins can cause cells that are destined to die by apoptosis to senesce and, conversely, cause cells that are destined to senesce to undergo apoptosis[30–32]. The senescence and apoptosis regulatory systems therefore communicate — probably through their common regulator, the p53 tumour suppressor protein[31]. The mechanisms by which senescent cells resist apoptosis are poorly understood. In some cells, resistance might be due to expression changes in proteins that inhibit, promote or implement apoptotic cell death[33,34]. In others, p53 might preferentially transactivate genes that arrest proliferation, rather than those that facilitate apoptosis[35].

Altered gene expression. Senescent cells show striking changes in gene expression, including changes in known cell-cycle inhibitors or activators[35–41]. Two cell-cycle inhibitors that are often expressed by senescent cells are the cyclin-dependent kinase inhibitors (CDKIs) p21 (also termed CDKN1a, p21Cip1, Waf1 or SDI1) and p16 (also termed CDKN2a or p16INK4a)[6,7]. These CDKIs are components of tumour-suppressor pathways that are governed by the p53 and retinoblastoma (pRB) proteins, respectively. p53 and pRB are transcriptional regulators, and the pathways they govern are frequently disrupted in cancer[42]. Both pathways can establish and maintain the growth arrest that is typical of senescence. p21 is induced directly by p53 (REFS 35,43) but the mechanisms that induce p16, a tumour suppressor in its own right, are incompletely understood[44]. Ultimately, p21 and p16 maintain pRB in a hypophosphorylated and active state but, as discussed below, their activities are not equivalent.

Senescent cells also repress genes that encode proteins that stimulate or facilitate cell-cycle progression (for example, replication-dependent histones, c-FOS, cyclin A, cyclin B and PCNA (proliferating cell nuclear antigen))[45–48]. Some of these genes are repressed because E2F, the transcription factor that induces them, is inactivated by pRB. In some senescent cells, E2F target genes are silenced by a pRB-dependent reorganization of chromatin into discrete foci that are termed senescence-associated heterochromatin foci (SAHFs)[45].

Interestingly, many changes in gene expression appear to be unrelated to the growth arrest. Many senescent cells overexpress genes that encode secreted proteins that can alter the tissue microenvironment[36–41]. For example, senescent fibroblasts overexpress proteins that remodel the extracellular matrix or mediate local inflammation. As discussed below, these findings raise the possibility that as senescent cells increase with age, they might contribute to age-related decrements in tissue structure and function[8,12]. The mechanisms that are responsible for the senescence-associated secretory phenotype are unknown.

Senescence markers. Several markers can identify senescent cells in culture and *in vivo*. However, no markers are exclusive to the senescent state. A poorly understood feature of these markers is that, aside from the decline in DNA replication, all of them require several days to develop.

An obvious marker for senescent cells is the lack of DNA replication, which is typically detected by the incorporation of 5-bromodeoxyuridine or ³H-thymidine, or by immunostaining for proteins such as PCNA and Ki-67. Of course, these markers do not distinguish between senescent cells and quiescent or differentiated post-mitotic cells. The first marker to be used for the more specific identification of senescent cells was the senescence-associated β-galactosidase (SA-βgal)[49]. This marker is detectable by histochemical staining in most senescent cells. However, it is also induced by stresses such as prolonged confluence in culture. The SA-βgal probably derives from the lysosomal β-galactosidase and reflects the increased lysosomal biogenesis that commonly occurs in senescent cells[50]. In addition, p16 — an important regulator of senescence — is now used to identify senescent cells[51]. p16 is expressed by many, but not all, senescent cells[52,53] and it is also expressed by some tumour cells, especially those that have lost pRB function[44]. Recently, three proteins were identified in a screen for genes that were expressed following oncogene-induced senescence, and the proteins were subsequently used to identify senescent cells: DEC1 (differentiated embryo-chondrocyte expressed-1), p15 (a CDKI) and DCR2 (decoy death receptor-2)[54]. The specificity and significance of these proteins for senescent cells are not yet clear, but they are promising additional markers.

Some senescent cells can also be identified by the cytological markers of SAHFs[45] and senescence-associated DNA-damage foci (SDFs)[16,20,55,56]. SAHFs are detected by the preferential binding of DNA dyes, such as

Figure 1 | **The senescent phenotype induced by multiple stimuli.** Mitotically competent cells respond to various stressors by undergoing cellular senescence. These stressors include dysfunctional telomeres, non-telomeric DNA damage, excessive mitogenic signals including those produced by oncogenes (which also cause DNA damage), non-genotoxic stress such as perturbations to chromatin organization and, probably, stresses with an as-yet-unknown etiology. The senescence response causes striking changes in cellular phenotype. These changes include an essentially permanent arrest of cell proliferation, development of resistance to apoptosis (in some cells), and an altered pattern of gene expression. The expression or appearance of senescence-associated markers such as senescence-associated β-galactosidase, p16, senescence-associated DNA-damage foci (SDFs) and senescence-associated heterochromatin foci (SAHFs) are neither universal nor exclusive to the senescent state and therefore are not shown.

4′,6-diamidino-2-phenylindole (DAPI), and the presence of certain heterochromatin-associated histone modifications (for example, H3 Lys9 methylation) and proteins (for example, heterochromatin protein-1 (HP1)). SAHFs also contain E2F target genes, which SAHFs are thought to silence. In mouse cells, pericentromeric chromatin also preferentially binds DAPI, contains modified histones and proteins found in SAHFs, and forms cytologically detectable foci. However, there is no evidence that these foci contain E2F target genes; indeed, they are also present in proliferating cells. Because pericentromeric foci are much more prominent in mouse cells than human cells[57,58], they can be mistaken for SAHFs. SDFs, by contrast, are present in senescent cells from mice and humans and contain proteins that are associated with DNA damage (for example, phosphorylated histone H2AX (γ-H2AX) and p53-binding protein-1 (53BP1)). As discussed below, these foci result from dysfunctional telomeres and other sources of DNA damage.

Causes of cellular senescence

What causes cells to senesce? The first clues came from understanding why normal human cells do not proliferate indefinitely in culture (BOX 1), but subsequent studies showed that senescence can be induced by many stimuli (FIG. 1).

Telomere-dependent senescence. Telomeres are stretches of repetitive DNA (5′-TTAGGG-3′ in vertebrates) and associated proteins that cap the ends of linear chromosomes and protect them from degradation or fusion by DNA-repair processes[59]. The precise telomeric structure is not known, but mammalian telomeres are thought to end in a large circular structure, termed a t-loop[60]. Because standard DNA polymerases cannot completely replicate DNA ends — a phenomenon called the end-replication problem — cells lose 50–200 base pairs of telomeric DNA during each S phase[61] (FIG. 2). Human telomeres range from a few kilobases to 10–15 kb in length, so many cell divisions are possible before the end-replication problem renders telomeres critically short and dysfunctional. Only one or a few such telomeres are sufficient to trigger senescence[62,63]. The end-replication problem is a major (but not the sole) reason why normal cells do not proliferate indefinitely (BOX 1).

Dysfunctional telomeres trigger a classical DNA-damage response (DDR)[16,55,56,64] (FIG. 3). The DDR enables cells to sense damaged DNA, particularly double-strand breaks (DSBs), and to respond by arresting cell-cycle progression and repairing the damage if possible. Although the severity of the damage is probably an important factor, little is known about how cells choose between transient DDR activation and the persistent DDR signalling that is evident in many senescent cells. Many proteins participate in the DDR, including protein kinases (for example, ataxia telangiectasia mutated (ATM) and checkpoint-2 (CHK2)), adaptor proteins (for example, 53BP1 and MDC1 (mediator of DNA damage checkpoint protein-1)) and chromatin modifiers (for example, γ-H2AX). Many of these proteins localize to the DNA-damage foci that are detected in senescent cells. In cells that senesce owing to dysfunctional telomeres, these foci also contain a subset of telomeres, suggesting that dysfunctional telomeres resemble DSBs.

The end-replication problem can be circumvented by telomerase. This enzyme contains a catalytic protein component (telomerase reverse transcriptase; TERT) and a template RNA component, and adds telomeric DNA repeats directly to chromosome ends[65]. Most normal cells do not express TERT, or express it at levels that are too low to prevent telomere shortening[66,67]. By contrast, germ-line cells and many cancer cells express TERT. Moreover, ectopic TERT expression in normal human cells simultaneously prevents telomere shortening and senescence caused by the end-replication problem[68]. However, telomerase cannot prevent senescence caused by non-telomeric DNA damage or other senescence inducers[69].

This point is demonstrated by the behaviour of many mouse cells. In contrast to most human cells, cells from laboratory mice have long telomeres (>20 kb) and many express telomerase. Nonetheless, many mouse cells senesce after only a few doublings under standard culture conditions. This arrest is due to the supraphysiological oxygen level (20%) that is used in standard culture protocols. A 20% oxygen level, to which mouse cells are much more sensitive than human cells, causes severe DNA damage and, in the absence of efficient repair
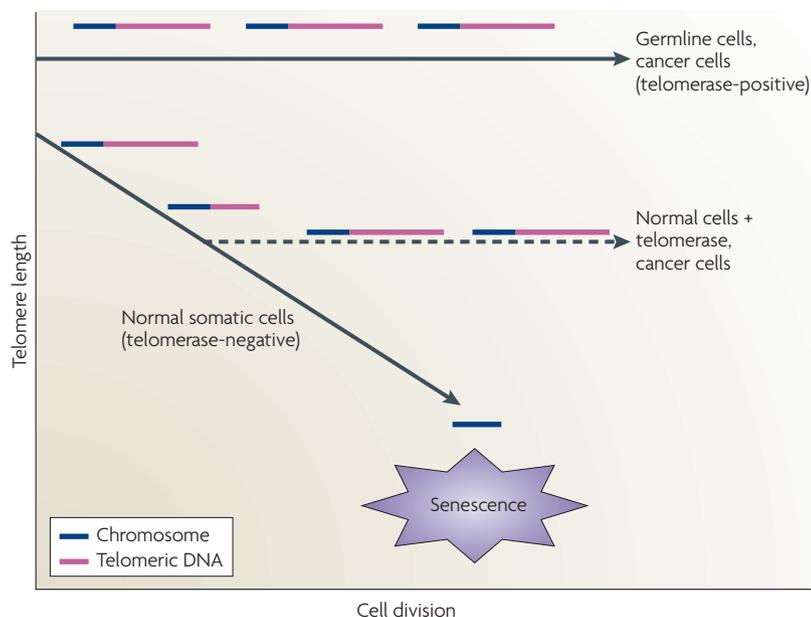
Figure 2 | **Telomere-dependent senescence.** Telomeres are stretches of a repetitive DNA sequence and associated proteins (magenta) that are located at the termini of linear chromosomes (blue). The telomeric ends form a circular protective cap termed the t-loop, which may help to prevent the termini from triggering a full DNA-damage response (DDR). Telomere lengths are maintained by the enzyme telomerase, which is expressed by cells that comprise the germline as well as many cancer cells. Most normal somatic cells do not express this enzyme, or express it only transiently or at levels that are too low to prevent telomere shortening caused by the end-replication problem. In such cells, telomere lengths decline with each cell cycle. Eventually, one or a few telomeres become sufficiently short and malfunction, presumably owing to loss of the protective protein–DNA structure. Dysfunctional telomeres trigger a DDR, to which cells respond by undergoing senescence.

mechanisms, DSBs. Both the damage and replicative failure are mitigated by culturing mouse cells at a (lower) physiological oxygen level[70].

DNA-damage-initiated senescence. Severe DNA damage that occurs anywhere in the genome — especially damage that creates DSBs — causes many cell types to undergo senescence[15,70]. In culture, such cells harbour SDFs for many weeks or longer (J.C. and F.d'A.d.F., unpublished observations). There is as yet no firm evidence that these persistent foci contain irreparable DSBs. Whatever their nature, they may provide constitutive signals to p53 to maintain the senescence growth arrest.

Both damage- and telomere-initiated senescence depend strongly on p53 and are usually accompanied by expression of p21 (REFS 15,16,55) (FIG. 4). However, in many cells, DNA damage and dysfunctional telomeres also induce p16, albeit with delayed kinetics. p16, then, provides a second barrier to prevent the growth of cells with severely damaged DNA or dysfunctional telomeres[52,71,72].

Many chemotherapeutic drugs cause severe DNA damage. As expected, such drugs induce senescence in normal cells. Surprisingly, they also induce senescence in some tumour cells, both in culture and *in vivo*[73]. Tumour cells with wild-type, as opposed to mutant, p53 are more likely to senesce in response to chemotherapy, at

least in cell culture and cancer-prone mouse models[74–76], consistent with a pivotal role for p53 in damage-induced senescence. Of practical importance, DNA-damaging therapies are more likely to be efficacious in tumours that senesce, compared with those that do not[73–76].

Senescence caused by chromatin perturbation. The chromatin state determines the extent to which genes are active (euchromatin) or silent (heterochromatin), and depends mainly on histone modifications (for example, acetylation and methylation). Interestingly, chemical histone deacetylase inhibition (HDAi), which promotes euchromatin formation, induces senescence[17,77]. The mechanism by which this occurs is poorly understood, and may differ depending on the species and cell type. For example, in human fibroblasts, HDAi sequentially induces p21 and p16 expression, and the senescence growth arrest critically depends on the presence of pRB. By contrast, in mouse fibroblasts, the p53 pathway is more important for the senescence response to HDAi (REF. 77). Because HDAi can induce ATM kinase activity[78], HDAi might cause senescence in some cells by initiating a p53-dependent DDR.

The finding that HDAi causes senescence appears to conflict with the role of heterochromatin and SAHFs in establishing and maintaining the senescent growth arrest[45]. Likewise, HDAi-induced senescence seems to be in conflict with the finding that downregulation of a histone acetyl transferase, which promotes heterochromatin formation, induces senescence[79]. It is not known how senescence can be triggered both by heterochromatin disruption and by activities that are associated with heterochromatin formation. Both manipulations cause extensive but incomplete changes in chromatin organization, so each may alter the expression of different critical genes, and the response may be cell-type specific. Understanding this paradox could be important, because HDAi holds promise for treating certain cancers[80].

Oncogene-induced senescence. Oncogenes are mutant versions of normal genes that have the potential to transform cells in conjunction with additional mutations. Normal cells respond to many oncogenes by undergoing senescence. This phenomenon was first observed when an oncogenic form of RAS, a cytoplasmic transducer of mitogenic signals, was expressed in normal human fibroblasts[18]. Subsequently, other members of the RAS signalling pathway (for example, RAF, MEK, MOS and BRAF), as well as pro-proliferative nuclear proteins (for example, E2F-1), were shown to cause senescence when overexpressed or expressed as oncogenic versions[22,81–83].

Because oncogenes that induce senescence stimulate cell growth, the senescence response may counteract excessive mitogenic stimulation, which puts cells at risk of oncogenic transformation. This idea is supported by the finding that mouse cells that are cultured in serum-free medium (which reduces the high mitogenic pressure of serum) resist RAS-induced senescence[84]. Likewise, some rodent cells do not replicatively senesce (BOX 1) in serum-free medium, which suggests that excessive mitogenic stimulation is responsible for their senescence[85,86].

Euchromatin
Chromatin that is in an open conformation and hence accessible. Also termed active chromatin.

Heterochromatin
Chromatin that is in a closed conformation and hence inaccessible. Also termed silent or inactive chromatin. Chromatin probably exists in many forms between the extremes of euchromatin and heterochromatin.
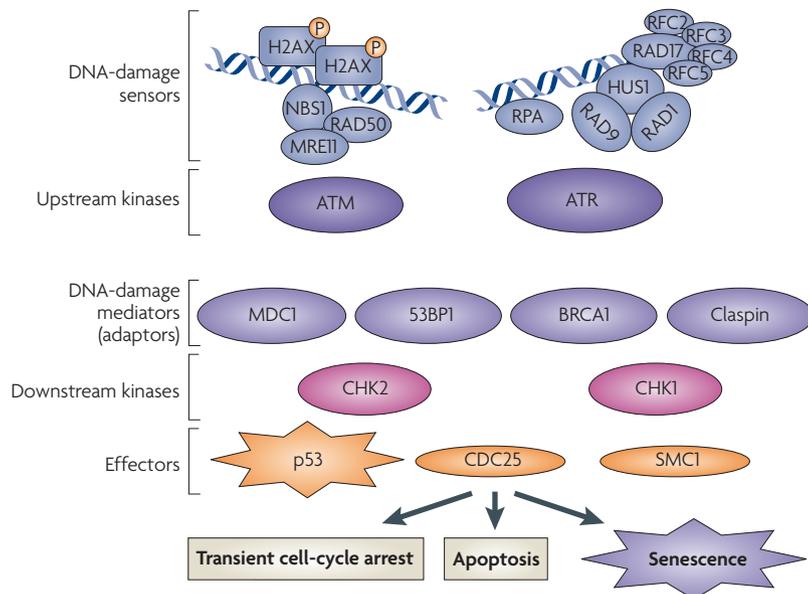
Figure 3 | **The DNA-damage response.** DNA damage in the form of DNA double-strand breaks and other DNA discontinuities is thought to be sensed by a host of factors such as replication protein A (RPA) and replication factor C (RFC)-like complexes (which contain the cell-cycle-checkpoint protein RAD17). These complexes recruit the 911 complex (RAD9–HUS1–RAD1). Damage is also sensed by the MRN complex (MRE11–RAD50–NBS1). Detection of DNA damage then leads to the activation of upstream protein kinases such as ataxia telangiectasia mutated (ATM) and ATM and Rad-3 related (ATR), which trigger immediate events such as phosphorylation of the histone variant H2AX. The modified chromatin recruits multiple proteins. Some of these proteins augment signalling by the upstream kinases, participate in transducing the damage signal and optimize repair activities by other proteins, the identity of which depends on the nature of the damage and position in the cell cycle (for example, the DNA end-stabilizing heterodimers Ku70/80, DNA ligases such as ligase IV, exonucleases such as MRE11, DNA helicases such as BLM). Several adaptor proteins, including MDC1, 53BP1, BRCA1 and claspin, orchestrate the orderly recruitment of DNA-damage response proteins, as well as the function of downstream kinases such as checkpoint-1 (CHK1) and CHK2, which propagate the damage signal to effector molecules such as SMC1, CDC25 and the tumour suppressor p53. The effector molecules halt cell-cycle progression, either transiently or permanently (senescence), or trigger cell death (apoptosis). 53BP1, p53-binding protein-1; BRCA1, breast cancer type-1 susceptibility protein; HUS1, hydroxyurea-sensitive-1 protein; MDC1, mediator of DNA damage checkpoint protein-1; MRE11, meiotic recombination-11 protein; NBS1, Nijmegen breakage syndrome-1 protein; SMC1, structural maintenance of chromosomes protein-1.

Is oncogene-induced senescence distinct from chromatin or telomere/damage-induced senescence? Perhaps not; for example, oncogenic RAS induces p16 and the formation of SAHFs[45,87,88]. Moreover, although oncogene-induced senescence does not entail telomere shortening, many oncogenes induce a robust DDR owing to the DNA damage that is caused by aberrant DNA replication. This DDR has a causal role in both the initiation and maintenance of oncogene-induced senescence because its experimental downregulation prevents senescence, allows cell proliferation and predisposes cells to oncogenic transformation[20,89].

Oncogene-induced senescence was first identified in cultured cells and does not occur in all cells[90,91], so is it physiologically relevant? Recent findings show that oncogenes elicit a senescence response that curtails the development of cancer[83,92–95]. In mice, strong mitogenic signals that are caused by activated oncogenes, or loss of the tumour suppressor protein PTEN (which dampens mitogenic signals), cause benign lesions that consist of senescent cells. Likewise, benign naevi in human skin contain cells that express oncogenic BRAF and are senescent. These findings suggest that oncogene-induced senescence occurs and suppresses tumorigenesis *in vivo*. Tumorigenesis requires additional mutations — notably in p53 or p16 — that prevent or perhaps reverse the senescence growth arrest[92,93,95].

Stress and other inducers of senescence. Sustained signalling by certain anti-proliferative cytokines, such as interferon-β, also causes senescence. Acute interferon-β stimulation reversibly arrests cell growth, but chronic stimulation increases intracellular oxygen radicals and elicits a p53-dependent DDR and senescence[96]. Likewise, chronic signalling by transforming growth factor-β, an inhibitor of epithelial cell proliferation, induces senescence by promoting p16–pRB-dependent heterochromatin formation[97,98].

Finally, the loosely defined phenomenon referred to as cell-culture stress, or 'culture shock', can induce p16-dependent, telomere-independent senescence. For example, human keratinocytes and mammary epithelial cells spontaneously express p16 and senesce with long telomeres under standard culture conditions. This does not occur when the cells are cultured on feeder layers ('lawns' of fibroblasts), but after many doublings on feeder layers, the cells eventually undergo telomere-dependent senescence[99]. These findings suggest that, in addition to hyperphysiological growth conditions, inadequate growth conditions also induce senescence.

Some cells lack a p16-dependent senescence response because the gene encoding p16 is silenced, often by DNA methylation[100,101]. Many human cell cultures, including fibroblasts, are heterogeneous and replicatively senesce as mosaics; some cells senesce due to the expression of p16 and others senesce due to telomere shortening and a p53-dependent DDR[16,52,53]. The stimuli that induce p16 are poorly understood. In some cells, oxidative stress induces p16 (REFS 70,102), but this is not always the case[53]. Oncogenic RAS can induce p16 by phosphorylating and activating ETS transcription factors[87], but expression of p16 is controlled by multiple factors, including the chromatin state[44,77,103].

The expression of p16 and a loss of p16 inducibility also occur *in vivo*. Expression of p16 increases with age in many murine tissues[51,104], and was recently shown to increase in murine haematopoietic, neuronal and pancreatic stem or progenitor cells *in vivo*[105–107]. This expression prevents stem-cell proliferation, possibly by inducing senescence. Moreover, human mammary epithelial cells spontaneously silence p16 via promoter methylation *in vivo*[108] such that, as in culture, the adult breast epithelial compartment is mosaic for the expression of p16.

Control by the p53 and p16–pRB pathways

The senescence growth arrest is established and maintained by the p53 and p16–pRB tumour suppressor pathways (FIG. 4). These pathways interact but can

independently halt cell-cycle progression. To some extent, they also respond to different stimuli. In addition, there are both cell-type-specific and species-specific differences in the propensity with which cells engage one or the other pathway, and in the ability of each pathway to induce senescence. Finally, although most cells senesce owing to engagement of the p53 pathway, p16–pRB pathway, or both, there are examples of senescence that appear to be independent of these pathways[21,83]. These examples raise the possibility of a p53- and p16–pRB-independent senescence pathway(s).

The p53 pathway. Stimuli that generate a DDR (for example, ionizing radiation and telomere dysfunction) induce senescence primarily through the p53 pathway. This pathway is regulated at multiple points by proteins such as the E3 ubiquitin-protein ligase HDM2 (MDM2 in mice), which facilitates p53 degradation, and the alternate-reading-frame protein (ARF), which inhibits HDM2 activity[42]. p21 is a crucial transcriptional target of p53 and mediator of p53-dependent senescence[109]. However, p21 also mediates a transient DNA-damage-induced growth arrest. So what determines whether cells senesce or arrest transiently? The answer is currently unknown. One possibility is that rapid DNA repair quickly terminates p53–p21 signalling, whereas slow, incomplete or faulty repair results in sustained signalling and senescence. Whatever the case, experimental reduction in p53, p21 or DDR proteins (for example, ATM or CHK2) prevents telomere- or damage-induced senescence, and, in some cells (for example, those that express little or no p16 or oncogenic RAS), it can even reverse the senescence growth arrest[20,52,55,64,109,110]. The proliferation of damaged cells with a reduced DDR or p53 function often cannot be sustained, however, because telomeres eventually become severely eroded, leading to a state of extensive genomic instability and cell death that is termed crisis or mitotic catastrophe (BOX 1). Rare mutational or epigenetic events can activate TERT expression or recombination mechanisms to elongate telomeres[111]. Such cells are at a high risk of malignant transformation because they can replicate indefinitely and harbour mutations and chromosomal abnormalities.

The DDR and p53 pathway provide a first line of defence against cancer by preventing the growth of cells with severely damaged DNA, which are at risk of developing and propagating oncogenic mutations[112,113]. However, the loss of p53 or an intact DDR — either before cells experience damage or after damage-induced senescence — usually leads to mitotic catastrophe, which acts as a second barrier that must be overcome by telomere stabilization[111].

The p16–pRB pathway. Stimuli that produce a DDR can also engage the p16–pRB pathway, but this usually occurs secondary to engagement of the p53 pathway[71,72]. Nonetheless, some senescence-inducing stimuli act primarily through the p16–pRB pathway. This is particularly true of epithelial cells, which are more prone than fibroblasts to inducing p16 and arresting proliferation, at least in culture. Furthermore, there are species-specific differences:
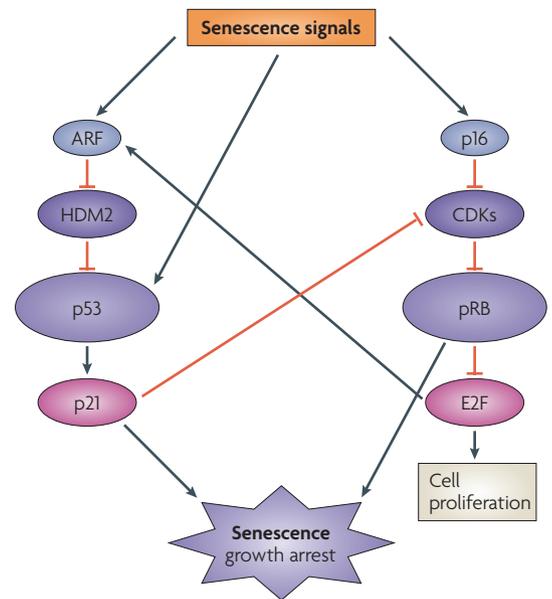

Figure 4 | **Senescence controlled by the p53 and p16–pRB pathways.** Senescence-inducing signals, including those that trigger a DNA-damage response (DDR), as well as many other stresses (FIG. 1), usually engage either the p53 or the p16–retinoblastoma protein (pRB) tumour suppressor pathways. Some signals, such as oncogenic RAS, engage both pathways. p53 is negatively regulated by the E3 ubiquitin-protein ligase HDM2 (MDM2 in mice), which facilitates its degradation, and HDM2 is negatively regulated by the alternate-reading-frame protein (ARF). Active p53 establishes the senescence growth arrest in part by inducing the expression of p21, a cyclin-dependent kinase (CDK) inhibitor that, among other activities, suppresses the phosphorylation and, hence, the inactivation of pRB. Senescence signals that engage the p16–pRB pathway generally do so by inducing the expression of p16, another CDK inhibitor that prevents pRB phosphorylation and inactivation. pRB halts cell proliferation by suppressing the activity of E2F, a transcription factor that stimulates the expression of genes that are required for cell-cycle progression. E2F can also curtail proliferation by inducing ARF expression, which engages the p53 pathway. So, there is reciprocal regulation between the p53 and p16–pRB pathways. Interactions among ARF, HDM2, p53, p21, CDKs, pRB and E2F also occur in other cell contexts — for example, during the DDR and reversible or transient growth arrest — so it not yet clear how senescence, as opposed to quiescence or transient growth arrest, is established. It is noteworthy, however, that at least in cell-culture studies, upregulation of p16 is not part of the immediate DDR and does not occur during transient growth arrests or quiescence.

for example, experimental disruption of telomeres primarily engages the p53 pathway in mouse cells but both the p53 and p16–pRB pathways in human cells[114].

Oncogenic RAS induces p16 expression by activating ETS transcription factors; ETS activity is counteracted by ID proteins[87], which are downregulated in senescent cells[115]. It is not clear how other senescence-causing stimuli induce p16 expression. One possible mechanism is the reduced expression of Polycomb INK4a repressors such

as BMI1 (REFS 53,116) and CBX7 (REF. 117). Consistent with this idea, BMI1 or CBX7 overexpression extends the replicative lifespan of human and mouse fibroblasts[53,103,117].

p16 and p21 are both CDKIs; hence, both can keep pRB in an active, hypophosphorylated form, thereby preventing E2F from transcribing genes that are needed for proliferation[42]. However, p16 and p21 are clearly not equivalent[118]. Cells that senesce solely due to p53–p21 activation can resume growth after inactivation of the p53 pathway, and do so for many cell doublings until crisis or mitotic catastrophe occurs[20,52,55,64,110]. However, although cells that senesce due to oncogenic RAS (which induces p16 expression) can resume limited proliferation[20], cells that fully engage the p16–pRB pathway for several days usually cannot resume growth even after inactivation of p53, pRB or p16 (REF. 52). Moreover, the loss of p16–pRB activity upregulates p53 and p21 expression in part because E2F also stimulates ARF expression[119,120]. Despite reciprocal regulation between the p53 and p16–pRB pathways (FIG. 4), there are differences in how cells respond when one or the other pathway mediates a senescence response.

The p16–pRB pathway is crucial for generating SAHFs, which silence the genes that are needed for proliferation[45]. SAHFs require several days to develop, during which time there are transient interactions among chromatin-modifying proteins such as HIRA (histone repressor A), ASF1a (anti-silencing function-1a) and HP1 (REF. 88). Ultimately, each SAHF contains portions of a single condensed chromosome, which is depleted for the linker histone H1 and enriched for HP1 and the histone variant macroH2A[121,122]. Like the growth arrest, once established, SAHFs no longer require p16 or pRB for maintenance[45]. These findings suggest that the p16–pRB pathway can establish self-maintaining senescence-associated heterochromatin. This activity may be due to the ability of pRB to complex with histone-modifying enzymes that form repressive chromatin[123]. Although SAHFs are not present in all senescent cells, the p16–pRB pathway might establish chromatin states that are functionally, if not cytologically, equivalent to SAHFs in cells that do not develop these structures.

Significance of senescence *in vivo*

Hayflick's observations[1] were made using cultured cells, and much of our current understanding of the causes and consequences of senescence still derives from cell cultures. Only during the past decade or so has cellular senescence been shown to occur and to be important *in vivo*.

Senescent cells **in vivo.** Senescence-associated markers (see above) have been used to identify senescent cells *in vivo*, with the caveat that none of these markers are exclusive to the senescent state. In rodents, primates and humans, senescent cells are found in many renewable tissues, including the vasculature, haematopoietic system, many epithelial organs and the stroma[12,49,51,124]. Notably, cells that express one or more senescence markers are relatively rare in young organisms, but their number increases with age. How abundant are senescent cells in aged organisms? Estimates vary widely depending on the study, species and tissue, ranging from <1% to >15%. It is difficult to know the cause of the senescence response from these studies. However, among the senescence markers that accumulate with age are SDFs that co-localize with telomeres, suggesting that, at least in some tissues, telomere dysfunction causes senescence *in vivo*.

Cells that express senescence markers are also found at sites of chronic age-related pathology, such as osteoarthritis and atherosclerosis[125–127]. Thus, senescent cells are associated with ageing and age-related diseases *in vivo*, as suggested by Hayflick's early experiments. In addition, senescent cells are associated with benign dysplastic or preneoplastic lesions[83,92–94] and benign prostatic hyperplasia[128], but not with malignant tumours. They are also found in normal and tumour tissues following DNA-damaging chemotherapy[73–76]. These findings support the second speculation from Hayflick's experiments — that cellular senescence suppresses the development of cancer. Senescent cells are therefore found at appropriate times and places for their proposed roles in tumour suppression and ageing.

Why do senescent cells accumulate *in vivo*? The answer to this question is not known. Recent findings suggest that senescent cells, at least those induced by acute p53 activation in murine tumour models, can be cleared by host mechanisms such as the immune system[129,130]. Virtually nothing is known about how senescent cells are recognized by the immune system, whether additional mechanisms clear them *in vivo* or whether clearance mechanisms change with age or in age-related diseases. Likewise, nothing is known about whether the senescent cells found *in vivo* have escaped clearance or are in the process of being cleared.

Cellular senescence and cancer. Senescence-inducing stimuli are potentially oncogenic, and cancer cells must acquire mutations that allow them to avoid telomere-dependent and oncogene-induced senescence[2,93,131–133]. These mutations typically occur in the p53 and p16–pRB pathways. Of course, these pathways have multiple activities, all of which may contribute to their tumour suppressor activities. Nonetheless, there are several instances in which loss of the senescence response appears to be a crucial, albeit insufficient, step in the development of cancer. For example, genetically engineered mice that are deficient in a histone methyltransferase or p53 contain cells that fail to senesce in response to appropriate stimuli; these mice are invariably cancer-prone[92,93,134]. Likewise, cells from patients with Li-Fraumeni syndrome, which carry mutations in p53 or CHK2 (REFS 135,136), overcome senescence much more readily than normal cells[137]; humans with these mutations are also cancer-prone. Finally, as noted earlier, some preneoplastic lesions contain large numbers of cells that express senescence markers, which suggests that a senescence response halts their progression to malignancy.

Cellular senescence probably suppresses tumorigenesis because cancer development requires cell proliferation[2], so any mechanism that stringently prevents cell growth
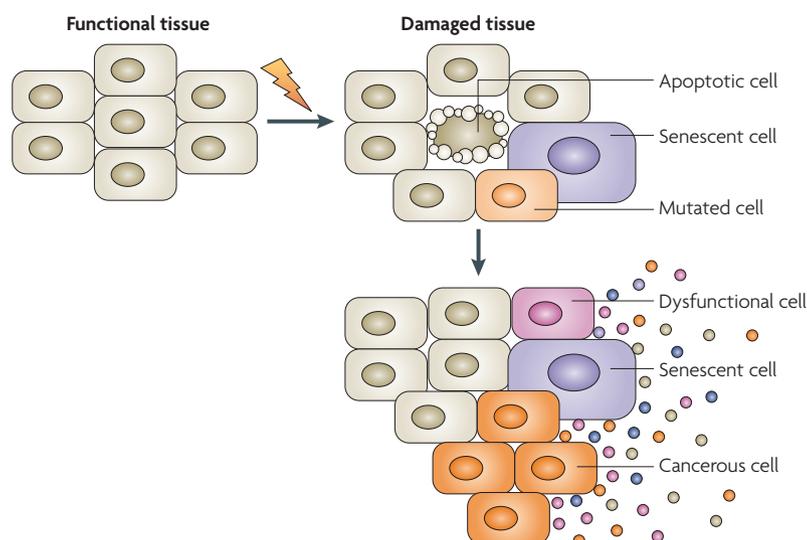
Figure 5 | **Potential deleterious effects of senescent cells.** Damage to cells within tissues can result in several outcomes. Of course, the damage may be completely repaired, restoring the cell and tissue to its pre-damaged state. Excessive or irreparable damage, however, can cause cell death (apoptosis), senescence or an oncogenic mutation. The division of a neighbouring cell, or a stem or progenitor cell, usually replaces apoptotic cells. Cell division, however, increases the risk of fixing DNA damage as an oncogenic mutation, leaving the tissue with pre-malignant or potentially malignant cells. Senescent cells, by contrast, may not be readily replaced; in any case, their number can increase with age. Senescent cells secrete various factors that can alter or inhibit the ability of neighbouring cells to function, resulting in dysfunctional cells. They can also stimulate the proliferation and malignant progression of nearby premalignant cells. Therefore, an accumulation of senescent cells can both compromise normal tissue function and facilitate cancer progression.

will, a priori, prevent cancer. However, failure to senesce is usually insufficient for malignant transformation. This is particularly true for replicative senescence; for example, telomerase prevents telomere-initiated senescence, but does not confer malignant properties on cells[138]. Likewise, the inactivation of p16, p21 or certain DDR genes — or even p53 or pRB (for example, by viral oncogenes) — increases the replicative lifespan of human cells but does not transform them *per se*[20,109,139]. Such cells usually enter crisis, from which rare replicatively immortal cells (which have overcome the end-replication problem) can arise[139]. Even then, the immortal cells may not be tumorigenic until they acquire mutations that activate mitogenic oncogenes such as RAS or inactivate tumour suppressors that dampen mitogenic signals such as PTEN[83,92,93,140]. Thus, genetic or epigenetic events that avert replicative senescence are necessary but insufficient for malignant tumorigenesis.

Senescence reversal can occur if cells senesce without fully engaging the p16–pRB pathway and subsequently lose p53 function[52]. It is not known whether this occurs *in vivo*. However, cells with silenced (methylated) p16 exist in apparently normal tissue[108]; should such cells senesce (for example, in response to telomere dysfunction or DNA damage) and subsequently lose p53 function, they could, in principle, resume proliferation. Likewise, senescent p16-negative cells in dysplastic naevi[83] could acquire a mutation that inactivates p53

function and revert to a proliferating state. Non-dividing cells can acquire mutations[4] so these scenarios are not impossible, but it is not clear whether they are plausible. Whatever the case, it is apparent that senescence poses a formidable but not insurmountable barrier to cancer progression.

Cellular senescence and ageing. The link between cellular senescence and ageing is more tentative than the link to cancer[12]. As noted earlier, the number of senescent cells increases with age, and senescent cells are present at sites of age-related pathology. Further, recent findings implicate p16-dependent senescence in three hallmarks of ageing — decrements in neurogenesis, haematopoiesis and pancreatic function[105–107]. p16 expression increases with age in the stem and progenitor cells of the mouse brain, bone marrow and pancreas, where it suppresses stem-cell proliferation and tissue regeneration. This rise in p16-positive stem cells is apparent in early middle-age. Strikingly, the age-related decline in stem-cell growth and tissue regeneration is substantially retarded in mice that have been genetically engineered to lack p16 expression. However, as expected, these mice die prematurely (in late middle-age) of cancer.

The age-dependent rise in p16-positive stem and progenitor cells is consistent with the idea that stem-cell senescence might at least partly explain the age-related decline in brain and bone-marrow function and the development of type II diabetes. However, it is not yet known whether the p16-positive stem and progenitor cells are in fact senescent. So, it is possible that both the tumour-suppressive and pro-ageing activities of p16 are partly due to growth suppression without senescence. Nonetheless, these findings suggest that the tumour-suppressive activity of p16 might be inextricably linked to its pro-ageing effects. A similar trade-off between tumour suppression and ageing is seen in mice with constitutively hyperactive forms of p53 — animals that express these forms are remarkably tumour-free, but show multiple signs of accelerated ageing[141,142], which are at least partly due to their increased sensitivity to senescence-inducing stimuli[142].

A second mechanism by which senescent cells might contribute to ageing comes from their altered pattern of gene expression — specifically, the upregulation of genes that encode extracellular-matrix-degrading enzymes, inflammatory cytokines and growth factors, which can affect the behaviour of neighbouring cells or even distal cells within tissues[12] (FIG. 5). These secreted factors can disrupt the normal tissue structure and function in cell-culture models (for example, the functional and morphological differentiation of mammary epithelial cells or epidermal keratinocytes)[143,144]. Moreover, factors secreted by senescent cells can stimulate the growth and angiogenic activity of nearby premalignant cells, both in culture and *in vivo*[145–149]. Therefore, senescent cells, which themselves cannot form tumours, may fuel the progression of nearby premalignant cells, thereby — ironically — facilitating the development of cancer in ageing organisms. Together, these findings support the idea that the senescence response is antagonistically

pleiotropic, and balances the benefits of suppressing cancer in young organisms against promoting the development of deleterious ageing phenotypes[8,12].

Future questions and directions

Despite the transition from cell-culture curiosity to potential regulator of cancer and ageing, cellular senescence remains enigmatic and continues to pose a host of questions. Precisely how do the p53 and p16–pRB pathways establish and, equally importantly, maintain the senescent growth arrest? Both of these tumour-suppressor pathways also cause transient or reversible cell-cycle arrests, so how are their activities modified by senescence-inducing signals? For that matter, how do cells 'decide' whether to undergo a transient growth arrest, senescence or apoptosis in response to damage or stress signals? The signals that induce p16, both in culture and *in vivo*, are especially obscure at present.

Likewise, little is known about the mechanisms that are responsible for the apparently deleterious senescent secretory phenotype. How and why does this phenotype develop? Finally, how does the senescence response balance tumour suppression, tissue regeneration and ageing phenotypes? Clearly, it would not be desirable to reverse the senescence growth arrest — this would allow damaged, stressed or oncogene-expressing cells to proliferate and therefore increase the risk of cancer. But will it be possible to eliminate the deleterious (pro-ageing) aspects of cellular senescence (for example, the senescent secretory phenotype) without reversing the tumour-suppressive growth arrest? The generation of engineered mice that carry additional copies of properly regulated p53 (REF. 150) or p16 and ARF[151], or have reduced activity of the negative p53 regulator MDM2 (REF. 152), lend hope to this possibility. These mice develop little cancer without signs of accelerated ageing. However, the lifespans of these mice were not significantly longer than control mice, despite cancer being a major cause of death in mice. It remains to be seen whether other, possibly non-lethal, age-related pathologies were accelerated or exacerbated. The existence of these mice also raises the possibility that there is (or can be) little or no trade-off between tumour suppression and longevity, if not between tumour suppression and ageing phenotypes or certain age-related pathologies. Whatever the case, it might be possible, through specific interventions, to ameliorate any antagonistically pleiotropic effects that tumour suppressors might have.

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Li-Fraumeni syndrome | type II diabetes
UniProtKB: http://ca.expasy.org/sprot
ATM | CHK2 | E2F | HDM2 | p16 | p21 | p53 | pRB | PTEN

FURTHER INFORMATION
Judith Campisi's homepages:
http://www.buckinstitute.org/site/index.php?option=com_content&task=view&id=113&Itemid=100
http://www.lbl.gov/lifesciences/labs/campisi_lab.html
Fabrizio d'Adda di Fagagna's homepage:
http://www.ifom-ieo-campus.it/research/dadda.php
ALL LINKS ARE ACTIVE IN THE ONLINE PDF.