Mechanisms of Ageing and Development 170 (2018) 2–9

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Review

Stress, cell senescence and organismal ageing

João Pedro de Magalhães[a,*,1], João F. Passos[b,1]

[a] Integrative Genomics of Ageing Group, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool L7 8TX, United Kingdom
[b] Institute for Cell and Molecular Biosciences & Newcastle University Institute for Ageing, Newcastle upon Tyne NE4 5PL, United Kingdom

ARTICLE INFO

Keywords:
Biogerontology
Drugs
Lifespan
Longevity
Telomeres

ABSTRACT

Cellular senescence was first described by Hayflick and Moorhead in the 1960s as the irreversible arrest of cells following prolonged cultivation. Telomere shortening is the key mechanism driving replicative senescence in human fibroblasts. Later, pioneering work by Olivier Toussaint and others showed that stress plays a major role in the induction of senescence *in vitro*, a phenomenon known as stress-induced premature senescence or SIPS. It is also now widely accepted that senescence plays a role *in vivo*. An emerging body of evidence from animal models, and particularly mice, has demonstrated an important role for senescence in several processes such as embryonic development, wound healing, tumour suppression and ageing. However, mostly due to a lack of availability of tissues and specific markers, less is known about the importance of cell senescence in humans. In this review, we summarize some of the key findings in the field of senescence, stress-induced senescence and telomeres. We focus particularly on the role of telomere dysfunction and senescence during the ageing process as well as potential interventions, including pharmacological approaches like telomerase activators and senolytics, to counteract their detrimental effects in ageing and disease.

1. Introduction

Because cells are the fundamental building blocks of humans and animals, it is clear that cellular changes contribute to the ageing process. A major open question, however, is the nature of those changes and how exactly they contribute to degeneration and disease in old age. In 1961, Leonard Hayflick and Paul Moorhead discovered that human cells can only divide a finite number of times in culture (Hayflick and Moorhead, 1961). The limited proliferative ability of human cells *in vitro*, known as replicative senescence (RS), has since become a major focus of research in biogerontology. Hayflick and Moorhead worked with fibroblasts, a cell type found in connective tissue, but RS has been found in other cell types (de Magalhaes and Toussaint, 2004c): keratinocytes, endothelial cells, lymphocytes, adrenocortical cells, vascular smooth muscle cells, chondrocytes, etc. In addition, RS is observed in cells derived from embryonic tissues, in cells from adults of all ages, and in cells taken from many animals: mice, chickens, Galapagos tortoises, etc. (Hayflick, 1994). The number of divisions cells undergo in culture varies considerably between cell types and species.

In addition to RS, a number of factors can accelerate and/or trigger cell senescence, including various forms of stress like oxidative stress. Depending on the dose of stressor used, a cell population will react in different ways. For instance, a high cytotoxic dosage will cause such an amount of damage that cellular biochemical activities decrease leading to cellular death by necrosis. The level of cumulative damage sustained by cells determines whether programmed cell death–apoptosis–can unfold or, if the damage is lower, senescence. Since a cellular population is not homogeneous, the dosage of the stressor will shift the percentage of cells executing each of the possible programs depending on the amount of stress, respectively, from no stress to high stress: cellular proliferation, senescence, apoptosis, and necrosis (Toussaint et al., 2002). Oncogenes such as *ras* can also induce senescence (Serrano et al., 1997), though oncogene-induced senescence will not be discussed here at length. The way sublethal stress can accelerate the appearance of the senescent phenotype in cells has been deemed as another form of cellular senescence, termed stress-induced premature senescence (SIPS) (Brack et al., 2000). The work of Olivier Toussaint focused primarily on senescence, and specifically replicative senescence and SIPS. Our aim here is to review the potential impact of senescence and SIPS on the ageing of whole organisms and humans in particular.

2. Of telomeres and senescence

During *in vitro* ageing, the telomeres shorten gradually in each subcultivation (Harley et al., 1990). Telomere shortening is the primary cause of RS in human fibroblasts, since it has been shown that ectopic

* Corresponding author.
E-mail addresses: jp@senescence.info (J.P. de Magalhães), joao.passos@newcastle.ac.uk (J.F. Passos).
[1] The authors contributed equally to this work.

http://dx.doi.org/10.1016/j.mad.2017.07.001
Received 28 March 2017; Received in revised form 17 June 2017; Accepted 4 July 2017
Available online 05 July 2017

expression of telomerase, an enzyme able to counteract telomere shortening, is able to bypass the senescence-induced arrest (Bodnar et al., 1998).

Telomeres are tandem TTAGGG repeats found at the ends of chromosomes, associated with several telomere-binding proteins. With each cell division telomeres shorten progressively, due to the so called "end-replication problem". This is due to the intrinsic inability of DNA polymerases to replicate the telomere C-rich lagging-strand. During the process of lagging-strand synthesis, RNA primers allow DNA polymerases to initiate DNA replication; however, upon removal of the last primer from the 3′ end, the newly synthesized strand will inevitably be a few nucleotides shorter (de Lange, 2005).

We now understand more clearly why the shortening of telomeres results in senescence. Telomeres are thought to form a lariat-like structure called telomere-loop (t-loop) which is stabilized by several telomere binding proteins collectively known as the "shelterin" complex (de Lange, 2005). It is thought that with each cell division, telomere shortening precipitates the loss of "shelterin" components and the destabilization of the t-loop conformation, resulting in exposure of the telomere end (Griffith et al., 1999). Consistent with this hypothesis, depletion of "shelterin" components such as TRF2 or POT1 leads to activation of a DNA damage response (DDR) at telomere ends (Takai et al., 2003; Wu et al., 2006). Moreover, it was shown that during RS, fibroblasts accumulate proteins involved in the DDR at telomeric regions, including γH2A.X, 53BP1, MDC1 and NBS1 (d'Adda di Fagagna et al., 2003). DDR activation can result in the activation of transcription factor p53 which is involved in a variety of processes including DNA repair and apoptosis, but most notably results in expression of cyclin-dependent kinase inhibitor p21, which together with activation of p16, are thought to be the major pathways in the induction of senescence (Beauséjour et al., 2003).

3. Is SIPS telomere-independent?

Traditionally, SIPS was thought to be distinct from RS due to the lack of involvement of telomere shortening (Parrinello et al., 2003). Exposure to different types of acute sub-lethal stresses such as oxidative stress and DNA damaging agents was shown to induce cellular senescence in different cell types at relatively short periods of time (ranging from 3 to 10 days) without or with modest telomere shortening. This led to the widespread idea that telomeres were not involved in this process, since considerable cell division would be required for telomeres to shorten until reaching a critical length. Moreover, mouse-derived embryonic fibroblasts, which have long telomeres and express the enzyme telomerase, were shown to undergo SIPS (Parrinello et al., 2003). Work by Olivier Toussaint also showed that telomerase expression in normal human fibroblasts does not protect against SIPS, suggesting that mechanisms other than telomere shortening must cause the appearance of the senescence phenotype (de Magalhães et al., 2002). SIPS was then suggested to be mediated by oxidative DNA damage and occurring independently of telomeres.

However, recent data has challenged the concept that SIPS is a telomere-independent process. Induction of SIPS is characterized initially by DNA damage, which occurs randomly all over the genome, and subsequent activation of a DDR. As the senescent phenotype develops, most of the DNA damage is repaired within 24 h; however, damage at telomeric regions remains unrepaired for months, contributing to a persistent and unresolved DNA damage response (Hewitt et al., 2012). Interestingly, these telomeric lesions occur irrespectively of telomere length and the presence or absence of the enzyme telomerase (Fumagalli et al., 2012; Hewitt et al., 2012). Thus, while SIPS induced by sub-lethal acute stress is not driven by the shortening of telomeres *per se*, in some circumstances SIPS can still be classified as a telomere-dependent process.

Mechanistically, there is a reason why telomeric regions differ from the rest of the genome in terms of repair. Telomere-binding proteins such as TRF2 have been shown to play a role in the inhibition of non-homologous end joining as a way to prevent telomere end-to-end fusions, precursors of genomic instability in cells. Similarly, when cells are exposed to oxidative or DNA damaging agents, if damage is induced in telomeric regions, TRF2 inhibits repair and contributes to an unresolved DDR which helps stabilize the senescence arrest (Fumagalli et al., 2012).

Damage at telomeric regions is not exclusive to cells *in vitro*. Normal mice show an age-dependent increase in telomeric-associated DNA damage foci (TAF) in the gut, liver and lung of mice, which occurs independently of telomere length (Birch et al., 2015; Hewitt et al., 2012). Telomere damage irrespectively of length has also been observed with age in hippocampal neurons and in the liver of baboons (Fumagalli et al., 2012). Moreover, analysis of individual telomeres in small airway epithelial cells in the lungs of Chronic Obstructive Pulmonary Disease (COPD) patients, which show increased senescence markers such as p16, has also revealed that damaged telomeres are not significantly shorter (Birch et al., 2015).

Telomere-length independent senescence was also observed in the context of oncogene-induced senescence. In melanocytic nevi it was shown that telomeres signalling a DDR were not shorter when compared to functional ones (Suram et al., 2012). Interestingly, studies have suggested that having long telomeres may in fact be disadvantageous. In a study where telomeres were elongated in human cancer cells, it was shown that cells with very long telomeres were more sensitive to ionising irradiation (Fairlie and Harrington, 2015).

4. The role of telomere-induced senescence *in vivo*

In vivo, telomere length is heterogeneous (Serra and von Zglinicki, 2002; Takubo et al., 2002). Telomere shortening *in vivo* has been reported (de Magalhaes and Toussaint, 2004c), for example in liver cells (Aikata et al., 2000), lymphocytes (Pan et al., 1997), skin cells (Lindsey et al., 1991), blood (Iwama et al., 1998), and colon mucosa (Hastie et al., 1990). For instance, telomere shortening appears to impact on the function of immune T cells, and telomerase activators can restore a more youthful functional profile (Effros, 2009). Weak correlations between telomere length and donor age have been reported (Allsopp et al., 1992; Kammori et al., 2002; Njajou et al., 2007), though some studies found no correlation at all (Mondello et al., 1999; Nwosu et al., 2005; Renault et al., 2002; Serra and von Zglinicki, 2002; Takubo et al., 2002). Overall, telomere shortening may occur in some tissues *in vivo* in association with certain pathologies and with age. An association between telomere length and mortality has been reported in people aged 60 and over (Cawthon et al., 2003), and telomere shortening appears to be accelerated in people living more stressful lives (Epel et al., 2004). While these results support the idea that telomere shortening is a marker of stress and age-related pathology, they do not prove that telomere shortening is a causal factor in ageing.

Probably, the best evidence for a causal role of telomeres in the ageing process derives from data from telomerase-deficient mice. Mice carrying a homozygous deletion of the RNA component of telomerase (mTERC) only experience accelerated age-related degeneration after being bred for several generations, which results in telomere shortening (Blasco et al., 1997). This in turn results in critically short telomeres which are responsible for an early onset of senescence and apoptosis (Lee et al., 1998) and reduced regenerative capacity of proliferative tissues such as the intestine and the hematopoietic system (Wong et al., 2003). Interestingly, deletion of p21 in these mice improves regenerative capacity of the intestine and hematopoietic stem cells (Choudhury et al., 2007). Reintroduction of telomerase in telomerase-deficient mice is able to revert the premature ageing phenotype observed in various tissues (Jaskelioff et al., 2011).

In terms of comparative biology, no connection appears to exist between the mean telomere length of cells and the longevity of mammalian species. Although humans are the longest-lived primate, they

have the shortest telomeres (Kakuo et al., 1999; Steinert et al., 2002). Mice have massively long telomeres and feature high telomerase activity in many organs, in contrast to humans (Prowse and Greider, 1995). Interestingly, inbred mice have long (Kipling and Cooke, 1990) while wild mice have short telomeres (Hemann and Greider, 2000). In rodents, telomerase activity correlates negatively with lifespan but does not correlate with longevity (Seluanov et al., 2007). The largest comparative study of telomeres and telomerase, involving over 60 mammalian species, found that smaller, short-lived species tend to have long telomeres and high levels of telomerase (Gomes et al., 2011). Taken together, it seems that the evolution of large body sizes and longevity is strongly associated with the evolution of short telomeres and telomerase suppression, presumably for tumour suppression (de Magalhaes, 2013; Gomes et al., 2011).

Altogether, these studies suggest that: 1) telomere length is not a limiting factor in an organism's lifespan, and 2) if senescence plays a detrimental role during ageing *in vivo* (discussed in the next section), this may occur independently of telomere length.

5. Do senescent cells accumulate with age in tissues?

For a long time it was debated whether the discovery made by Hayflick and Moorhead had any physiological relevance or was merely an artefact of cells grown in relatively artificial culture conditions. Hayflick and Moorhead proposed that senescence may represent ageing, however, recent data has revealed that this view is too simplistic, since senescence has been shown to play multiple important physiological roles, such as: tumour suppression (Serrano et al., 1997), tissue repair and wound healing (Demaria et al., 2014; Krizhanovsky et al., 2008), embryonic development (Muñoz-Espín et al., 2013; Storer et al., 2013) and age-related degeneration (Baker et al., 2011). Senescent cells also appear to have benefits in promoting insulin secretion by pancreatic beta cells (Helman et al., 2016). In addition, senescent cells have been detected in the context of many different age-related diseases, including atherosclerosis, lung disease, diabetes and many others (Munoz-Espin and Serrano, 2014).

Given the multitude of functions of senescent cells, which can be of a positive or negative nature depending on the context, it has been argued that there may be different types of senescence rather than a universal phenotype. For instance, senescence during embryonic development occurs transiently, since senescent cells are rapidly removed by the immune system after executing their role, and is not associated with the activation of a DDR (Muñoz-Espín et al., 2013). In contrast, during ageing, senescent cells are thought to be persistent, induced by random molecular damage and associated with the activation of a DDR (Munoz-Espin and Serrano, 2014). Recent work has demonstrated that senescent cells are able to attract (potentially via the secretion of chemokines) different immune cells, including Natural Killer cells, macrophages and T-lymphocytes which are involved in their specific clearance from tissues (Kang et al., 2011; Krizhanovsky et al., 2008; Xue et al., 2007). It is possible that persistence of senescent cells in tissues during ageing and age-related diseases is a consequence of the inability of the immune system to clear senescent cells – in view of the well reported decline of the immune system with age – however this has not yet been experimentally tested.

Do senescent cells accumulate with age? One of the main challenges to the study of senescence *in vivo* has been the absence of a universal marker that can unequivocally identify senescent cells. The most widely-used marker is the presence of senescence-associated β-galactosidase (SA-β-gal) activity. The enzyme β-galactosidase, a lysosomal hydrolase, is normally active at pH 4, but in senescent cells it often happens for β-galactosidase to be active at pH 6, a shift that can be detected with a simple biochemical assay. Both *in vitro* and *in vivo*, the percentage of cells positive for SA β-gal increases with, respectively, population doublings and age (Dimri et al., 1995). However, there are major limitations to the use of this marker, since SA-β-Gal staining can also be detected in immortalized cells and quiescent cells induced by both confluency and serum starvation (Cristofalo, 2005). Also, it has been suggested that a major limitation of using SA β-gal staining *in vivo* is a false-positive signal from macrophages and other pro-inflammatory cells (Hall et al., 2016). In addition, since it requires fresh tissues, its detection is not straightforward technically and has more than often generated conflicting results.

Given the challenge of identifying a specific marker able to identify senescent cells, most researchers currently rely on a multiple marker approach. Indeed, several markers have been identified which are closely associated with cellular senescence, including absence of proliferation markers (Lawless et al., 2010), changes in heterochromatin (Narita et al., 2003), telomere-associated DNA damage (Hewitt et al., 2012), expression of cyclin-dependent kinase inhibitors p21, p16 (Baker et al., 2016), and senescence-associated distension of satellites (Swanson et al., 2013). In a variety of mouse tissues, it is clear that most of these markers increase with age; however, given the fact that most of these markers are not exclusive for senescent cells, the exact frequency of senescent cells in older tissues is still unknown. Furthermore, given the limited availability of tissues, little is known about the accumulation of senescent cells with age in healthy humans.

Interestingly, many senescence markers have also been found in post-mitotic tissues such as neurons (Jurk et al., 2012), adipocytes (Baker et al., 2011; Minamino et al., 2009) and osteocytes (Farr et al., 2016), which goes against the dogma that senescence is restricted to proliferating cells. It is possible that with ageing, senescence-inducing pathways (which play roles in tumour suppression and during development) can be inadvertently switched on during ageing of post-mitotic cells. However, given that the primary characteristic of senescence is a permanent cell-cycle arrest, the consequences of the activation of these pathways in post-mitotic cells are still not understood.

6. Senescent cells can cause tissue disruption and/or degeneration

Apart from cell-cycle arrest, senescent cells have been shown to experience dramatic changes in terms of gene expression, metabolism, epigenome and, importantly, have a distinct secretome profile, known as the Senescence-Associated Secretory Phenotype (SASP) (Coppé et al., 2008). The SASP includes pro-inflammatory cytokines as well as chemokines, growth factors and extracellular matrix-degrading proteins which may impact on the tissue microenvironment. Indeed, work by Olivier Toussaint revealed overexpression of cytokines (e.g., IL6) and matrix metalloproteinase in late passage fibroblasts (de Magalhaes et al., 2004). The SASP is thought to have evolved as a way for senescent cells to communicate with the immune system (potentially to facilitate their own clearance), but also as an extracellular signal to promote the regeneration of tissues through the stimulation of nearby progenitor cells.

It has been shown that a "chronic" SASP is able to induce senescence in adjacent young cells, contributing to tissue dysfunction (Acosta et al., 2013; Jurk et al., 2014) and paradoxically tumorigenesis (Krtolica et al., 2001). Repeated stimulation of WI-38 human fibroblasts with pro-inflammatory cytokines interleukin-1 α (IL-1α) or tumour necrosis factor-α (TNF-α) induces SIPS (Dumont et al., 2000). These cytokines' circulating levels increase *in vivo* (Lio et al., 2003), favouring inflammation and perhaps contributing to SIPS *in vivo*; senescent cells might then also contribute to increase inflammatory levels, creating a positive feedback loop (Fig. 1). Senescent cells also show mitochondrial dysfunction and increased production of Reactive Oxygen Species (ROS) (Passos et al., 2010, 2007). ROS-derived from senescent cells have also been shown to induce DNA damage in neighbouring proliferating cells (Nelson et al., 2012). Because senescent cells can secrete pro-inflammatory cytokines and other factors that disrupt the tissue microenvironment, they may contribute to disruption of cell and tissue function. Even a small percentage of senescent cells, in fact, may interfere with tissue homeostasis and function (Shay and Wright, 2000).

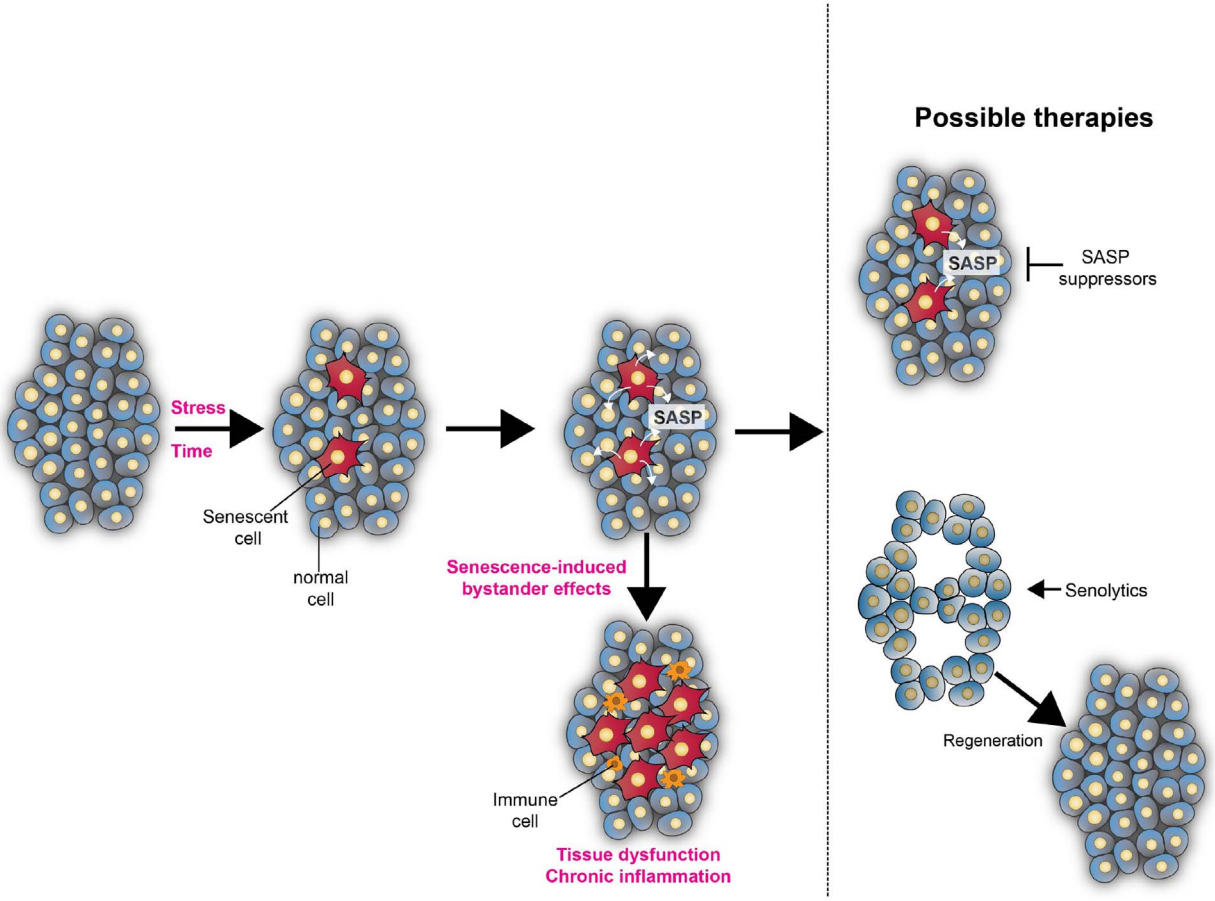

Fig. 1. During the ageing process, exposure to intrinsic and extrinsic stresses can lead to the accumulation of senescent cells. Senescent cells in tissues (if not cleared by the immune system) produce a SASP which can, over time, induce senescence in neighbouring young cells, resulting in further accumulation of senescent cells. Senescent cells contribute to tissue dysfunction via induction of the SASP. Therapeutically, two main approaches are currently being tested to counteract the detrimental effects of senescence during ageing: 1) drugs which suppress the SASP- this approach allows senescent cells to remain in tissues but aims to prevent its associated bystander effects; 2) drugs that specifically kill senescent cells, named "senolytics".

Studies using genetically-modified mice found that genetic clearance of senescent cells delays ageing-associated disorders in old mice (Baker et al., 2016, 2011; Xu et al., 2015). Initially this was observed in progeroid mice that accumulate more senescent cells than normally (Baker et al., 2011). Clearance of senescent cells also did not extend lifespan in progeroid mice, which Baker et al. claim is due to their mice dying primarily of heart disease that is not affected by the treatment. More recently, using the same genetic approach, the same group found that removing senescent cells in normal mice preserves health in some tissues, though not in others, protects from cancer and extends median (but not maximum) lifespan (Baker et al., 2016). Other studies have shown beneficial effects of genetic clearance of senescent cells: Elimination of p16 positive cells was shown to enhance adipogenesis and improve metabolic function in old mice (Xu et al., 2015), and clearance of naturally occurring p19Arf-positive cells restored lung compliance, structure, and elasticity in aged mice (Hashimoto et al., 2016). Therefore, these landmark studies provide evidence that senescent cells can promote age-related phenotypes, at least in a subset of organs.

7. Genetics of ageing and links to senescence

As mentioned above, senescent cells likely accumulate in some tissues and may contribute to organ dysfunction, yet whether they are causal factors in ageing remains a subject of debate. Some genetic interventions that alter mammalian ageing appear to influence tissue homeostasis by affecting senescence, cell proliferation, and cell death, yet such evidence is circumstantial (de Magalhaes and Faragher, 2008). Evidence from genetic manipulation experiments of players involved in telomeric signal transduction is mixed (de Magalhaes, 2004). Increasing the dosage in mice of INK4a/ARF (the gene coding the mouse homolog of p16) offers resistance against cancer but does not affect ageing (Matheu et al., 2004). Another study found that INK4a/ARF induction in mice results in premature senescence and inhibits cell proliferation but does not induce cell senescence (Boquoi et al., 2015). There is also evidence that p53 may influence ageing in mice (Donehower, 2002), but it is not clear the same is true for humans. Likewise, disruption of p63, a homologue of p53, appears to accelerate ageing in mice (Keyes et al., 2005), yet human defects in p63 do not (Celli et al., 1999). Mouse strains with increased levels of p53 and INK4a/ARF are long-lived (Matheu et al., 2007), though it is unclear whether their ageing process is altered. Arguably the strongest evidence for a role of telomerase in ageing comes from telomerase-overexpressing mice also engineered to resist cancer via enhanced expression of p53 and INK4a/ARF, as these mice are long-lived (Tomas-Loba et al., 2008). Even though it is not clear whether ageing is delayed in these animals or the exact mechanisms, these findings point towards some level of protection from age-related degeneration via optimization of pathways associated with telomeres and RS. Telomerase gene therapy in old mice also modestly increased lifespan (Bernardes de Jesus et al., 2012). It should be noted, however, that telomerase may have functions independent of telomere elongation, such as in protecting mitochondria from stress (Ahmed et al., 2008). As abovementioned, another study showed that telomerase reactivation reverses degeneration in mice (Jaskelioff et al., 2011). However, this study was conducted in animals that have no

telomerase to begin with and thus develop a number of pathologies. Benefits from reactivating telomerase in mice that become sick for lack of telomerase are hardly surprising.

Olivier Toussaint was also involved in establishing databases of ageing-related genes (de Magalhaes et al., 2005) and in developing bioinformatics methods for studying ageing (de Magalhaes and Toussaint, 2004b). Of note, we performed the first systematic analysis of ageing-related genes (de Magalhaes and Toussaint, 2004a). More recently a new database of genes associated with cell senescence, entitled CellAge (http://genomics.senescence.info/cells/), has been developed to facilitate system-level analysis of cell senescence. Furthermore, a gene expression meta-analysis across mammalian tissues and species found signatures of senescent cells in aged tissues (de Magalhaes et al., 2009). In humans, one study measured changes in telomere length in over 4000 people over 10 years but found no association with mortality or morbidity (Weischer et al., 2014). However, human epidemiological data suggest a causal role of both short and very long telomeres in cancer, heart disease and other age-related diseases (Codd et al., 2013), so some impact of telomere shortening in age-related diseases besides cancer cannot be excluded.

8. Pharmacological prospects by targeting senescence

Some have argued that if telomerase can avoid ageing in cells *in vitro*, maybe it can be used to combat human ageing (Fossel, 1996). A number of companies and labs are developing telomerase-based therapies to fight ageing and at least one product, a natural product-derived telomerase activator called TA-65, is already available. One study reported that taking TA-65 may result in the decline of senescent immune system cells in patients (Harley et al., 2011). TA-65 can also increase telomerase levels in some mouse tissues and was reported to improve health indicators in mice but it did not increase mean or maximum lifespan (de Jesus et al., 2011).

Even though our knowledge of telomerase is still imperfect, it is questionable whether telomerase-activating therapies will succeed in retarding human ageing (de Magalhaes and Toussaint, 2004c). Firstly, mice expressing lots of telomerase do not live longer (Artandi et al., 2002). Moreover, telomerase is important in cellular proliferation, yet many of our organs, such as the brain, are mostly composed of cells that do not proliferate. Hence, telomerase will do little to alleviate ageing in these tissues. Lastly, there is ample evidence that telomerase favours tumorigenesis and so telomerase-based therapies may foster cancer development. Although research on telomerase is still in its infancy, there are questions about the efficiency and long-term safety of telomerase-based anti-ageing therapies. The fact that TA-65 can increase telomerase levels but does not extend lifespan in mice (de Jesus et al., 2011) is in line with this reasoning.

Some companies are also selling telomere measurements to estimate biological age. Although telomere shortening may be a marker of certain diseases, there is no evidence at present that telomere length is a better indicator of biological age than chronological age.

Another strategy which has proven promising is the use of drugs that can specifically eliminate senescent cells and are commonly called “senolytics” (Fig. 1). Several drugs have been shown to eliminate senescent cells *in vivo* and have beneficial effects in models of ageing and age-related disease. Dasatinib and quercetin (D + Q) when given in combination have been shown to reduce senescent cells in aged, radiation-exposed and progeroid mice, improving several healthspan parameters (Zhu et al., 2015). Moreover, D + Q administration was shown to improve vasomotor function in aged and hypercholesterolaemic mice (Roos et al., 2016), improve lung function in a mouse model of idiopathic pulmonary fibrosis (Schafer et al., 2017) and in mouse models of hepatic steatosis (Ogrodnik et al., 2017). Inhibitors of the BCL-2 family, such as ABT-263 (navitoclax) and ABT-737, have been shown to reduce senescent hematopoietic and muscle stem cells in radiation-exposed and aged mice (Chang et al., 2016) and senescent cells in the lung and epidermis (Yosef et al., 2016). Further highlighting the potential of targeting senescence as a way to counteract age-related diseases, recent work in mice has shown that genetic elimination of p16-positive senescent cells ameliorated atherosclerosis (Childs et al., 2016) and osteoarthritis (Jeon et al., 2017). While these strategies have been shown to have beneficial effects during mouse ageing *in vivo*, it is still unclear what are the effects of these therapies on young tissues, as discussed in (Birch and Passos, 2017). Recently, a FOXO4 peptide that perturbs the FOXO4 interaction with p53 was shown to clear senescent cells and improve healthspan in aged and progeroid mice (Baar et al., 2017).

Finally, since the SASP has been proposed to be an inducer of tissue dysfunction during ageing, another potential strategy to counteract the negative effects of senescent cells is to identify SASP-suppressing drugs. While it is still unclear whether the SASP specifically contributes to ageing, several studies suggest that its inhibition can improve lifespan and healthspan. For instance, inhibition of the mTOR pathway (using the drug rapamycin), which has been shown to extend lifespan in a variety of model organisms (de Magalhaes et al., 2012; Harrison et al., 2009), is an effective SASP suppressor (Correia-Melo et al., 2016; Herranz et al., 2015; Laberge et al., 2015). Inhibition of the JAK pathway (using the drug ruxolitinib) has been shown to suppress the SASP and alleviate age-related tissue dysfunction (Xu et al., 2015). Inhibition of NF-κB signalling (the main transcription factor regulating the SASP) both genetically and pharmacologically, has been shown to prevent age-related deterioration in progeroid mouse models (Osorio et al., 2012). However, given the fact that the SASP is required in certain physiological contexts, it is still unclear whether targeting the SASP would be a viable anti-ageing therapy.

9. Concluding remarks

While there is little evidence to suggest that cells running out of divisions are a major factor in ageing, it is possible that stress and various insults are contributors to senescence *in vivo*. Even a small fraction of senescent cells in organs may impair tissue renewal and homeostasis, decrease organ function, and contribute to the ageing phenotype, as shown by the studies genetically ablating senescent cells. While our knowledge about senescence *in vivo* has increased exponentially in the last decade, this is mostly through work using laboratory mice, which have known limitations (de Magalhaes, 2014). As such, one major challenge in the field is to determine levels of senescent cells in human tissues and whether they contribute to ageing and/or pathologies in humans. Furthermore, given the diverse functions of senescent cells in processes such as repair, wound healing, cancer, development and ageing, we still need to better characterize senescence *in vivo* in these different contexts. Finally, we still know very little about *in vivo* rates of occurrence and turnover of senescent cells. Therefore, in spite of recent advances in our understanding of senescence, telomeres and SIPS, many questions remain and these will be timely and important areas of research for years to come.

Acknowledgements

JPM is grateful for the opportunity to conduct his doctoral studies with Olivier Toussaint from whom he learned so much. Work in JFP lab is funded by BBSRC. Work in the JPM lab is supported by the Wellcome Trust (104978/Z/14/Z), the Leverhulme Trust (RPG-2016-015), LongeCity and the Methuselah Foundation.

References

Acosta, J.C., Banito, A., Wuestefeld, T., Georgilis, A., Janich, P., Morton, J.P., Athineos, D., Kang, T.-W., Lasitschka, F., Andrulis, M., Pascual, G., Morris, K.J., Khan, S., Jin, H., Dharmalingam, G., Snijders, A.P., Carroll, T., Capper, D., Pritchard, C., Inman, G.J., Longerich, T., Sansom, O.J., Benitah, S.A., Zender, L., Gil, J.s., 2013. A complex

secretory program orchestrated by the inflammasome controls paracrine senescence. Nat. Cell Biol. 15, 978–990.
Ahmed, S., Passos, J.F., Birket, M.J., Beckmann, T., Brings, S., Peters, H., Birch-Machin, M.A., von Zglinicki, T., Saretzki, G., 2008. Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. J. Cell Sci. 121, 1046–1053.
Aikata, H., Takaishi, H., Kawakami, Y., Takahashi, S., Kitamoto, M., Nakanishi, T., Nakamura, Y., Shimamoto, F., Kajiyama, G., Ide, T., 2000. Telomere reduction in human liver tissues with age and chronic inflammation. Exp. Cell Res. 256, 578–582.
Allsopp, R.C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E.V., Futcher, A.B., Greider, C.W., Harley, C.B., 1992. Telomere length predicts replicative capacity of human fibroblasts. Proc. Natl. Acad. Sci. U. S. A. 89, 10114–10118.
Artandi, S.E., Alson, S., Tietze, M.K., Sharpless, N.E., Ye, S., Greenberg, R.A., Castrillon, D.H., Horner, J.W., Weiler, S.R., Carrasco, R.D., DePinho, R.A., 2002. Constitutive telomerase expression promotes mammary carcinomas in aging mice. Proc. Natl. Acad. Sci. U. S. A. 99, 8191–8196.
Baar, M.P., Brandt, R.M.C., Putavet, D.A., Klein, J.D.D., Derks, K.W.J., Bourgeois, B.R.M., Stryeck, S., Rijksen, Y., van Willigenburg, H., Feijtel, D.A., van der Pluijm, I., Essers, J., van Cappellen, W.A., van Ijcken, W.F., Houtsmuller, A.B., Pothof, J., de Bruin, R.W.F., Madl, T., Hoeijmakers, J.H.J., Campisi, J., de Keizer, P.L.J., 2017. Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. Cell 169, 132–147 e116.
Baker, D.J., Wijshake, T., Tchkonia, T., LeBrasseur, N.K., Childs, B.G., van de Sluis, B., Kirkland, J.L., van Deursen, J.M., 2011. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479, 232–236.
Baker, D.J., Childs, B.G., Durik, M., Wijers, M.E., Sieben, C.J., Zhong, J.A., Saltness, R., Jeganathan, K.B., Verzosa, G.C., Pezeshki, A., Khazaie, K., Miller, J.D., van Deursen, J.M., 2016. Naturally occurring p16Ink4a-positive cells shorten healthy lifespan. Nature 530, 184–189.
Beauséjour, C.M., Krtolica, A., Galimi, F., Narita, M., Lowe, S.W., Yaswen, P., Campisi, J., 2003. Reversal of human cellular senescence: roles of the p53 and p16 pathways. EMBO J. 22, 4212–4222.
Bernardes de Jesus, B., Vera, E., Schneeberger, K., Tejera, A.M., Ayuso, E., Bosch, F., Blasco, M.A., 2012. Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. EMBO Mol. Med. 4, 691–704.
Birch, J., Passos, J.F., 2017. Targeting the SASP to combat ageing: mitochondria as possible intracellular allies? BioEssays 1600235-n/a.
Birch, J., Anderson, R.K., Correia-Melo, C., Jurk, D., Hewitt, G., Marques, F.M., Green, N.J., Moisey, E., Birrell, M.A., Belvisi, M.G., Black, F., Taylor, J.J., Fisher, A.J., De Soyza, A., Passos, J.F., 2015. DNA damage response at telomeres contributes to lung aging and chronic obstructive pulmonary disease. Am. J. Physiol.—Lung Cell. Mol. Physiol. 309, L1124–L1137.
Blasco, M.A., Lee, H.-W., Hande, M.P., Samper, E., Lansdorp, P.M., DePinho, R.A., Greider, C.W., 1997. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 91, 25–34.
Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S., Wright, W.E., 1998. Extension of life-span by introduction of telomerase into normal human cells. Science 279, 349–352.
Boquoi, A., Arora, S., Chen, T., Litwin, S., Koh, J., Enders, G.H., 2015. Reversible cell cycle inhibition and premature aging features imposed by conditional expression of p16Ink4a. Aging Cell 14, 139–147.
Brack, C., Lithgow, G., Osiewacz, H., Toussaint, O., 2000. EMBO WORKSHOP REPORT: molecular and cellular gerontology serpiano, Switzerland, September 18–22, 1999. EMBO J. 19, 1929–1934.
Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A., Kerber, R.A., 2003. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 361, 393–395.
Celli, J., Duijf, P., Hamel, B.C., Bamshad, M., Kramer, B., Smits, A.P., Newbury-Ecob, R., Hennekam, R.C., Van Buggenhout, G., van Haeringen, A., Woods, C.G., van Essen, A.J., de Waal, R., Vriend, G., Haber, D.A., Yang, A., McKeon, F., Brunner, H.G., van Bokhoven, H., 1999. Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. Cell 99, 143–153.
Chang, J., Wang, Y., Shao, L., Laberge, R.-M., Demaria, M., Campisi, J., Janakiraman, K., Sharpless, N.E., Ding, S., Feng, W., Luo, Y., Wang, X., Aykin-Burns, N., Krager, K., Ponnappan, U., Hauer-Jensen, M., Meng, A., Zhou, D., 2016. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat. Med. 22, 78–83.
Childs, B.G., Baker, D.J., Wijshake, T., Conover, C.A., Campisi, J., van Deursen, J.M., 2016. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. Science 354, 472.
Choudhury, A.R., Ju, Z., Djojosubroto, M.W., Schienke, A., Lechel, A., Schaetzlein, S., Jiang, H., Stepczynska, A., Wang, C., Buer, J., Lee, H.-W., von Zglinicki, T., Ganser, A., Schirmacher, P., Nakauchi, H., Rudolph, K.L., 2007. Cdkn1a deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation. Nat. Genet. 39, 99–105.
Codd, V., Nelson, C.P., Albrecht, E., Mangino, M., Deelen, J., Buxton, J.L., Hottenga, J.J., Fischer, K., Esko, T., Surakka, I., Broer, L., Nyholt, D.R., Mateo Leach, I., Salo, P., Hagg, S., Matthews, M.K., Palmen, J., Norata, G.D., O'Reilly, P.F., Saleheen, D., Amin, N., Balmforth, A.J., Beekman, M., de Boer, R.A., Bohringer, S., Braund, P.S., Burton, P.R., de Craen, A.J., Denniff, M., Dong, Y., Douroudis, K., Dubinina, E., Eriksson, J.G., Garlaschelli, K., Guo, D., Hartikainen, A.L., Henders, A.K., Houwing-Duistermaat, J.J., Kananen, L., Karssen, L.C., Kettunen, J., Klopp, N., Lagou, V., van Leeuwen, E.M., Madden, P.A., Magi, R., Magnusson, P.K., Mannisto, S., McCarthy, M.I., Medland, S.E., Mihailov, E., Montgomery, G.W., Oostra, B.A., Palotie, A., Peters, A., Pollard, H., Pouta, A., Prokopenko, I., Ripatti, S., Salomaa, V., Suchiman, H.E., Valdes, A.M., Verweij, N., Vinuela, A., Wang, X., Wichmann, H.E., Widen, E., Willemsen, G., Wright, M.J., Xia, K., Xiao, X., van Veldhuisen, D.J., Catapano, A.L., Tobin, M.D., Hall, A.S., Blakemore, A.I., van Gilst, W.H., Zhu, H., Consortium, C., Erdmann, J., Reilly, M.P., Kathiresan, S., Schunkert, H., Talmud, P.J., Pedersen, N.L., Perola, M., Ouwehand, W., Kaprio, J., Martin, N.G., van Duijn, C.M., Hovatta, I., Gieger, C., Metspalu, A., Boomsma, D.I., Jarvelin, M.R., Slagboom, P.E., Thompson, J.R., Spector, T.D., van der Harst, P., Samani, N.J., et al., 2013. Identification of seven loci affecting mean telomere length and their association with disease. Nat. Genet. 45, 422–427.
Coppé, J.P., Patil, C.K., Rodier, F., Sun, Y., Muñoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.-Y., Campisi, J., 2008. Senescence-associated secretory phenotypes reveal cell-Nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. 6, e301.
Correia-Melo, C., Marques, F.D.M., Anderson, R., Hewitt, G., Hewitt, R., Cole, J., Carroll, B.M., Miwa, S., Birch, J., Merz, A., Rushton, M.D., Charles, M., Jurk, D., Tait, S.W.G., Czapiewski, R., Greaves, L., Nelson, G., Bohlooly-Y, M., Rodriguez-Cuenca, S., Vidal-Puig, A., Mann, D., Saretzki, G., Quarato, G., Green, D.R., Adams, P.D., von Zglinicki, T., Korolchuk, V.I., Passos, J.F., 2016. Mitochondria are required for pro-ageing features of the senescent phenotype. EMBO J. 35, 724–742.
Cristofalo, V.J., 2005. SA [beta] gal staining: biomarker or delusion. Exp. Gerontol. 40, 836–838.
d'Adda di Fagagna, F., Reaper, P.M., Clay-Farrace, L., Fiegler, H., Carr, P., von Zglinicki, T., Saretzki, G., Carter, N.P., Jackson, S.P., 2003. A DNA damage checkpoint response in telomere-initiated senescence. Nature 426, 194–198.
de Jesus, B.B., Schneeberger, K., Vera, E., Tejera, A., Harley, C.B., Blasco, M.A., 2011. The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. Aging Cell 10, 604–621.
de Lange, T., 2005. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev. 19, 2100–2110.
de Magalhães, J.P., Chainiaux, F., Remacle, J., Toussaint, O., 2002. Stress-induced premature senescence in BJ and hTERT-BJ1 human foreskin fibroblasts. FEBS Lett. 523, 157–162.
de Magalhaes, J.P., Faragher, R.G., 2008. Cell divisions and mammalian aging: integrative biology insights from genes that regulate longevity. BioEssays 30, 567–578.
de Magalhaes, J.P., Toussaint, O., 2004a. GenAge: a genomic and proteomic network map of human ageing. FEBS Lett. 571, 243–247.
de Magalhaes, J.P., Toussaint, O., 2004b. How bioinformatics can help reverse engineer human aging. Ageing Res. Rev. 3, 125–141.
de Magalhaes, J.P., Toussaint, O., 2004c. Telomeres and telomerase: a modern fountain of youth? Rejuvenation Res. 7, 126–133.
de Magalhaes, J.P., Chainiaux, F., de Longueville, F., Mainfroid, V., Migeot, V., Marcq, L., Remacle, J., Salmon, M., Toussaint, O., 2004. Gene expression and regulation in H2O2-induced premature senescence of human foreskin fibroblasts expressing or not telomerase. Exp. Gerontol. 39, 1379–1389.
de Magalhaes, J.P., Costa, J., Toussaint, O., 2005. HAGR: the human ageing genomic resources. Nucleic Acids Res. 33, D537–543.
de Magalhaes, J.P., Curado, J., Church, G.M., 2009. Meta-analysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics 25, 875–881.
de Magalhaes, J.P., Wuttke, D., Wood, S.H., Plank, M., Vora, C., 2012. Genome-environment interactions that modulate aging: powerful targets for drug discovery. Pharmacol. Rev. 64, 88–101.
de Magalhaes, J.P., 2004. From cells to ageing: a review of models and mechanisms of cellular senescence and their impact on human ageing. Exp. Cell Res. 300, 1–10.
de Magalhaes, J.P., 2013. How ageing processes influence cancer. Nat. Rev. Cancer 13, 357–365.
de Magalhaes, J.P., 2014. Why genes extending lifespan in model organisms have not been consistently associated with human longevity and what it means to translation research. ABBV Cell Cycle 13, 2671–2673.
Demaria, M., Ohtani, N., Youssef Sameh, A., Rodier, F., Toussaint, W., Mitchell James, R., Laberge, R.-M., Vijg, J., Van Steeg, H., Dollé Martijn, E.T., Hoeijmakers Jan, H.J., de Bruin, A., Hara, E., Campisi, J., 2014. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev. Cell 31, 722–733.
Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., Pereira-Smith, O., 1995. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. U. S. A. 92, 9363–9367.
Donehower, L.A., 2002. Does p53 affect organismal aging. J. Cell. Physiol. 192, 23–33.
Dumont, P., Balbeur, L., Remacle, J., Toussaint, O., 2000. Appearance of biomarkers of in vitro ageing after successive stimulation of WI-38 fibroblasts with IL-1alpha and TNF-alpha: senescence associated beta-galactosidase activity and morphotype transition. J. Anat. 197 (Pt 4), 529–537.
Effros, R.B., 2008. Kleemeier Award Lecture 2008—the canary in the coal mine: telomeres and human healthspan. J. Gerontol. A. Biol. Sci. Med. Sci. 64, 511–515.
Epel, E.S., Blackburn, E.H., Lin, J., Dhabhar, F.S., Adler, N.E., Morrow, J.D., Cawthon, R.M., 2004. Accelerated telomere shortening in response to life stress. Proc. Natl. Acad. Sci. U. S. A. 101, 17312–17315.
Fairlie, J., Harrington, L., 2015. Enforced telomere elongation increases the sensitivity of human tumour cells to ionizing radiation. DNA Repair 25, 54–59.
Farr, J.N., Fraser, D.G., Wang, H., Jaehn, K., Ogrodnik, M.B., Weivoda, M.M., Drake, M.T., Tchkonia, T., LeBrasseur, N.K., Kirkland, J.L., Bonewald, L.F., Pignolo, R.J., Monroe, D.G., Khosla, S., 2016. Identification of senescent cells in the bone microenvironment. J. Bone Miner. Res. 31, 1920–1929.
Fossel, M., 1996. Reversing Human Aging. William Morrow and Company, New York.
Fumagalli, M., Rossiello, F., Clerici, M., Barozzi, S., Cittaro, D., Kaplunov, J.M., Bucci, G., Dobreva, M., Matti, V., Beausejour, C.M., Herbig, U., Longhese, M.P., d'Adda di Fagagna, F., 2012. Telomeric DNA damage is irreparable and causes persistent DNA-

damage-response activation. Nat. Cell Biol. 14, 355–365.
Gomes, N.M., Ryder, O.A., Houck, M.L., Charter, S.J., Walker, W., Forsyth, N.R., Austad, S.N., Venditti, C., Pagel, M., Shay, J.W., Wright, W.E., 2011. Comparative biology of mammalian telomeres: hypotheses on ancestral states and the roles of telomeres in longevity determination. Aging Cell 10, 761–768.
Griffith, J.D., Comeau, L., Rosenfield, S., Stansel, R.M., Bianchi, A., Moss, H., de Lange, T., 1999. Mammalian telomeres end in a large duplex loop. Cell 97, 503–514.
Hall, B.M., Balan, V., Gleiberman, A.S., Strom, E., Krasnov, P., Virtuoso, L.P., Rydkina, E., Vujcic, S., Balan, K., Gitlin, I., Leonova, K., Polinsky, A., Chernova, O.B., Gudkov, A.V., 2016. Aging of mice is associated with p16(Ink4a)- and β-galactosidase-positive macrophage accumulation that can be induced in young mice by senescent cells. Aging (Milano) 8, 1294–1315.
Harley, C.B., Futcher, A.B., Greider, C.W., 1990. Telomeres shorten during ageing of human fibroblasts. Nature 345, 458–460.
Harley, C.B., Liu, W., Blasco, M., Vera, E., Andrews, W.H., Briggs, L.A., Raffaele, J.M., 2011. A natural product telomerase activator as part of a health maintenance program. Rejuvenation Res. 14, 45–56.
Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., Flurkey, K., Nadon, N.L., Wilkinson, J.E., Frenkel, K., Carter, C.S., Pahor, M., Javors, M.A., Fernandez, E., Miller, R.A., 2009. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460, 392–395.
Hashimoto, M., Asai, A., Kawagishi, H., Mikawa, R., Iwashita, Y., Kanayama, K., Sugimoto, K., Sato, T., Maruyama, M., Sugimoto, M., 2016. Elimination of p19(ARF)-expressing cells enhances pulmonary function in mice. JCI Insight 1, e87732.
Hastie, N.D., Dempster, M., Dunlop, M.G., Thompson, A.M., Green, D.K., Allshire, R.C., 1990. Telomere reduction in human colorectal carcinoma and with ageing. Nature 346, 866–868.
Hayflick, L., Moorhead, P.S., 1961. The serial cultivation of human diploid cell strains. Exp. Cell Res. 25, 585–621.
Hayflick, L., 1994. How and Why We Age. Ballantine Books, New York.
Helman, A., Klochendler, A., Azazmeh, N., Gabai, Y., Horwitz, E., Anzi, S., Swisa, A., Condiotti, R., Granit, R.Z., Nevo, Y., Fixler, Y., Shreibman, D., Zamir, A., Tornovsky-Babeay, S., Dai, C., Glaser, B., Powers, A.C., Shapiro, A.M., Magnuson, M.A., Dor, Y., Ben-Porath, I., 2016. p16(Ink4a)-induced senescence of pancreatic beta cells enhances insulin secretion. Nat. Med. 22, 412–420.
Hemann, M.T., Greider, C.W., 2000. Wild-derived inbred mouse strains have short telomeres. Nucleic Acids Res. 28, 4474–4478.
Herranz, N., Gallage, S., Mellone, M., Wuestefeld, T., Klotz, S., Hanley, C.J., Raguz, S., Acosta, J.C., Innes, A.J., Banito, A., Georgilis, A., Montoya, A., Wolter, K., Dharmalingam, G., Faull, P., Carroll, T., Martinez-Barbera, J.P., Cutillas, P., Reisinger, F., Heikenwalder, M., Miller, R.A., Withers, D., Zender, L., Thomas, G.J., Gil, J., 2015. mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. Nat. Cell Biol. 17, 1205–1217.
Hewitt, G., Jurk, D., Marques, F.D.M., Correia-Melo, C., Hardy, T., Gackowska, A., Anderson, R., Taschuk, M., Mann, J., Passos, J.F., 2012. Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence. Nat. Commun. 3, 708.
Iwama, H., Ohyashiki, K., Ohyashiki, J.H., Hayashi, S., Yahata, N., Ando, K., Toyama, K., Hoshika, A., Takasaki, M., Mori, M., Shay, J.W., 1998. Telomeric length and telomerase activity vary with age in peripheral blood cells obtained from normal individuals. Hum. Genet. 102, 397–402.
Jaskelioff, M., Muller, F.L., Paik, J.-H., Thomas, E., Jiang, S., Adams, A.C., Sahin, E., Kost-Alimova, M., Protopopov, A., Cadinanos, J., Horner, J.W., Maratos-Flier, E., DePinho, R.A., 2011. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature 469, 102–106.
Jeon, O.H., Kim, C., Laberge, R.-M., Demaria, M., Rathod, S., Vasserot, A.P., Chung, J.W., Kim, D.H., Poon, Y., David, N., Baker, D.J., van Deursen, J.M., Campisi, J., Elisseeff, J.H., 2017. Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. Nat. Med.
Jurk, D., Wang, C., Miwa, S., Maddick, M., Korolchuk, V., Tsolou, A., Gonos, E.S., Thrasivoulou, C., Jill Saffrey, M., Cameron, K., von Zglinicki, T., 2012. Postmitotic neurons develop a p21-dependent senescence-like phenotype driven by a DNA damage response. Aging Cell 11, 996–1004.
Jurk, D., Wilson, C., Passos, J.F., Oakley, F., Correia-Melo, C., Greaves, L., Saretzki, G., Fox, C., Lawless, C., Anderson, R., Hewitt, G., Pender, S.L.F., Fullard, N., Nelson, G., Mann, J., van de Sluis, B., Mann, D.A., von Zglinicki, T., 2014. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nat. Commun. 2.
Kakuo, S., Asaoka, K., Ide, T., 1999. Human is a unique species among primates in terms of telomere length. Biochem. Biophys. Res. Commun. 263, 308–314.
Kammori, M., Nakamura, K.I., Kawahara, M., Mimura, Y., Kaminishi, M., Takubo, K., 2002. Telomere shortening with aging in human thyroid and parathyroid tissue. Exp. Gerontol. 37, 513–521.
Kang, T.-W., Yevsa, T., Woller, N., Hoenicke, L., Wuestefeld, T., Dauch, D., Hohmeyer, A., Gereke, M., Rudalska, R., Potapova, A., Iken, M., Vucur, M., Weiss, S., Heikenwalder, M., Khan, S., Gil, J., Bruder, D., Manns, M., Schirmacher, P., Tacke, F., Ott, M., Luedde, T., Longerich, T., Kubicka, S., Zender, L., 2011. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature 479, 547–551.
Keyes, W.M., Wu, Y., Vogel, H., Guo, X., Lowe, S.W., Mills, A.A., 2005. p63 deficiency activates a program of cellular senescence and leads to accelerated aging. Genes Dev. 19, 1986–1999.
Kipling, D., Cooke, H.J., 1990. Hypervariable ultra-long telomeres in mice. Nature 347, 400–402.
Krizhanovsky, V., Yon, M., Dickins, R.A., Hearn, S., Simon, J., Miething, C., Yee, H., Zender, L., Lowe, S.W., 2008. Senescence of activated stellate cells limits liver fibrosis. Cell 134, 657–667.
Krtolica, A., Parrinello, S., Lockett, S., Desprez, P.-Y., Campisi, J., 2001. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc. Natl. Acad. Sci. U. S. A. 98, 12072–12077.
Laberge, R.-M., Sun, Y., Orjalo, A.V., Patil, C.K., Freund, A., Zhou, L., Curran, S.C., Davalos, A.R., Wilson-Edell, K.A., Liu, S., Limbad, C., Demaria, M., Li, P., Hubbard, G.B., Ikeno, Y., Javors, M., Desprez, P.-Y., Benz, C.C., Kapahi, P., Nelson, P.S., Campisi, J., 2015. MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. Nat. Cell Biol. 17, 1049–1061.
Lawless, C., Wang, C., Jurk, D., Merz, A., von Zglinicki, T., Passos, J.F., 2010. Quantitative assessment of markers for cell senescence. Exp. Gerontol. 45, 772–778.
Lee, H.-W., Blasco, M.A., Gottlieb, G.J., Horner, J.W., Greider, C.W., DePinho, R.A., 1998. Essential role of mouse telomerase in highly proliferative organs. Nature 392, 569–574.
Lindsey, J., McGill, N.I., Lindsey, L.A., Green, D.K., Cooke, H.J., 1991. In vivo loss of telomeric repeats with age in humans. Mutat. Res. 256, 45–48.
Lio, D., Scola, L., Crivello, A., Colonna-Romano, G., Candore, G., Bonafe, M., Cavallone, L., Marchegiani, F., Olivieri, F., Franceschi, C., Caruso, C., 2003. Inflammation, genetics, and longevity: further studies on the protective effects in men of IL-10 -1082 promoter SNP and its interaction with TNF-alpha -308 promoter SNP. J. Med. Genet. 40, 296–299.
Matheu, A., Pantoja, C., Efeyan, A., Criado, L.M., Martin-Caballero, J., Flores, J.M., Klatt, P., Serrano, M., 2004. Increased gene dosage of Ink4a/Arf results in cancer resistance and normal aging. Genes Dev. 18, 2736–2746.
Matheu, A., Maraver, A., Klatt, P., Flores, I., Garcia-Cao, I., Borras, C., Flores, J.M., Vina, J., Blasco, M.A., Serrano, M., 2007. Delayed ageing through damage protection by the Arf/p53 pathway. Nature 448, 375–379.
Minamino, T., Orimo, M., Shimizu, I., Kunieda, T., Yokoyama, M., Ito, T., Nojima, A., Nabetani, A., Oike, Y., Matsubara, H., Ishikawa, F., Komuro, I., 2009. A crucial role for adipose tissue p53 in the regulation of insulin resistance. Nat. Med. 15, 1082–1087.
Mondello, C., Petropoulou, C., Monti, D., Gonos, E.S., Franceschi, C., Nuzzo, F., 1999. Telomere length in fibroblasts and blood cells from healthy centenarians. Exp. Cell Res. 248, 234–242.
Muñoz-Espín, D., Cañamero, M., Maraver, A., Gómez-López, G., Contreras, J., Murillo-Cuesta, S., Rodríguez-Baeza, A., Varela-Nieto, I., Ruberte, J., Collado, M., Serrano, M., 2013. Programmed cell senescence during mammalian embryonic development. Cell 155, 1104–1118.
Munoz-Espin, D., Serrano, M., 2014. Cellular senescence: from physiology to pathology. Nat. Rev. Mol. Cell Biol. 15, 482–496.
Narita, M., Nuñez, S., Heard, E., Narita, M., Lin, A.W., Hearn, S.A., Spector, D.L., Hannon, G.J., Lowe, S.W., 2003. Rb-Mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 113, 703–716.
Nelson, G., Wordsworth, J., Wang, C., Jurk, D., Lawless, C., Martin-Ruiz, C., von Zglinicki, T., 2012. A senescent cell bystander effect: senescence-induced senescence. Aging Cell 11, 345–349.
Njajou, O.T., Cawthon, R.M., Damcott, C.M., Wu, S.H., Ott, S., Garant, M.J., Blackburn, E.H., Mitchell, B.D., Shuldiner, A.R., Hsueh, W.C., 2007. Telomere length is paternally inherited and is associated with parental lifespan. Proc. Natl. Acad. Sci. U. S. A. 104, 12135–12139.
Nwosu, B.U., Nilsson, O., Mitchum Jr., R.D., Coco, M., Barnes, K.M., Baron, J., 2005. Lack of telomere shortening with age in mouse resting zone chondrocytes. Horm. Res. 63, 125–128.
Ogrodnik, M., Miwa, S., Tchkonia, T., Tiniakos, D., Wilson, C.L., Lahat, A., Day, C., Burt, A., Palmer, A., Anstee, Q.M., Nagaraja Grellscheid, S., Hoeijmakers, J.H.J., Barnhoorn, S., Mann, D., Bird, T.G., Vermeij, W.P., Kirkland, J.L., Passos, J.F., von Zglinicki, T., Jurk, D., 2017. Cellular senescence drives age-dependent hepatic steatosis. Nat. Commun. 15691.
Osorio, F.G., Barcena, C., Soria-Valles, C., Ramsay, A.J., de Carlos, F., Cobo, J., Fueyo, A., Freije, J.M.P., Lopez-Otin, C., 2012. Nuclear lamina defects cause ATM-dependent NF-Î°B activation and link accelerated aging to a systemic inflammatory response. Genes Dev. 26, 2311–2324.
Pan, C., Xue, B.H., Ellis, T.M., Peace, D.J., Diaz, M.O., 1997. Changes in telomerase activity and telomere length during human T lymphocyte senescence. Exp. Cell Res. 231, 346–353.
Parrinello, S., Samper, E., Krtolica, A., Goldstein, J., Melov, S., Campisi, J., 2003. Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. Nat. Cell Biol. 5, 741–747.
Passos, J.F., Saretzki, G., Ahmed, S., Nelson, G., Richter, T., Peters, H., Wappler, I., Birkett, M., Harold, G., Schaeuble, K., Birch-Machin, M., Kirkwood, T., von Zglinicki, T., 2007. Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. PLoS Biol. 5, e110.
Passos, J.F., Nelson, G., Wang, C., Richter, T., Simillion, C., Proctor, C.J., Miwa, S., Olijslagers, S., Hallinan, J., Wipat, A., Saretzki, G., Rudolph, K.L., Kirkwood, T.B.L., von Zglinicki, T., 2010. Feedback between p21 and reactive oxygen production is necessary for cell senescence. Mol. Syst. Biol. 6, 347.
Prowse, K.R., Greider, C.W., 1995. Developmental and tissue-specific regulation of mouse telomerase and telomere length. Proc. Natl. Acad. Sci. U. S. A. 92, 4818–4822.
Renault, V., Thornell, L.E., Eriksson, P.O., Butler-Browne, G., Mouly, V., Thorne, L.E., 2002. Regenerative potential of human skeletal muscle during aging. Aging Cell 1, 132–139.
Roos, C.M., Zhang, B., Palmer, A.K., Ogrodnik, M.B., Pirtskhalava, T., Thalji, N.M., Hagler, M., Jurk, D., Smith, L.A., Casaclang-Verzosa, G., Zhu, Y., Schafer, M.J., Tchkonia, T., Kirkland, J.L., Miller, J.D., 2016. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. Aging Cell 15, 973–977.
Schafer, M.J., White, T.A., Iijima, K., Haak, A.J., Ligresti, G., Atkinson, E.J., Oberg, A.L., Birch, J., Salmonowicz, H., Zhu, Y., Mazula, D.L., Brooks, R.W., Fuhrmann-

Stroissnigg, H., Pirtskhalava, T., Prakash, Y.S., Tchkonia, T., Robbins, P.D., Aubry, M.C., Passos, J.F., Kirkland, J.L., Tschumperlin, D.J., Kita, H., LeBrasseur, N.K., 2017. Cellular senescence mediates fibrotic pulmonary disease. Nat. Commun. 8, 14532.

Seluanov, A., Chen, Z., Hine, C., Sasahara, T.H., Ribeiro, A.A., Catania, K.C., Presgraves, D.C., Gorbunova, V., 2007. Telomerase activity coevolves with body mass not lifespan. Aging Cell 6, 45–52.

Serra, V., von Zglinicki, T., 2002. Human fibroblasts in vitro senesce with a donor-specific telomere length. FEBS Lett. 516, 71–74.

Serrano, M., Lin, A.W., McCurrach, M.E., Beach, D., Lowe, S.W., 1997. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 88, 593–602.

Shay, J.W., Wright, W.E., 2000. Hayflick, his limit, and cellular ageing. Nat. Rev. Mol. Cell Biol. 1, 72–76.

Steinert, S., White, D.M., Zou, Y., Shay, J.W., Wright, W.E., 2002. Telomere biology and cellular aging in nonhuman primate cells. Exp. Cell Res. 272, 146–152.

Storer, M., Mas, A., Robert-Moreno, A., Pecoraro, M., Ortells, M.C., Di Giacomo, V., Yosef, R., Pilpel, N., Krizhanovsky, V., Sharpe, J., Keyes William, M., 2013. Senescence is a developmental mechanism that contributes to embryonic growth and patterning. Cell 155, 1119–1130.

Suram, A., Kaplunov, J., Patel, P.L., Ruan, H., Cerutti, A., Boccardi, V., Fumagalli, M., Di Micco, R., Mirani, N., Gurung, R.L., Hande, M.P., d/, 'Adda di Fagagna, F., Herbig, U., 2012. Oncogene-induced telomere dysfunction enforces cellular senescence in human cancer precursor lesions. EMBO J. 31, 2839–2851.

Swanson, E.C., Manning, B., Zhang, H., Lawrence, J.B., 2013. Higher-order unfolding of satellite heterochromatin is a consistent and early event in cell senescence. J. Cell Biol. 203, 929–942.

Takai, H., Smogorzewska, A., de Lange, T., 2003. DNA damage foci at dysfunctional telomeres. Curr. Biol. 13, 1549–1556.

Takubo, K., Izumiyama-Shimomura, N., Honma, N., Sawabe, M., Arai, T., Kato, M., Oshimura, M., Nakamura, K.I., 2002. Telomere lengths are characteristic in each human individual. Exp. Gerontol. 37, 523–531.

Tomas-Loba, A., Flores, I., Fernandez-Marcos, P.J., Cayuela, M.L., Maraver, A., Tejera, A., Borras, C., Matheu, A., Klatt, P., Flores, J.M., Vina, J., Serrano, M., Blasco, M.A., 2008. Telomerase reverse transcriptase delays aging in cancer-resistant mice. Cell 135, 609–622.

Toussaint, O., Dumont, P., Remacle, J., Dierick, J.F., Pascal, T., Frippiat, C., Magalhaes, J.P., Zdanov, S., Chainiaux, F., 2002. Stress-induced premature senescence or stress-induced senescence-like phenotype: one in vivo reality, two possible definitions? Sci. World J. 2, 230–247.

Weischer, M., Bojesen, S.E., Nordestgaard, B.G., 2014. Telomere shortening unrelated to smoking, body weight, physical activity, and alcohol intake: 4,576 general population individuals with repeat measurements 10 years apart. PLoS Genet. 10, e1004191.

Wong, K.-K., Maser, R.S., Bachoo, R.M., Menon, J., Carrasco, D.R., Gu, Y., Alt, F.W., DePinho, R.A., 2003. Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. Nature 421, 643–648.

Wu, L., Multani, A.S., He, H., Cosme-Blanco, W., Deng, Y., Deng, J.M., Bachilo, O., Pathak, S., Tahara, H., Bailey, S.M., Deng, Y., Behringer, R.R., Chang, S., 2006. Pot1 deficiency initiates DNA damage checkpoint activation and aberrant homologous recombination at telomeres. Cell 126, 49–62.

Xu, M., Palmer, A.K., Ding, H., Weivoda, M.M., Pirtskhalava, T., White, T.A., Sepe, A., Johnson, K.O., Stout, M.B., Giorgadze, N., Jensen, M.D., LeBrasseur, N.K., Tchkonia, T., Kirkland, J.L., 2015. Targeting senescent cells enhances adipogenesis and metabolic function in old age. eLife 4, e12997.

Xue, W., Zender, L., Miething, C., Dickins, R.A., Hernando, E., Krizhanovsky, V., Cordon-Cardo, C., Lowe, S.W., 2007. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 445, 656–660.

Yosef, R., Pilpel, N., Tokarsky-Amiel, R., Biran, A., Ovadya, Y., Cohen, S., Vadai, E., Dassa, L., Shahar, E., Condiotti, R., Ben-Porath, I., Krizhanovsky, V., 2016. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. Nat. Commun. 7, 11190.

Zhu, Y., Tchkonia, T., Pirtskhalava, T., Gower, A.C., Ding, H., Giorgadze, N., Palmer, A.K., Ikeno, Y., Hubbard, G.B., Lenburg, M., O'Hara, S.P., LaRusso, N.F., Miller, J.D., Roos, C.M., Verzosa, G.C., LeBrasseur, N.K., Wren, J.D., Farr, J.N., Khosla, S., Stout, M.B., McGowan, S.J., Fuhrmann-Stroissnigg, H., Gurkar, A.U., Zhao, J., Colangelo, D., Dorronsoro, A., Ling, Y.Y., Barghouthy, A.S., Navarro, D.C., Sano, T., Robbins, P.D., Niedernhofer, L.J., Kirkland, J.L., 2015. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell 14, 644–658.