Methodological Reviews discuss methods that are of broad interest to the community of cardiovascular investigators and that enable a better understanding of cardiovascular biology, particularly recent technologies in which the methods are still in flux and/or not widely known. It is hoped that these articles, written by recognized experts, will be useful to all investigators, but especially to early-career investigators.

Assessing Cell and Organ Senescence Biomarkers

Bruno Bernardes de Jesus, Maria A. Blasco

Abstract: **A major goal in cancer and aging research is to discriminate the biochemical modifications that happen locally that could account for the healthiness or malignancy of tissues. Senescence is one general antiproliferative cellular process that acts as a strong barrier for cancer progression, playing a crucial role in aging. Here, we focus on the current methods to assess cellular senescence, discriminating the advantages and disadvantages of several senescence biomarkers. (*Circ Res*. 2012;111:97-109.)**

Key Words: biomarkers ■ senescence ■ telomeres

Senescence, from the Latin word *senex*, meaning "growing old," is a process characterized by a flat and large cellular morphology, an irreversible proliferative arrest, and a differential expression of genes, including upregulation of cell-cycle–negative modulators. Replicative senescence was first described by Hayflick and Moorhead in 1961.[1] They observed that human fibroblasts enter in an irreversible state characterized by an exhaustion of replicative potential after a determined number of in vitro duplications. Speculations were made that this process could explain organismal aging. Although the accurate speculations have been confirmed, demonstrating that accumulation of senescent cells could impact on organismal aging and contribute to the appearance of age-related pathologies,[2] cellular senescence is, currently, also accepted as an important general antiproliferative cellular process that acts as a strong barrier for cancer progression.[3] Telomere shortening is currently established as one of the major mechanisms leading to senescence,[4,5] although it can be reached through several nontelomeric pathways involving cytokines, oncogenes, persistent DNA damage activation, or in vitro cell culture shock[6] (the so-called stress-induced premature senescence[7,8] or stress or aberrant signaling-induced senescence[9,10]). Because of the dual role of senescence either in tumor protection or in aging progression, a clear method of identification is central for understanding their role, in either normal or pathological conditions.

Telomere-dependent replicative senescence and stress-induced premature senescence act through the modulation of a convergent group of proteins including p53 and Rb[11] (Figure 1). Telomere shortening, either through replication cycles (the so-called end-replication problem)[12] or attributable to telomere uncapping,[13] leads to the recognition of telomere ends as DNA breaks, resulting in DNA damage–dependent phosphorylation and stabilization of p53,[14,15] which activate a cascade response including the transcriptional activation of the cyclin-dependent kinase inhibitor $p21^{CIP1}$.[16] However, stress-induced senescence works mainly through the activation of $p16^{INK4a}$ (p16), which acts in a telomere length–independent way.[17] Either p21 (through CyclinE/Cdk2 inhibition) or p16 (through CyclinD/Cdk4,6 inhibition) leads to a common response involving the inhibition of Rb, which results in the inactivation of the E2F transcription factor and target genes.[11] The importance of the Rb or p53 factors in senescence could be assessed from the condition in which any of these two proteins is disrupted. It was demonstrated in vitro and in vivo that interfering with these pathways prevents senescence[18–21] (although the relative contribution of each factor depends on several variants such as stress type and cell strain[22–24]) or, on the contrary, high expression levels induce senescence in different cell types.[25]

Telomere-Dependent Senescence and Aging

Telomeres are complex DNA repeats found at the chromosome ends that ensure genomic stability.[14,26–33] In mamma-

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Non-standard Abbreviations and Acronyms

DAPI	4′,6-diamidino-2-phenylindole
DDR	DNA damage response
HMGA	high-mobility group A
OIS	oncogene-induced senescence
PML	promyelocytic leukemia
Pot1	protection of telomeres 1
Rap1	repressor-activator protein 1
SAHF	senescence-associated heterochromatin foci
Terc	telomerase RNA component
Tert	telomerase reverse-transcriptase
Tin2	TRF1-interacting nuclear protein 2
TRF1	telomeric repeat binding factor 1
TRF2	telomeric repeat binding factor 2

lian cells, they are constituted by TTAGGG repeats, which could vary from few to tens of kb pairs between species.[12] Telomeres are protected by a complex of six proteins, named shelterin. Shelterin includes TRF1, TRF2, Pot1-TPP1 heterodimer, Rap1, and Tin2[34] (Figure 2). During each cell replication cycle, telomeres shorten because of the end-replication problem, a situation aggravated by the shortage of telomerase in the majority of the cells constituting adult tissues (Figure 3). Telomerase is constituted by a catalytic unit with reverse-transcriptase activity (Tert) and RNA component (Terc) that serves as template for telomere extension.[35] Telomerase compensates the telomere attrition through a concerted action of its two subunits. Telomerase is the main cellular protein responsible for telomere maintenance and elongation. Telomerase activity is, however, silenced in most cells of adult tissues and is almost only present in adult stem cell compartments.[36,37] Nevertheless, this activity in the stem cell compartments is not enough to counteract age-dependent telomere shortening. From studies in vitro, the shortening rate was described to be between 30 bp and 120 bp per cycle.[38,39] The same tendency was observed in vivo, when

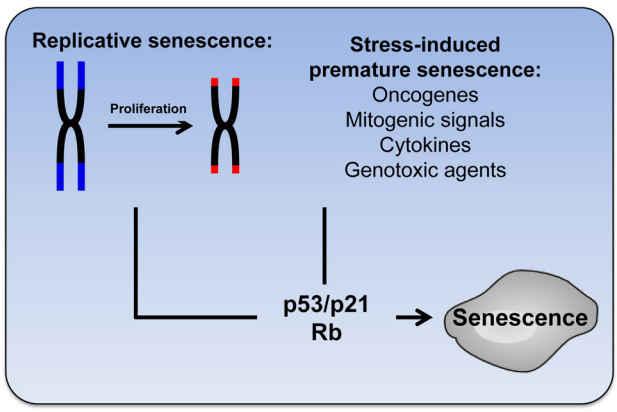

Figure 1. Conditions leading to senescence in cells and tissues. Telomere shortening–dependent senescence or stress-induced senescence (in a telomere length–independent scenario) is summarized, demonstrating how they share some of the main senescence effectors, the axis p53/p21 and Rb.

Table. Senescence Biomarkers

Markers	References
SA-β-Gal	83, 91, 92, 118, 212
p53/p21	91, 213, 214
DEC1/DEC2	92
p16	83, 92, 212, 215
ARF	91, 92, 215
p15	92, 216
SAHF	Review 134
H1/macroH2A/H3.3/H3metLys9	132, 143, 217, 218
Asf1a/HIRA	143
HP1/HMGA	132, 142
SASP/SMS	
IL-6/IL-8	159
Telomere-induced foci/DDR	29, 31, 178–180, 183

DDR indicates DNA damage response; IL, interleukin; SA-β-Gal, senescence-associated β-Gal; SAHF, senescence-associated heterochromatin foci; SASP, senescence-associated secretory phenotype; SMS, senescence-messaging secretome.

comparisons of tissues from old and young humans demonstrated time-dependent telomere shortening.[40] A similar correlation was observed between telomere shortening and pathological conditions. There are several hints linking the progressive telomere shortening to the onset of the aging phenotypes observed with lifetime progression (this topic will be further detailed in the next section). Accelerated aging or, at least, some of its characteristics at the tissue level can be recapitulated by mutations in the core telomerase complex or some of its binding proteins (for a comprehensive review see Martinez et al[34]). Also, external factors that seemed to negatively impact on healthy aging (such as obesity, lack of exercise, or stress) were demonstrated to have a mirror impact on telomere length.[40,41]

Having a severe impact on cell division, telomeres and telomerase are gatekeepers for cancer progression. Many of the human cancers are positive for telomerase,[42] although they do not present telomere lengthening. It has been observed that tumors present shorter telomeres compared with healthy tissues.[43–48] Senescence arises from short telomeres acting as tumor suppressors, when genomic stability is conserved.[12,13,28,49–54] However, some exceptions were perceived to contradict this observation, in the presence of a p53 knockout background or together with overexpression of TRF2, a shelterin component; it was observed that short telomeres could evolve to chromosomal aberrations, genomic instability, and, ultimately, tumor formation.[13,28,55–57]

Further supporting the role of telomerase in aging and cancer, telomerase-deficient mice models present an unusual resistance to induction of tumors, concomitant with the presence of premature aging signs, and a short lifespan that could be worsened by subsequent crosses of mice with short telomeres.[37,49,53,58–61] Although laboratory mice (*Mus musculus,* C57Bl6) present much longer telomeres compared with humans, it was observed that they can similarly recapitulate age-dependent telomere shortening in vivo ([36] and Vera E, Bernardes de Jesus B, Foronda M; Blasco MA, unpublished

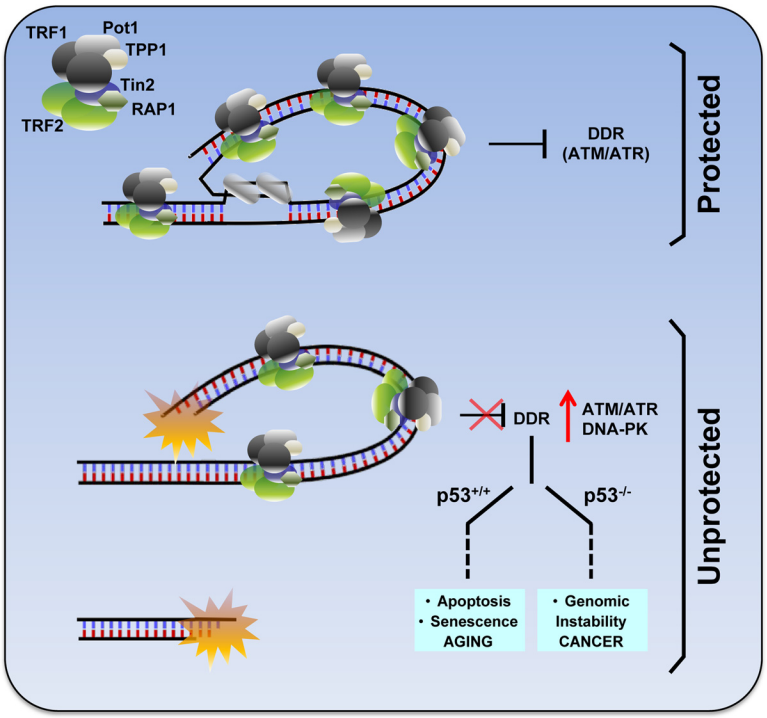

Figure 2. Telomeres structure and senescence. Telomeres form a protective structure (T-loop) that is covered by a complex of different proteins named shelterin (and other proteins not detailed here[12]). Telomere shortening and uncapping are believed to result in senescence.[2,10] Although telomere shortening involves uncapping, it was observed that uncapping per se could result in a senescent condition without modifications in the telomere length.[35,211] Short telomeres or telomeres that lost the aptitude to form the T-loops could lead to the activation of DNA damage response (DDR) and trigger senescence.[12,29]

data, 2012). However, mice overexpressing telomerase were shown to present an increased lifespan when protected from tumors through overexpression of tumor suppressors.[62] Moreover, recently it was demonstrated that reintroduction of telomerase in a telomerase-deficient model of accelerated aging could reverse some of the aging phenotypes, and that telomerase stimulation in adult organisms could result in beneficial metabolic improvements, without cancer.[63,64]

Because telomerase expression in adult tissues is confined to the pool of stem cells, the role of telomerase in cancer and aging could result from an imbalance in the stem cell pool. Old organisms experiencing short telomeres in the pools of stem cells could experience an inability to regenerate tissues. The capacity to regenerate hair or skin, which is directly dependent of the hair bulb and interfollicular pools of stem cells, is compromised in telomerase-deficient mice.[56] In this situation, p53, through the CDK inhibitor $p21^{CIP1}$, acts as the major checkpoint controlling the destiny of these stem cells with short telomeres. As previously noted, in the absence of p53, stem cells with short telomeres are recognized as normal cells and this could result in tumors formation. Although there is well-established importance of telomerase in the pool of stem cell, it also was observed that tissues with low-turnover could likewise experience telomere shortening.[65] This is believed to be related to cell division and accumulation of damage in the genome through intrinsic or extrinsic pathways. Moreover, there is recent evidence linking a pivotal role for telomerase in low-turnover tissues, such as the heart,[66] through a concerted action in mitochondrial biogenesis. To further settle the association between short telomeres and aging, the presence of senescent cells was confirmed in the liver of telomerase null mice.[67,68] The imbalance of the pool of stem cells, together with the presence of senescent cells in adult tissues, could contribute to the telomere-dependent cancer and aging involvement. Whether the pool of stem cells also experience senescence or apoptosis is still unclear.

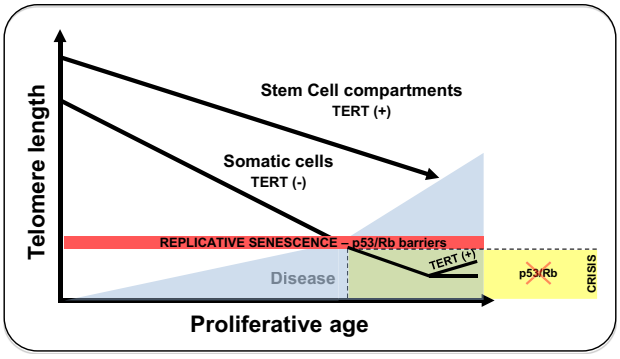

Figure 3. Changes in telomere length. Somatic tissues (usually telomerase negative), including stem cell compartments (which are telomerase-positive), experience a consistent telomere shortening with proliferative time (cell division or increasing age). Telomere attrition has been proposed and is now believed to be related to the onset of age-related pathologies. The stem cell compartments experience an attenuation of telomere shortening because of the fact that this group of cells is positive for telomerase. A certain telomere size (or uncapping) could result in a senescent condition, although only if the barriers p53 and Rb are fully functional. Mutations in any of these barriers result in subsequent shortening and major genomic aberrations ("crisis"[58]), which, concomitant with activation of telomerase, could be the ignition of and fueling of cancer.

Telomeres as Indicators of Biological Aging

As previously described, telomere shortening or modifications in the telomere capping structure could lead to a DNA damage response at the telomeres ends, end-to-end chromosome fusion, and cell-cycle arrest or apoptosis[12] (Figure 2). Short telomeres are commonly found in several pathological

conditions and are an indicator and probably a cause of the disease onset and outcomes. As an example, the load of short telomeres was shown to be linked with diseases from the central nervous system. Although the brain barely experiences a replicative-associated telomere shortening, at least at the same rate as other proliferative tissues, recent evidence has indicated that telomeres could play a crucial role in brain biology.[63] Telomeres are shortened in patients with neurodegenerative diseases such as dementia or Alzheimer disease.[69,70] Telomerase overexpression in a telomerase-negative background demonstrated rescue of brain pathologies associated with aging progression attributable to telomere shortening.[63] Although postmitotic neurons are not candidates for telomere elongation through replication, the recent association between telomeres and mitochondria could unveil novel roles for telomerase in avoidance of telomere shortening in low-turnover tissues.[66,71–73] Telomere shortening also had great impact on cellular immunology; in particular, new telomerase activators were shown to boost the immune system of humans and mice.[74–76] This could be related to the capacity of telomerase to mobilize the pools of stem cells, namely, in this particular situation, the hematopoietic stem cell niches. According to this last point, it has been observed that patients carrying rare human disorders characterized by mutations in telomerase or shelterin components, such as dyskeratosis congenital or aplastic anemia, present an unusual increase in the percentage of short telomeres and, among other particular phenotypes, have development of complete bone marrow failure in the first or second decade of life, which results in the major cause of death.[12,77,78] Moreover, to reinforce the role of telomerase in hematopoietic plasticity, elderly humans presenting with anemia usually demonstrate shorter systemic telomeres.[79] Telomere shortening also correlates with cardiovascular diseases. Despite the established importance of telomere shortening and senescence in the aging phenotypes, there are still methodological issues on telomere lengthening measurements and short telomeres thresholds. These issues are particularly delineated in the next sections, particularly what new advances have been made in telomere analysis focusing on new methodologies that permit reliable telomere assessments either systemically or at the tissue and/or cellular levels.

Oncogene-Induced Senescence

One particular form of nontelomeric stimulus that could lead to senescence involves an unusual activation of oncogenes.[6,11] It was first observed in cultured human cells subjected to overexpression of oncogenic Ras,[80] a cytoplasmic mitogenic signal. After, several other proteins linked to the Ras pathway or other cellular pathways involved in proliferation were shown to lead to oncogene-induced senescence (OIS) when overexpressed.[81–83] Endogenous expression levels of oncogenes do not directly result in OIS.[84,85] Oncogene loading and activity required for senescence are still unknown and, hypothetically, a stochastic accumulation of mutations could be needed for OIS formation.[86] Being activated after oncogenic signals, OIS was thought to have a role in protecting tissues from uncontrolled proliferative stimulus and is identified as a potent anticancer mechanism in vivo. For example, B-RAF or N-RAS (kinases involved in the mitogen-activated protein kinase pathway) are commonly found in benign nevi,[87–89] which concomitantly present markers of senescence such as high expression levels of p16.[83,90] Other benign lesions resulting from tumor suppressor mutations also are enriched in senescent cells.[91] It is believed that senescence is activated in intermediate state where the tumor started but did not reach full malignancy, which usually coincides with the normal function of the major barriers p53 and p16. High percentages of senescent cells have been found in benign or premalignant tumors but not in fully malignant neoplasias of both humans and mice.[92]

Regardless of the many differences between OIS and other senescence programmed pathways (telomere/DNA damage response [DDR]-dependent or those induced through chromatin modifications), there are also common platforms linking these processes. One of them comprises the inactivation of the general transcription factor E2F, which is activated downstream of several oncogenes and is involved in the formation of specific outcomes of DDR activation.[93] Also, it is well-characterized that oncogene activation leads to abnormal replication stress, which could be associated with replication-dependent DNA damage.[94,95] Moreover, disruption of the DNA damage response or absence of DNA replication prevents OIS, suggesting that it could be a consequence of DNA damage.[94,96,97] Confirming OIS and DDR association, premalignant tumors present an exacerbated activation of the DNA damage response.[95]

Senescence in Myocardial Aging

Cardiovascular disease is one of the leading causes of death in developed countries. Understanding how age modulates heart decline is crucial for preventive strategies. The aged heart presents acute alterations in its constitution, particularly a functional loss of cardiomyocytes and other supporting cells, because of clearance of dysfunctional cells through different mechanisms.[98,99] This loss of cells is paralleled by a reduction of the heart regenerative capacity with age progression.[100] Similar to other tissues, aged heart presents an increase of senescent cells (positive for p16, p21, p53, and presenting short telomeres), which, together with the aforementioned characteristics, results in the development of cardiac failure.[101,102] Senescence was shown to have adverse effects in heart healing after fibrosis through reduction of the postinfarction inflammatory response, which could account for the high risk of infarction in elderly patients.[103] It must be noted, however, the scarceness of data supporting many of the senescence methods described hereafter in in vivo conditions and, in particular, in low-turnover tissues such as the heart.[104]

Telomere size has long-been associated with cardiovascular disease. Animal models lacking Tert or Terc have development of cardiovascular dysfunction.[105] This involves p53 activation attributable to short telomeres and seems to comprise, among other things, mitochondrial dysfunction,[28,66,106] something previously observed to be compromised in aged hearts.[107–109] However, Tert overexpression prolongs the division capacity of cardiomyocytes.[110] Several hints link telomere biology with heart dysfunction, particularly the lack

of regenerative capacity observed in aged cardiomyocytes and heart stem cells and the association between short telomeres and high risk of cardiovascular disease in healthy humans.[111,112] Short telomeres were found in patients with several heart conditions, further characterized as valuable predictors for upcoming heart problems. Furthermore, cardiovascular diseases have been causally linked to the inherent percentage of short telomeres[105,113] (a comprehensive review demonstrating the importance of telomeres on cardiovascular aging is provided elsewhere[111]). A review of the impact of short telomeres on heart function including the novel roles for telomerase in mitochondrial modulation is provide elsewhere,[112] confirming that telomere attrition, senescence, and cell death associate with the development of cardiac problems in humans.

Assessing short telomeres, p53, and senescence in heart are valuable indicators for predicting heart functionality. Clearance of senescent cells has been shown to delay dysfunction of several tissues and to extend lifespan, and this possibly could be extended to the heart.[2] Also, new therapies based on transplantation of multipotent stem cells and mobilization of endogenous heart stem cells or, recently, in the "reprogramming" of adult cardiac fibroblast into cardiomyocytes have been recently described as potential therapeutic strategies.[114–116] Modulation of telomerase activity in the aged wild-type heart remains unknown, although telomerase expression strategies increase the functionality of several organs,[62,117] including the heart of a model of premature aging caused by accelerated telomere shortening attributable to telomerase deficiency.[63] Novel strategies relying on the mobilization of stem cells and in the assessment of dysfunctional or senescent cells in the heart will be valuable approaches for healing the aged heart.

Assessing Cellular and Tissue Senescence: Senescence Biomarkers

As previously detailed, the importance of senescence as a "terminal" cellular condition has led to the identification of several senescence biomarkers (Table). Senescent cells have been present in different tissues from different organisms, so the correct description and sensitivity for these markers are of utmost importance in the study of cancer and aging. Senescent cells are characterized by the incapacity of DNA synthesis; however, the assays for these measurements, such as BrdUrd (5-bromo-2-deoxyuridine) or ^{3}H-thymidine incorporation,[6] are nonspecific because they cannot distinguish between quiescent and senescence cells. Still, and as described in the next sections, when used together with senescence specific markers they could be of notable importance.

Senescence-Associated β-Galactosidase

One of the best-characterized and simplified methods to measure senescence in vitro and in vivo is the β-galactosidase (β-Gal) assay, which measures β-Gal activity expressed by senescent cells that can be detectable at pH 6.0 by immunohistochemistry.[118,119] This marker was shown to be present in senescent cells only, and not in presenescent or quiescent fibroblasts or keratinocytes, and is a reliable marker for senescent cell detection in several organisms and conditions.[92,120–125] Demonstrating the correlation between senescence and aging, senescence-associated β-Gal (SA-β-Gal) was shown to increase in an age-dependent manner in skin samples of human donors.[118] Despite being (together with cellular morphology) one of the most common marks of senescence, β-Gal activity has shown background reaction to senescent-independent conditions such cell contact inhibition or high cellular confluence.[126]

SA-β-Gal has not been associated with any major pathway identified in senescent cells or has been a requisite to reach the cellular senescent state. It is thought that it derives from the increased lysosomal content of senescent cells.[127] SA-β-Gal protocol involves the staining (cells or tissues) with X-Gal (5-Bromo-4-chloro-Indoly-β-D-Galactoside or other fluorescent analog such as FDG),[118,128] a chromogenic substrate of β-Gal. X-Gal is cleaved by β-Gal, resulting in an insoluble blue dye.

SA-β-Gal was confirmed as a senescent marker in a replicative senescent protocol or through senescence-induced methods involving DNA damage agents, oncogenic signals, or overexpression of tumor suppressors such p16 and ARF.[129]

Senescence-Associated Heterochromatin Foci and Promyelocytic Leukemia Protein Nuclear Bodies

A pivotal marker of senescence is senescence-associated heterochromatin foci (SAHF). The concept of irreversibility in senescence has been subjected to several suggestions. It has been noticed in seminal studies that senescence cells could not return to a cycling state, even after clearance of the senescence-inducing factor,[81,130] although exceptions have been described.[20,131] Only recent observations lead to a mechanism that could somehow elucidate such a condition. Senescent cells present a characteristic heterochromatin condensation structure involving the formation of heterochromatic foci.[132] These foci can be visible under microscopy and are characterized by condensed regions of DNA/chromatin, which appear as DAPI clusters. SAHF are known to silence and repress several E2F-regulated genes such as MCM3, PCNA, or Cyclin A,[132–134] and are known to be triggered by several pathways involving p16 or p53 activation.[134]

SAHF regions do not present transcription starting sites and are enriched in chromatin repression marks, namely recruitment of different isoforms of HP1, macroH2A (a repressive variant of histone H2A), and trimethylation of lysine 9 in histone 3 (H3K9me3); moreover, SAHF present an exclusion of permissive chromatin marks such as the acetylation of lysine 9 or methylation of lysine 4 in histone 3[134] and a resistance to nuclease digestion, indicating a highly compacted chromatin structure. Recent results demonstrated that SAHF induced by oncogenic stress restrains DDR-favoring senescence despite apoptosis.[135]

SAHF formation involves the concerted action of several protein complexes. It was initially characterized as a response to oncogenic Ras signaling and telomere dysfunction, where the active form of Rb was identified, has one of the SAHF switches. Plasminogen activator inhibitor-1[136] and the canonical Wnt pathway (antagonist)[137] were described as additional modulators of senescence and SAHF formation in mouse and human fibroblasts. Enforcing the relation between the antagonistic effects of Wnt in cell senescence, telomerase is

usually downregulated in postmitotic tissues and it has been shown to be a specific regulator of Wnt target genes,[138] although there is still debate regarding such a direct correlation.[139] Several other complexes have been identified as being involved in the SAHF formation. Among them are the histone chaperones Asf1 and HIRA[140,141] and the HMGA protein complexes.[142] Ectopically, expression of any of these factors leads to SAHF formation and induces senescence; contrary, specific knockdown of HMGA or Asf1 prevents SAHF formation and results in an impairment of senescence.[142,143]

Although not all the different cellular models present SAHF when senescent (a situation that seems to be dependent on the pathway activated), in line with the previous information SAHF are induced through several stress stimulus and contribute to the accomplishment of a senescent condition. Several proteins that accumulate at SAHF are then valuable markers of senescence. HMGA, HIRA, HP1, or H3K9me3 have been positively colocalized at SAHF regions in human fibroblasts, although only in an OIS background with Ras^{V12} and with strict dependence on p16.[134]

SAHF not only is a hallmark of in vitro culture senescence but can also be a valuable marker in vivo. Collado et al[92] observed that activation of $K\text{-}Ras^{V12}$ transgenic mice have development of a plethora of tumors in the lungs. These tumors present an increased activation of p16 together with HP1, which is a SAHF marker. Of note, such observations were correlated with premalignant tumors and not malignant neoplasias, further confirming the importance of senescence in E2F repression and tumor stagnancy.

Promyelocytic leukemia protein (PML) is a tumor suppressor gene that was first identified in 1991 as a translocation partner of retinoic acid in promyelocytic leukemia.[144,145] It was observed that PML expression results in a senescent state[146,147] and is involved in the cellular senescence response to oncogenic stimulus, such as Ras.[146,148,149] Inactivation of PML in mouse cells results in a cancer-prone phenotype.[150] Being a strong growth suppressor, disruption of PML in vivo confirmed an increase of tumors in models of induced carcinogenesis.[150,151] PML is expressed ubiquitously and is usually accumulated at PML nuclear bodies, which are structures found at somatic cells that increase in size with several cellular stresses and the senescent state. PML involvement in senescence acts through the Rb and p53 pathways, two proteins known to interact with PML and to accumulate in PML nuclear bodies. The stabilization of p53 and Rb seems to be supported through independent pathways.[152–154] Cellular senescence is characterized by high expression levels of PML,[146,155] which regulate the activity of E2F transcription factors. After oncogenic-induced senescence through Ras activation, in an Rb-dependent process, E2F transcription factors accumulate at PML nuclear bodies. Moreover, it was observed that PML levels in senescent cells are dependent on the DNA damage signal and accumulate near unrepaired DNA damage regions, which could indicate that senescence is linked to the incapacity of the cells to deal with unrepaired lesions in the genome.[156] Further linking senescence to premalignancies, PML bodies were found frequently in benign prostate tumors but not in prostate cancers[153]; moreover, PML nuclear bodies are absent in cancer cells.[157,158]

Secretory Phenotype

Senescent cells experience huge modifications not only at the morphological level but also at the gene regulation and transcriptome levels. One of the most well-characterized changes is the so-called senescence-associated secretory phenotype (SASP)[159,160] or senescence-messaging secretome (SMS),[161] which is characterized by the secretion of inflammatory signals resembling a local immune response. These changes assign a strong capacity to senescent cells to interact with the environment through the gene expression and secretion of several proteins involved in the inflammation process. Among the >40 intercellular signaling factors identified are the soluble inflammatory cytokines, chemokines, and proteases (a comprehensive review is provided elsewhere[162]). Secreted factors include proteins involved in major cellular pathways, such the Wnt, transforming growth factor-β, or IGF1.[161] SASP not only is a hallmark of senescence cells but also can support the senescence condition. Such is the case of the secreted inflammatory cytokines (interleukin-6) or chemokines (interleukin-8)[163,164]; however, reduction of the DDR components ATM, CHK2, and NBS1 averts the release of some SASP components.[160] SASP formation involves the concerted action of senescence-activated transcription factors (such as NF-κB or C/EBPβ), DDR signaling, and differential regulation of microRNA expression.[162] As described, proinflammatory cytokines could support senescence and, oddly, could favor cancer progression and tissue degeneration, seemingly against the anticancer role of senescence cells.

The modification of the surrounding space through the expression of several secreted proteins has led to the observation that despite the well-characterized role of senescence in tumor suppression, senescence could play a role in assisting tumor biogenesis. Inflammation per se has been linked to cancer predisposition,[165] and the proinflammatory response resulting from senescence could produce similar consequences. Senescent cells via SASP were shown to promote malignant phenotypes in vivo.[159,166] Senescent cells were also demonstrated to trigger epithelial to mesenchymal transition and invasiveness,[167] or to promote tumorigenesis of epithelial cells.[168] The inflammatory response carried by SASP seems to be dependent on a functional p53 in a condition in which its absence favors and amplifies SASP.[159] The tumorigenic capacity of p53-deficient cells could be favored by increased SASP activation. SASP could be similarly mediating the age-related pathologies associated with increased senescence.[3,169,170] Some of the secreted proteins could act in a cell-nonautonomous way to strengthen the cell-cycle arrest. Inflammation not only is involved in cancer susceptibility but also plays major roles in aging-related diseases. Inflammation has been documented in patients with Alzheimer disease who present an increased expression of interleukin-1 and interleukin-6.[169,171,172] Atherosclerosis also has been demonstrated to be dependent on an inflammatory response, which is the basis of disease onset.[173] Moreover, a well-characterized association has been established between inflammation and metabolic syndrome, such as insulin resistance and type 2 diabetes.[169] Adipose tissues of obese subjects were shown to have high levels of cytokines, a situation possibly linked to the accumulation of senescent

cells. In mice, obesity in the context of type 2 diabetes has been shown to increase oxidative damage and senescence-like phenotype.[174] Finding and characterizing new SASP factors and elucidating which factors and how these factors affect tumorigenesis or aging will allow a better understanding of this specific marker of senescence.

Telomere Shortening and DDR

As previously noted, telomere shortening is one of the best-described causes of senescence. From in vitro studies it was observed that senescence was reached when telomeres reach half of their original size,[38,175] a length that permits a stable cellular physiology. Shorter telomeres can be potentially reached when the barriers p53 and Rb are bypassed. In this situation, the genomic stability is no longer conserved and the cell experiences major chromosomal aberrations, a situation termed "crisis,"[58] which could result in cancer (Figure 3). Telomere size is not the only factor believed to play a role in senescence; telomere uncapping has been shown to guide the same ending. Short telomeres could be recognized as DNA double-strand breaks, which are a form of cellular damage. Nevertheless, DNA strand breaks that could arise from other nontelomeric regions could also induce senescence.[176] Telomeres not only could be recognized as double-strand breaks but also are believed to be cellular sensors of several forms of DNA damage. Telomere shortening could be accelerated with oxidative damage, a type of DNA damage that increases with aging.[177] Moreover, oxidative damage was shown to be similarly capable of activating cellular checkpoints and to lead to apoptosis or senescence in a telomere-independent way.[7] DNA repair proteins accumulate at aberrant telomeres and trigger senescence through $p21^{CIP1}$, ATM, and p53-dependent pathways.[178] Telomere-induced foci are then characteristic marks of this cellular state and colocalize with H2AX in senescence and/or aging conditions.[179]

DNA damage response involves the concerted action of several protein complexes that take place at the DNA lesion. These complexes include γ-H2AX, ATM, ATR, 53BP1, MRN, and CHK1/2 and eventually lead to the activation of p53 and apoptosis or senescence. In line with the idea that DDR is concomitant with senescence and could lead to irreversible cell-cycle blockage, some proteins involved in the DNA repair machinery were shown to colocalize with other "traditional" senescence markers, such as β-Gal.[180,181] However, H2AX foci were shown to increase in senescent cells in culture and in the skin of old primates or aged human lymphocytes, demonstrating the relation between senescence, DDR, and aging.[179,182]

H2AX foci could present two distinct typologies, a transient staining characteristic of repaired lesions and a more constant type characteristic of persistent, nonrepaired lesions. This last situation is characteristic of DDR-depended senescence and is referred to as DNA segments with chromatin alterations reinforcing senescence.[183] DNA segments with chromatin alterations reinforcing senescence enclose several proteins activated in DDR,[29,160,178] as well as telomere-induced foci,[29,178] which distinguish them from "normal" H2AX foci.[183] The detection of these structures could be assessed by immunohistochemistry against the phosphorylated form of H2AX and 53BP1 (p53-binding protein 1, a tumor suppressor and checkpoint activator that acts downstream of H2AX).[184–186] Several other proteins that accumulate at DDR foci also could be markers of senescence; nevertheless, these markers are not fully competent senescence markers per se. Telomere-induced foci, for instance, identify senescent cells in vivo, but they also could mark presenescent cells.[187,188] Although DDR foci proteins are not senescence-specific markers, when used in combination with other methods they could be a valuable tool to characterize senescence in aging and cancer.

To unveil the role of telomeres in senescence, meticulous tools are needed to assess telomere length, structure, and integrity (Figure 4). Several methods have been described in the past decades; however, only recent advances permitted using telomere measuring in an accurate and consistent way. Here, we discuss only two methodologies for telomere measurement that permit assessment of telomere dynamics in vivo and in tissue at the cellular level, and all are based on quantitative fluorescence in situ hybridization technique.[189] Quantitative fluorescence in situ hybridization technique is based on the use of a fluorescent-labeled peptide nucleic acid $(CCCTAA)_3$) probe with a high affinity for the denatured telomere repeat sequence (TTAGGG). Telomere signals are detected through quantification of the fluorescent signal, relative to standards of known telomere length. Quantification is processed through automated software, turning the quantification into a consistent and unbiased process. A strong signal is strictly correlated with longer telomeres. Quantitative fluorescence in situ hybridization permits obtaining reliable data at the cellular level and is a strong technique for massive data acquisitions or for tissue partitioning that, together with secondary immunostaining, permits studying the telomere length in specific cell compartments.[36] New variants of the quantitative fluorescence in situ hybridization have been recently developed, particularly the high-throughput quantitative fluorescence in situ hybridization,[40,190] which allow the rapid acquisition of the telomere size from many samples, usually starting from total blood. High-throughput quantitative fluorescence in situ hybridization technique permits studying telomere dynamics in vivo, because a small blood sample is enough to establish peripheral blood mononucleocytes, which will serve as the basis for telomere measurements. Apart from the aforementioned advantages, both techniques permit measuring the percentage of critically short telomeres, which are believed to be the major cause of cellular dysfunction,[60,191,192] something that is missing from other quantitative measurement techniques of telomere length. Making telomere measurements fast and reliable, this technique permits to establish correlations between habits and telomere size, allowing the identification of the so-called biological age compared with the chronological age. A consistent telomere shortening and increase in the percentage of short telomeres were confirmed in mice and in humans over time, demonstrating the importance of telomeres in aging progression[36,193] (Vera E, Bernardes de Jesus B, Foronda M, Blasco MA, unpublished data, 2012). In addition, lifestyle factors such as diet, smoking, or lack of activity also

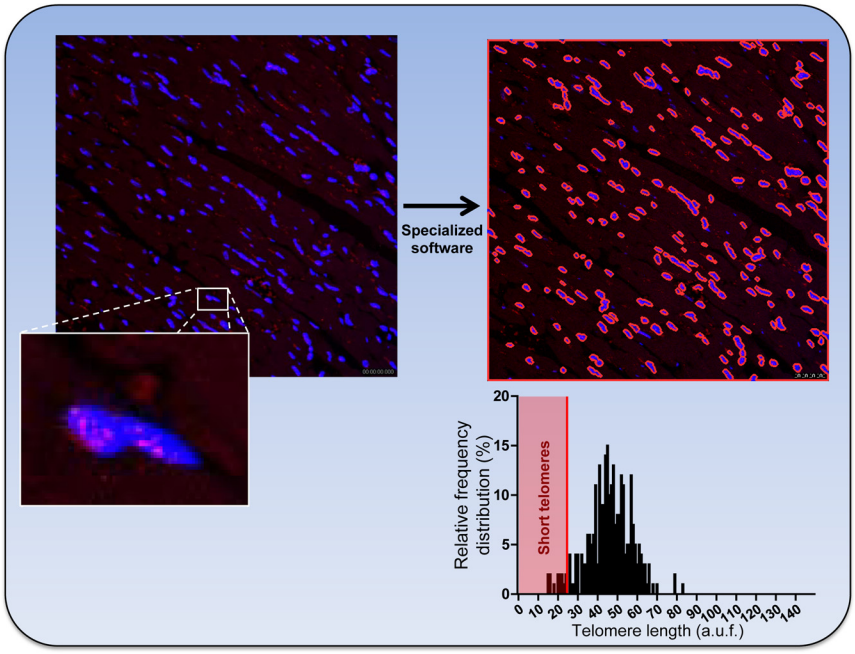

Figure 4. Assessing mean and short telomeres. Work flow of the quantitative fluorescence in situ hybridization technique in a tissue sample (sample from heart). Cells from formalin-fixed slides are hybridized to a fluorescent peptide nucleic acid probe against telomeric repeats. Telomere fluorescence is captured through a confocal microscope (usually a laser scanning microscope using a 40× or 63× oil immersion objectives). The maximal projections of z-stacks generated through advanced fluorescence software (LAS) are used for data calculations. Images are analyzed with specialized software, such as the Definiens XD software package (Definiens Developer Cell software version XD 1.2; Definiens AG). Several fields are analyzed in an automated way in which the DAPI (blue) signal is used to define the nuclear area and the fluorescent peptide nucleic acid telomeric probe (Cy3-red) screen is used to detect single telomeric signal inside of each nuclei. Representative quantification example of quantitative fluorescence in situ hybridization is represented (graph) where the percentage of short telomeres (telomeres <50% of mean intensity) is highlighted.

could be involved in telomere shortening and in an increased rate of age-associated pathologies.[194,195] Assessing telomere dynamics in vivo could be used as an indicator of how aging is progressing, and therapies that impact on telomeres are believed to impact on health[64] and, ultimately, lifespan.[62,63]

Rb and p53

Rb/p16 and p53/ARF are major cellular pathways involved in cellular arrest and oncogene-induced senescence. Mutations in any of these two tumor suppressors result in the bypass of the senescent signaling and lead to an uncontrolled growth pulse, usually a hallmark of cancer.[86] Virtually all cancers that reach malignancy have mutations in p53 and/or p16 pathways, demonstrating the significance of these tumor suppressors in cancer protection. Nevertheless, some tumors could undergo senescence in vivo and have a response to chemotherapy agents or restoration of the tumor suppressor barriers.[196] It was observed that restoring p53 results in the relapse of most of the tumors associated to p53 ablation or oncogenic induced (lymphomas, sarcomas and carcinomas), some of them through a senescent response involving the immunity system.[197,198] Drugs inducing p53 are obviously promising for cancer treatment.[199] The importance of these pathways explains the intense focus they gained and deserved in the past years as markers for tumor progression and/or relapse and senescence. Increased levels of p16, p15, ARF, p53, or p21 are commonly induced in senescent cells and have been identified as consistent OIS markers in vivo, both in humans and mice. The p53-mediated senescence is activated through DDR-dependent signals. It is regulated and regulates several proteins involved in the senescence response, creating in some situations a feedback loop. One of the most important targets of p53 is p21, a protein crucial for p53-dependent senescence response. Similar to reduction of p53, ablation of p21 prevents senescence[131,200,201] and favors an unrestrained proliferation of cells. This could result in a faster telomere shortening and subsequent chromosomal instability. p53 and the DDR are primary barriers for cancer progression, preventing the proliferation of genetically unstable cells.[202,203] On the contrary, a cancer-prone phenotype results from the ablation of p53 in a background with short telomeres or TRF1 deletion.[28,55–57,204] p16/Rb also could be activated by DDR, although secondarily to p53. Nevertheless, p16 pathway could represent the major switch to senescence in some situations. The major response to oncogenic Ras involves the activation of p16, although the mechanism is not fully known but involves the polycomb group proteins BMI1, CBX7, and CBX8, which are capable of regulating the p16 locus and delay senescence.[205–208] The involvement of these two pathways in cancer progression and senescence, together with the presence of functional detection methodologies, made targets of these two pathways common markers for in vivo and in vitro assessments.

Other Senescence Biomarkers

Collado et al[92] have described new OIS markers through DNA microarray screening of genetic changes that took place in HRAS-induced or KRAS-induced OIS but not with inhibition of senescence. A short list of these markers (for instance, $p15^{INK4b}$, DCR2, and DEC1) were further confirmed to colocalize with the "traditional" senescence markers SA-β-Gal and SAHF.[129] These markers also present an increased pattern in premalignant lesions demonstrating the same patterns of senescent cells.

Other markers have been described in the past years; however, their validity still needs to be confirmed in vivo. For example, there are hints associating autophagy with OIS, although the precise involvement remains unclear. Previously it was observed that autophagy increases OIS and contributes to cell-cycle arrest.[209]

Conclusions

The identification of senescence in vitro and in vivo further deepened our knowledge of two important research areas, cancer and aging. Although enormous progress has been made in the past years in senescence markers, understanding how senescence could affect the organism in pathological and nonpathological conditions is only possible if we could unveil, control, and predict the cellular basis of senescence. Although many markers have been identified in the past decades, alone none of these is truly a senescence indicator per se. Many of them are identified in specific conditions, and whether they can be reliably used in a widespread context including different tissues and models is unknown. Senescence biomarkers should be applicable for use in vivo because of the role of increased senescence in age-related diseases or the diminished cell-cycle blockage in the passage of a premalignant-to-malignant transformation. Senescence markers ultimately are cancer and aging markers and are valuable tools for the study of degenerative diseases and cancer.

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M.A.B. is a co-founder of Life Length, SL, a biotechnology company that commercializes telomere length tests.

References

1. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res*. 1961;25:585–621.
2. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature*. 2011;479:232–236.
3. Vijg J, Campisi J. Puzzles, promises and a cure for ageing. *Nature*. 2008;454:1065–1071.
4. Olovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol*. 1973;41:181–190.
5. Hayflick L. Mortality and immortality at the cellular level. A review. *Biochemistry (Mosc)*. 1997;62:1180–1190.
6. Campisi J, d'Adda di Fagagna F. Cellular senescence: When bad things happen to good cells. *Nat Rev Mol Cell Biol*. 2007;8:729–740.
7. Serrano M, Blasco MA. Putting the stress on senescence. *Curr Opin Cell Biol*. 2001;13:748–753.
8. Toussaint O, Medrano EE, von Zglinicki T. Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. *Exp Gerontol*. 2000;35:927–945.
9. Drayton S, Peters G. Immortalisation and transformation revisited. *Curr Opin Genet Dev*. 2002;12:98–104.
10. Wright WE, Shay JW. Historical claims and current interpretations of replicative aging. *Nat Biotechnol*. 2002;20:682–688.
11. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. *Cell*. 2007;130:223–233.
12. Blasco MA. Telomeres and human disease: Ageing, cancer and beyond. *Nat Rev Genet*. 2005;6:611–622.
13. Blanco R, Munoz P, Flores JM, Klatt P, Blasco MA. Telomerase abrogation dramatically accelerates TRF2-induced epithelial carcinogenesis. *Genes Dev*. 2007;21:206–220.
14. de Lange T. Shelterin: The protein complex that shapes and safeguards human telomeres. *Genes Dev*. 2005;19:2100–2110.
15. von Zglinicki T, Martin-Ruiz CM. Telomeres as biomarkers for ageing and age-related diseases. *Curr Mol Med*. 2005;5:197–203.
16. Choudhury AR, Ju Z, Djojosubroto MW, et al. Cdkn1a deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation. *Nat Genet*. 2007;39:99–105.
17. Serrano M, Lee H, Chin L, Cordon-Cardo C, Beach D, DePinho RA. Role of the Ink4a locus in tumor suppression and cell mortality. *Cell*. 1996;85:27–37.
18. Harvey M, Sands AT, Weiss RS, Hegi ME, Wiseman RW, Pantazis P, Giovanella BC, Tainsky MA, Bradley A, Donehower LA. In vitro growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene*. 1993;8:2457–2467.
19. Sage J, Mulligan GJ, Attardi LD, Miller A, Chen S, Williams B, Theodorou E, Jacks T. Targeted disruption of the three Rb-related genes leads to loss of G(1) control and immortalization. *Genes Dev*. 2000;14:3037–3050.
20. Dirac AM, Bernards R. Reversal of senescence in mouse fibroblasts through lentiviral suppression of p53. *J Biol Chem*. 2003;278:11731–11734.
21. Itahana K, Zou Y, Itahana Y, Martinez JL, Beausejour C, Jacobs JJ, Van Lohuizen M, Band V, Campisi J, Dimri GP. Control of the replicative life span of human fibroblasts by p16 and the polycomb protein Bmi-1. *Mol Cell Biol*. 2003;23:389–401.
22. Iwasa H, Han J, Ishikawa F. Mitogen-activated protein kinase p38 defines the common senescence-signalling pathway. *Genes Cells*. 2003;8:131–144.
23. Shay JW, Pereira-Smith OM, Wright WE. A role for both RB and p53 in the regulation of human cellular senescence. *Exp Cell Res*. 1991;196:33–39.
24. Smogorzewska A, de Lange T. Different telomere damage signaling pathways in human and mouse cells. *EMBO J*. 2002;21:4338–4348.
25. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. *Cancer Cell*. 2002;2:103–112.
26. van Steensel B, Smogorzewska A, de Lange T. TRF2 protects human telomeres from end-to-end fusions. *Cell*. 1998;92:401–413.
27. Celli GB, de Lange T. DNA processing is not required for ATM-mediated telomere damage response after TRF2 deletion. *Nat Cell Biol*. 2005;7:712–718.
28. Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ, Greider CW, DePinho RA. p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell*. 1999;97:527–538.
29. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP. A DNA damage checkpoint response in telomere-initiated senescence. *Nature*. 2003;426:194–198.
30. Karlseder J, Broccoli D, Dai Y, Hardy S, de Lange T. p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. *Science*. 1999;283:1321–1325.
31. Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Curr Biol*. 2003;13:1549–1556.
32. Blasco MA. The epigenetic regulation of mammalian telomeres. *Nat Rev Genet*. 2007;8:299–309.
33. Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annu Rev Genet*. 2008;42:301–334.
34. Martinez P, Blasco MA. Telomeric and extra-telomeric roles for telomerase and the telomere-binding proteins. *Nat Rev Cancer*. 2011;11:161–176.
35. Blackburn EH. Switching and signaling at the telomere. *Cell*. 2001;106:661–673.
36. Flores I, Canela A, Vera E, Tejera A, Cotsarelis G, Blasco MA. The longest telomeres: A general signature of adult stem cell compartments. *Genes Dev*. 2008;22:654–667.
37. Lee HW, Blasco MA, Gottlieb GJ, Horner JW II, Greider CW, DePinho RA. Essential role of mouse telomerase in highly proliferative organs. *Nature*. 1998;392:569–574.
38. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature*. 1990;345:458–460.

39. Vaziri H, Schachter F, Uchida I, Wei L, Zhu X, Effros R, Cohen D, Harley CB. Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. *Am J Hum Genet*. 1993;52:661–667.
40. Canela A, Vera E, Klatt P, Blasco MA. High-throughput telomere length quantification by FISH and its application to human population studies. *Proc Natl Acad Sci U S A*. 2007;104:5300–5305.
41. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM. Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci U S A*. 2004;101:17312–17315.
42. Shay JW, Wright WE. Telomerase therapeutics for cancer: Challenges and new directions. *Nat Rev Drug Discov*. 2006;5:577–584.
43. Engelhardt M, Drullinsky P, Guillem J, Moore MA. Telomerase and telomere length in the development and progression of premalignant lesions to colorectal cancer. *Clin Cancer Res*. 1997;3:1931–1941.
44. Engelhardt M, Albanell J, Drullinsky P, Han W, Guillem J, Scher HI, Reuter V, Moore MA. Relative contribution of normal and neoplastic cells determines telomerase activity and telomere length in primary cancers of the prostate, colon, and sarcoma. *Clin Cancer Res*. 1997;3:1849–1857.
45. Odagiri E, Kanada N, Jibiki K, Demura R, Aikawa E, Demura H. Reduction of telomeric length and c-erbB-2 gene amplification in human breast cancer, fibroadenoma, and gynecomastia. Relationship to histologic grade and clinical parameters. *Cancer*. 1994;73:2978–2984.
46. Meeker AK, Argani P. Telomere shortening occurs early during breast tumorigenesis: A cause of chromosome destabilization underlying malignant transformation? *J Mammary Gland Biol Neoplasia*. 2004;9:285–296.
47. Meeker AK, Hicks JL, Iacobuzio-Donahue CA, Montgomery EA, Westra WH, Chan TY, Ronnett BM, De Marzo AM. Telomere length abnormalities occur early in the initiation of epithelial carcinogenesis. *Clin Cancer Res*. 2004;10:3317–3326.
48. van Heek NT, Meeker AK, Kern SE, Yeo CJ, Lillemoe KD, Cameron JL, Offerhaus GJ, Hicks JL, Wilentz RE, Goggins MG, De Marzo AM, Hruban RH, Maitra A. Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. *Am J Pathol*. 2002;161:1541–1547.
49. Gonzalez-Suarez E, Samper E, Flores JM, Blasco MA. Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. *Nat Genet*. 2000;26:114–117.
50. Blasco MA, Hahn WC. Evolving views of telomerase and cancer. *Trends Cell Biol*. 2003;13:289–294.
51. Martinez P, Siegl-Cachedenier I, Flores JM, Blasco MA. MSH2 deficiency abolishes the anticancer and pro-aging activity of short telomeres. *Aging Cell*. 2009;8:2–17.
52. Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, DePinho RA. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature*. 2000;406:641–645.
53. Feldser DM, Greider CW. Short telomeres limit tumor progression in vivo by inducing senescence. *Cancer Cell*. 2007;11:461–469.
54. Sedivy JM. Telomeres limit cancer growth by inducing senescence: Long-sought in vivo evidence obtained. *Cancer Cell*. 2007;11:389–391.
55. Artandi SE, DePinho RA. A critical role for telomeres in suppressing and facilitating carcinogenesis. *Curr Opin Genet Dev*. 2000;10:39–46.
56. Flores I, Blasco MA. A p53-dependent response limits epidermal stem cell functionality and organismal size in mice with short telomeres. *PLoS One*. 2009;4:e4934.
57. Begus-Nahrmann Y, Lechel A, Obenauf AC, Nalapareddy K, Peit E, Hoffmann E, Schlaudraff F, Liss B, Schirmacher P, Kestler H, Danenberg E, Barker N, Clevers H, Speicher MR, Rudolph KL. p53 deletion impairs clearance of chromosomal-instable stem cells in aging telomere-dysfunctional mice. *Nat Genet*. 2009;41:1138–1143.
58. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell*. 1997;91:25–34.
59. Greenberg RA, Chin L, Femino A, Lee KH, Gottlieb GJ, Singer RH, Greider CW, DePinho RA. Short dysfunctional telomeres impair tumorigenesis in the INK4a(delta2/3) cancer-prone mouse. *Cell*. 1999;97:515–525.
60. Herrera E, Samper E, Martin-Caballero J, Flores JM, Lee HW, Blasco MA. Disease states associated with telomerase deficiency appear earlier in mice with short telomeres. *EMBO J*. 1999;18:2950–2960.
61. Samper E, Fernandez P, Eguia R, Martin-Rivera L, Bernad A, Blasco MA, Aracil M. Long-term repopulating ability of telomerase-deficient murine hematopoietic stem cells. *Blood*. 2002;99:2767–2775.
62. Tomas-Loba A, Flores I, Fernandez-Marcos PJ, Cayuela ML, Maraver A, Tejera A, Borras C, Matheu A, Klatt P, Flores JM, Vina J, Serrano M, Blasco MA. Telomerase reverse transcriptase delays aging in cancer-resistant mice. *Cell*. 2008;135:609–622.
63. Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, Sahin E, Kost-Alimova M, Protopopov A, Cadinanos J, Horner JW, Maratos-Flier E, Depinho RA. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature*. 2011;469:102–106.
64. de Jesus BB, Schneeberger K, Vera E, Tejera A, Harley CB, Blasco MA. The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. *Aging Cell*. 2011;10:604–621.
65. Takubo K, Aida J, Izumiyama-Shimomura N, Ishikawa N, Sawabe M, Kurabayashi R, Shiraishi H, Arai T, Nakamura K. Changes of telomere length with aging. *Geriatr Gerontol Int*. 2010;10(Suppl 1):S197–S206.
66. Sahin E, Colla S, Liesa M, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature*. 2011;470:359–365.
67. Wiemann SU, Satyanarayana A, Tsahuridu M, Tillmann HL, Zender L, Klempnauer J, Flemming P, Franco S, Blasco MA, Manns MP, Rudolph KL. Hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis. *FASEB J*. 2002;16:935–942.
68. Rudolph KL, Chang S, Millard M, Schreiber-Agus N, DePinho RA. Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. *Science*. 2000;287:1253–1258.
69. Honig LS, Schupf N, Lee JH, Tang MX, Mayeux R. Shorter telomeres are associated with mortality in those with APOE epsilon4 and dementia. *Ann Neurol*. 2006;60:181–187.
70. Panossian LA, Porter VR, Valenzuela HF, Zhu X, Reback E, Masterman D, Cummings JL, Effros RB. Telomere shortening in T cells correlates with Alzheimer's disease status. *Neurobiol Aging*. 2003;24:77–84.
71. Haendeler J, Drose S, Buchner N, Jakob S, Altschmied J, Goy C, Spyridopoulos I, Zeiher AM, Brandt U, Dimmeler S. Mitochondrial telomerase reverse transcriptase binds to and protects mitochondrial DNA and function from damage. *Arterioscler Thromb Vasc Biol*. 2009;29:929–935.
72. Passos JF, Saretzki G, Ahmed S, Nelson G, Richter T, Peters H, Wappler I, Birket MJ, Harold G, Schaeuble K, Birch-Machin MA, Kirkwood TB, von Zglinicki T. Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. *PLoS Biol*. 2007;5:e110.
73. Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature*. 2010;464:520–528.
74. Fauce SR, Jamieson BD, Chin AC, Mitsuyasu RT, Parish ST, Ng HL, Kitchen CM, Yang OO, Harley CB, Effros RB. Telomerase-based pharmacologic enhancement of antiviral function of human CD8+ T lymphocytes. *J Immunol*. 2008;181:7400–7406.
75. Harley CB, Liu W, Blasco M, Vera E, Andrews WH, Briggs LA, Raffaele JM. A natural product telomerase activator as part of a health maintenance program. *Rejuvenation Res*. 2011;14:45–56.
76. Dagarag M, Evazyan T, Rao N, Effros RB. Genetic manipulation of telomerase in HIV-specific CD8+ T cells: Enhanced antiviral functions accompany the increased proliferative potential and telomere length stabilization. *J Immunol*. 2004;173:6303–6311.
77. Carroll KA, Ly H. Telomere dysfunction in human diseases: The long and short of it! *Int J Clin Exp Pathol.* 2009;2:528–543.
78. Savage SA, Calado RT, Xin ZT, Ly H, Young NS, Chanock SJ. Genetic variation in telomeric repeat binding factors 1 and 2 in aplastic anemia. *Exp Hematol*. 2006;34:664–671.
79. Calado RT, Young NS. Telomere maintenance and human bone marrow failure. *Blood*. 2008;111:4446–4455.
80. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell*. 1997;88:593–602.
81. Zhu J, Woods D, McMahon M, Bishop JM. Senescence of human fibroblasts induced by oncogenic Raf. *Genes Dev*. 1998;12:2997–3007.
82. Lin AW, Barradas M, Stone JC, van Aelst L, Serrano M, Lowe SW. Premature senescence involving p53 and p16 is activated in response to constitutive MEK/MAPK mitogenic signaling. *Genes Dev*. 1998;12:3008–3019.
83. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ, Peeper DS. BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature*. 2005;436:720–724.
84. Guerra C, Mijimolle N, Dhawahir A, Dubus P, Barradas M, Serrano M, Campuzano V, Barbacid M. Tumor induction by an endogenous K-ras

oncogene is highly dependent on cellular context. *Cancer Cell*. 2003;4: 111–120.
85. Tuveson DA, Shaw AT, Willis NA, et al. Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell*. 2004;5:375–387.
86. Collado M, Serrano M. The power and the promise of oncogene-induced senescence markers. *Nat Rev Cancer*. 2006;6:472–476.
87. Bauer J, Curtin JA, Pinkel D, Bastian BC. Congenital melanocytic nevi frequently harbor NRAS mutations but no BRAF mutations. *J Invest Dermatol*. 2007;127:179–182.
88. Papp T, Pemsel H, Zimmermann R, Bastrop R, Weiss DG, Schiffmann D. Mutational analysis of the N-ras, p53, p16INK4a, CDK4, and MC1R genes in human congenital melanocytic naevi. *J Med Genet*. 1999;36: 610–614.
89. Pollock PM, Harper UL, Hansen KS, et al. High frequency of BRAF mutations in nevi. *Nat Genet*. 2003;33:19–20.
90. Gray-Schopfer VC, Cheong SC, Chong H, Chow J, Moss T, Abdel-Malek ZA, Marais R, Wynford-Thomas D, Bennett DC. Cellular senescence in naevi and immortalisation in melanoma: A role for p16? *Br J Cancer*. 2006;95:496–505.
91. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon-Cardo C, Pandolfi PP. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature*. 2005;436:725–730.
92. Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, Benguria A, Zaballos A, Flores JM, Barbacid M, Beach D, Serrano M. Tumour biology: Senescence in premalignant tumours. *Nature*. 2005; 436:642.
93. Dimri GP, Campisi J. Molecular and cell biology of replicative senescence. *Cold Spring Harb Symp Quant Biol*. 1994;59:67–73.
94. Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, Schurra C, Garre' M, Nuciforo PG, Bensimon A, Maestro R, Pelicci PG, d'Adda di Fagagna F. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature*. 2006;444: 638–642.
95. Nuciforo PG, Luise C, Capra M, Pelosi G, d'Adda di Fagagna F. Complex engagement of DNA damage response pathways in human cancer and in lung tumor progression. *Carcinogenesis*. 2007;28: 2082–2088.
96. Bartkova J, Rezaei N, Liontos M, et al. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature*. 2006;444:633–637.
97. Mallette FA, Gaumont-Leclerc MF, Ferbeyre G. The DNA damage signaling pathway is a critical mediator of oncogene-induced senescence. *Genes Dev*. 2007;21:43–48.
98. Olivetti G, Melissari M, Capasso JM, Anversa P. Cardiomyopathy of the aging human heart. Myocyte loss and reactive cellular hypertrophy. *Circ Res*. 1991;68:1560–1568.
99. Chien KR, Karsenty G. Longevity and lineages: Toward the integrative biology of degenerative diseases in heart, muscle, and bone. *Cell*. 2005; 120:533–544.
100. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabe-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, Jovinge S, Frisen J. Evidence for cardiomyocyte renewal in humans. *Science*. 2009;324:98–102.
101. Chimenti C, Kajstura J, Torella D, Urbanek K, Heleniak H, Colussi C, Di Meglio F, Nadal-Ginard B, Frustaci A, Leri A, Maseri A, Anversa P. Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. *Circ Res*. 2003;93:604–613.
102. Torella D, Rota M, Nurzynska D, et al. Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. *Circ Res*. 2004;94:514–524.
103. Bujak M, Kweon HJ, Chatila K, Li N, Taffet G, Frangogiannis NG. Aging-related defects are associated with adverse cardiac remodeling in a mouse model of reperfused myocardial infarction. *J Am Coll Cardiol*. 2008;51:1384–1392.
104. Hwang ES, Yoon G, Kang HT. A comparative analysis of the cell biology of senescence and aging. *Cell Mol Life Sci*. 2009;66:2503–2524.
105. Leri A, Franco S, Zacheo A, Barlucchi L, Chimenti S, Limana F, Nadal-Ginard B, Kajstura J, Anversa P, Blasco MA. Ablation of telomerase and telomere loss leads to cardiac dilatation and heart failure associated with p53 upregulation. *EMBO J*. 2003;22:131–139.
106. Finkel T. Telomeres and mitochondrial function. *Circ Res*. 2011;108: 903–904.
107. Ozawa T. Genetic and functional changes in mitochondria associated with aging. *Physiol Rev*. 1997;77:425–464.
108. Sachs HG, Colgan JA, Lazarus ML. Ultrastructure of the aging myocardium: A morphometric approach. *Am J Anat*. 1977;150:63–71.
109. Wallace DC, Shoffner JM, Trounce I, Brown MD, Ballinger SW, Corral-Debrinski M, Horton T, Jun AS, Lott MT. Mitochondrial DNA mutations in human degenerative diseases and aging. *Biochim Biophys Acta*. 1995;1271:141–151.
110. Oh H, Taffet GE, Youker KA, Entman ML, Overbeek PA, Michael LH, Schneider MD. Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. *Proc Natl Acad Sci U S A*. 2001;98:10308–10313.
111. De Meyer T, Rietzschel ER, De Buyzere ML, Van Criekinge W, Bekaert S. Telomere length and cardiovascular aging: The means to the ends? *Ageing Res Rev*. 2011;10:297–303.
112. Moslehi J, Depinho RA, Sahin E. Telomeres and mitochondria in the aging heart. *Circ Res*. 2012;110:1226–1237.
113. Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CJ, Samani NJ. Telomere length, risk of coronary heart disease, and statin treatment in the west of scotland primary prevention study: A nested case-control study. *Lancet*. 2007;369: 107–114.
114. Laflamme MA, Murry CE. Heart regeneration. *Nature*. 2011;473: 326–335.
115. Smart N, Bollini S, Dube KN, Vieira JM, Zhou B, Davidson S, Yellon D, Riegler J, Price AN, Lythgoe MF, Pu WT, Riley PR. De novo cardiomyocytes from within the activated adult heart after injury. *Nature*. 2011;474:640–644.
116. Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, Conway SJ, Fu JD, Srivastava D. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature*. 2012 Apr 18 [Epub ahead of print].
117. Bernardes de Jesus B, Vera E, Schneeberger K, Tejera AM, Ayuso E, Bosch F, Blasco MA. Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. *EMBO Mol Med*. 2012 May 15 [Epub ahead of print].
118. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A*. 1995;92:9363–9367.
119. Itahana K, Campisi J, Dimri GP. Methods to detect biomarkers of cellular senescence: The senescence-associated beta-galactosidase assay. *Methods Mol Biol*. 2007;371:21–31.
120. Krishnamurthy J, Torrice C, Ramsey MR, Kovalev GI, Al-Regaiey K, Su L, Sharpless NE. Ink4a/Arf expression is a biomarker of aging. *J Clin Invest*. 2004;114:1299–1307.
121. Cao L, Li W, Kim S, Brodie SG, Deng CX. Senescence, aging, and malignant transformation mediated by p53 in mice lacking the Brca1 full-length isoform. *Genes Dev*. 2003;17:201–213.
122. Sun LQ, Lee DW, Zhang Q, Xiao W, Raabe EH, Meeker A, Miao D, Huso DL, Arceci RJ. Growth retardation and premature aging phenotypes in mice with disruption of the SNF2-like gene, PASG. *Genes Dev*. 2004;18:1035–1046.
123. Castro P, Giri D, Lamb D, Ittmann M. Cellular senescence in the pathogenesis of benign prostatic hyperplasia. *Prostate*. 2003;55:30–38.
124. Mishima K, Handa JT, Aotaki-Keen A, Lutty GA, Morse LS, Hjelmeland LM. Senescence-associated beta-galactosidase histochemistry for the primate eye. *Invest Ophthalmol Vis Sci*. 1999;40: 1590–1593.
125. Melk A, Schmidt BM, Takeuchi O, Sawitzki B, Rayner DC, Halloran PF. Expression of p16INK4a and other cell cycle regulator and senescence associated genes in aging human kidney. *Kidney Int*. 2004; 65:510–520.
126. Severino J, Allen RG, Balin S, Balin A, Cristofalo VJ. Is beta-galactosidase staining a marker of senescence in vitro and in vivo? *Exp Cell Res*. 2000;257:162–171.
127. Kurz DJ, Decary S, Hong Y, Erusalimsky JD. Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. *J Cell Sci*. 2000;113(Pt 20):3613–3622.
128. Yang NC, Hu ML. A fluorimetric method using fluorescein di-beta-D-galactopyranoside for quantifying the senescence-associated beta-galactosidase activity in human foreskin fibroblast Hs68 cells. *Anal Biochem*. 2004;325:337–343.

129. Collado M, Serrano M. Senescence in tumours: Evidence from mice and humans. *Nat Rev Cancer*. 2010;10:51–57.
130. Kato D, Miyazawa K, Ruas M, Starborg M, Wada I, Oka T, Sakai T, Peters G, Hara E. Features of replicative senescence induced by direct addition of antennapedia-p16INK4a fusion protein to human diploid fibroblasts. *FEBS Lett*. 1998;427:203–208.
131. Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, Campisi J. Reversal of human cellular senescence: Roles of the p53 and p16 pathways. *EMBO J*. 2003;22:4212–4222.
132. Narita M, Nunez S, Heard E, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell*. 2003;113:703–716.
133. Zhang R, Chen W, Adams PD. Molecular dissection of formation of senescence-associated heterochromatin foci. *Mol Cell Biol*. 2007;27:2343–2358.
134. Funayama R, Ishikawa F. Cellular senescence and chromatin structure. *Chromosoma*. 2007;116:431–440.
135. Di Micco R, Sulli G, Dobreva M, et al. Interplay between oncogene-induced DNA damage response and heterochromatin in senescence and cancer. *Nat Cell Biol*. 2011;13:292–302.
136. Kortlever RM, Higgins PJ, Bernards R. Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. *Nat Cell Biol*. 2006;8:877–884.
137. Ye X, Zerlanko B, Kennedy A, Banumathy G, Zhang R, Adams PD. Downregulation of Wnt signaling is a trigger for formation of facultative heterochromatin and onset of cell senescence in primary human cells. *Mol Cell*. 2007;27:183–196.
138. Park JI, Venteicher AS, Hong JY, Choi J, Jun S, Shkreli M, Chang W, Meng Z, Cheung P, Ji H, McLaughlin M, Veenstra TD, Nusse R, McCrea PD, Artandi SE. Telomerase modulates Wnt signalling by association with target gene chromatin. *Nature*. 2009;460:66–72.
139. Strong MA, Vidal-Cardenas SL, Karim B, Yu H, Guo N, Greider CW. Phenotypes in mTERT+/- and mTERT-/- mice are due to short telomeres, not telomere-independent functions of telomerase reverse transcriptase. *Mol Cell Biol*. 2011;31:2369–2379.
140. Mousson F, Ochsenbein F, Mann C. The histone chaperone Asf1 at the crossroads of chromatin and DNA checkpoint pathways. *Chromosoma*. 2007;116:79–93.
141. Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y. Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. *Cell*. 2004;116:51–61.
142. Narita M, Krizhanovsky V, Nunez S, Chicas A, Hearn SA, Myers MP, Lowe SW. A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. *Cell*. 2006;126:503–514.
143. Zhang R, Poustovoitov MV, Ye X, Santos HA, Chen W, Daganzo SM, Erzberger JP, Serebriiskii IG, Canutescu AA, Dunbrack RL, Pehrson JR, Berger JM, Kaufman PD, Adams PD. Formation of Macroh2a-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. *Dev Cell*. 2005;8:19–30.
144. de The H, Lavau C, Marchio A, Chomienne C, Degos L, Dejean A. The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell*. 1991;66:675–684.
145. Kakizuka A, Miller WH Jr, Umesono K, Warrell RP Jr, Frankel SR, Murty VV, Dmitrovsky E, Evans RM. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. *Cell*. 1991;66:663–674.
146. Ferbeyre G, de Stanchina E, Querido E, Baptiste N, Prives C, Lowe SW. PML is induced by oncogenic ras and promotes premature senescence. *Genes Dev*. 2000;14:2015–2027.
147. Bischof O, Kim SH, Irving J, Beresten S, Ellis NA, Campisi J. Regulation and localization of the Bloom syndrome protein in response to DNA damage. *J Cell Biol*. 2001;153:367–380.
148. Pearson M, Carbone R, Sebastiani C, Cioce M, Fagioli M, Saito S, Higashimoto Y, Appella E, Minucci S, Pandolfi PP, Pelicci PG. PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. *Nature*. 2000;406:207–210.
149. de Stanchina E, Querido E, Narita M, Davuluri RV, Pandolfi PP, Ferbeyre G, Lowe SW. PML is a direct p53 target that modulates p53 effector functions. *Mol Cell*. 2004;13:523–535.
150. Wang ZG, Delva L, Gaboli M, Rivi R, Giorgio M, Cordon-Cardo C, Grosveld F, Pandolfi PP. Role of PML in cell growth and the retinoic acid pathway. *Science*. 1998;279:1547–1551.
151. Gurrieri C, Nafa K, Merghoub T, Bernardi R, Capodieci P, Biondi A, Nimer S, Douer D, Cordon-Cardo C, Gallagher R, Pandolfi PP. Mutations of the PML tumor suppressor gene in acute promyelocytic leukemia. *Blood*. 2004;103:2358–2362.
152. Bischof O, Kirsh O, Pearson M, Itahana K, Pelicci PG, Dejean A. Deconstructing PML-induced premature senescence. *EMBO J*. 2002;21:3358–3369.
153. Vernier M, Bourdeau V, Gaumont-Leclerc MF, Moiseeva O, Begin V, Saad F, Mes-Masson AM, Ferbeyre G. Regulation of E2Fs and senescence by PML nuclear bodies. *Genes Dev*. 2011;25:41–50.
154. Borden KL. Pondering the promyelocytic leukemia protein (PML) puzzle: Possible functions for pml nuclear bodies. *Mol Cell Biol*. 2002;22:5259–5269.
155. Bourdeau V, Baudry D, Ferbeyre G. PML links aberrant cytokine signaling and oncogenic stress to cellular senescence. *Front Biosci*. 2009;14:475–485.
156. Dellaire G, Bazett-Jones DP. PML nuclear bodies: Dynamic sensors of DNA damage and cellular stress. *Bioessays*. 2004;26:963–977.
157. Shen TH, Lin HK, Scaglioni PP, Yung TM, Pandolfi PP. The mechanisms of PML-nuclear body formation. *Mol Cell*. 2006;24:331–339.
158. Gurrieri C, Capodieci P, Bernardi R, Scaglioni PP, Nafa K, Rush LJ, Verbel DA, Cordon-Cardo C, Pandolfi PP. Loss of the tumor suppressor PML in human cancers of multiple histologic origins. *J Natl Cancer Inst*. 2004;96:269–279.
159. Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, Nelson PS, Desprez PY, Campisi J. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol*. 2008;6:2853–2868.
160. Rodier F, Coppe JP, Patil CK, Hoeijmakers WA, Munoz DP, Raza SR, Freund A, Campeau E, Davalos AR, Campisi J. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol*. 2009;11:973–979.
161. Kuilman T, Peeper DS. Senescence-messaging secretome: SMS-ing cellular stress. *Nat Rev Cancer*. 2009;9:81–94.
162. Davalos AR, Coppe JP, Campisi J, Desprez PY. Senescent cells as a source of inflammatory factors for tumor progression. *Cancer Metastasis Rev*. 2010;29:273–283.
163. Kuilman T, Michaloglou C, Vredeveld LC, Douma S, van Doorn R, Desmet CJ, Aarden LA, Mooi WJ, Peeper DS. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell*. 2008;133:1019–1031.
164. Young AR, Narita M. SASP reflects senescence. *EMBO Rep*. 2009;10:228–230.
165. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008;454:436–444.
166. Liu D, Hornsby PJ. Senescent human fibroblasts increase the early growth of xenograft tumors via matrix metalloproteinase secretion. *Cancer Res*. 2007;67:3117–3126.
167. Smit MA, Peeper DS. Epithelial-mesenchymal transition and senescence: Two cancer-related processes are crossing paths. *Aging (Albany NY)*. 2010;2:735–741.
168. Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: A link between cancer and aging. *Proc Natl Acad Sci U S A*. 2001;98:12072–12077.
169. Sikora E, Arendt T, Bennett M, Narita M. Impact of cellular senescence signature on ageing research. *Ageing Res Rev*. 2011;10:146–152.
170. Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, Carter C, Yu BP, Leeuwenburgh C. Molecular inflammation: Underpinnings of aging and age-related diseases. *Ageing Res Rev*. 2009;8:18–30.
171. Gupta A, Pansari K. Inflammation and Alzheimer's disease. *Int J Clin Pract*. 2003;57:36–39.
172. Finch CE, Morgan TE. Systemic inflammation, infection, ApoE alleles, and Alzheimer disease: A position paper. *Curr Alzheimer Res*. 2007;4:185–189.
173. Vasto S, Candore G, Balistreri CR, Caruso M, Colonna-Romano G, Grimaldi MP, Listi F, Nuzzo D, Lio D, Caruso C. Inflammatory networks in ageing, age-related diseases and longevity. *Mech Ageing Dev*. 2007;128:83–91.
174. Sone H, Kagawa Y. Pancreatic beta cell senescence contributes to the pathogenesis of type 2 diabetes in high-fat diet-induced diabetic mice. *Diabetologia*. 2005;48:58–67.
175. Allsopp RC, Harley CB. Evidence for a critical telomere length in senescent human fibroblasts. *Exp Cell Res*. 1995;219:130–136.

176. Robles SJ, Adami GR. Agents that cause DNA double strand breaks lead to p16INK4a enrichment and the premature senescence of normal fibroblasts. *Oncogene*. 1998;16:1113–1123.
177. von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci*. 2002;27:339–344.
178. Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol Cell*. 2004;14:501–513.
179. Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM. Cellular senescence in aging primates. *Science*. 2006;311:1257.
180. Wang C, Jurk D, Maddick M, Nelson G, Martin-Ruiz C, von Zglinicki T. DNA damage response and cellular senescence in tissues of aging mice. *Aging Cell*. 2009;8:311–323.
181. Elyada E, Pribluda A, Goldstein RE, et al. CKIalpha ablation highlights a critical role for p53 in invasiveness control. *Nature*. 2011;470:409–413.
182. Sedelnikova OA, Horikawa I, Redon C, Nakamura A, Zimonjic DB, Popescu NC, Bonner WM. Delayed kinetics of DNA double-strand break processing in normal and pathological aging. *Aging Cell*. 2008;7:89–100.
183. Rodier F, Munoz DP, Teachenor R, Chu V, Le O, Bhaumik D, Coppe JP, Campeau E, Beausejour CM, Kim SH, Davalos AR, Campisi J. DNA-SCARS: Distinct nuclear structures that sustain damage-induced senescence growth arrest and inflammatory cytokine secretion. *J Cell Sci*. 2011;124:68–81.
184. Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, Bonner RF, Bonner WM, Nussenzweig A. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. *Nat Cell Biol*. 2003;5:675–679.
185. Huyen Y, Zgheib O, Ditullio RA Jr, Gorgoulis VG, Zacharatos P, Petty TJ, Sheston EA, Mellert HS, Stavridi ES, Halazonetis TD. Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. *Nature*. 2004;432:406–411.
186. Meier A, Fiegler H, Munoz P, Ellis P, Rigler D, Langford C, Blasco MA, Carter N, Jackson SP. Spreading of mammalian DNA-damage response factors studied by ChIP-chip at damaged telomeres. *EMBO J*. 2007;26:2707–2718.
187. Beliveau A, Bassett E, Lo AT, Garbe J, Rubio MA, Bissell MJ, Campisi J, Yaswen P. p53-dependent integration of telomere and growth factor deprivation signals. *Proc Natl Acad Sci U S A*. 2007;104:4431–4436.
188. Verdun RE, Crabbe L, Haggblom C, Karlseder J. Functional human telomeres are recognized as DNA damage in G2 of the cell cycle. *Mol Cell*. 2005;20:551–561.
189. Poon SS, Lansdorp PM. Measurements of telomere length on individual chromosomes by image cytometry. *Methods Cell Biol*. 2001;64:69–96.
190. Canela A, Klatt P, Blasco MA. Telomere length analysis. *Methods Mol Biol*. 2007;371:45–72.
191. Hemann MT, Strong MA, Hao LY, Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell*. 2001;107:67–77.
192. Hao LY, Armanios M, Strong MA, Karim B, Feldser DM, Huso D, Greider CW. Short telomeres, even in the presence of telomerase, limit tissue renewal capacity. *Cell*. 2005;123:1121–1131.
193. Blasco MA. Telomere length, stem cells and aging. *Nat Chem Biol*. 2007;3:640–649.
194. Ornish D, Lin J, Daubenmier J, Weidner G, Epel E, Kemp C, Magbanua MJ, Marlin R, Yglecias L, Carroll PR, Blackburn EH. Increased telomerase activity and comprehensive lifestyle changes: A pilot study. *Lancet Oncol*. 2008;9:1048–1057.
195. Monaghan P, Haussmann MF. Do telomere dynamics link lifestyle and lifespan? *Trends Ecol Evol*. 2006;21:47–53.
196. Roninson IB. Tumor cell senescence in cancer treatment. *Cancer Res*. 2003;63:2705–2715.
197. Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, Newman J, Reczek EE, Weissleder R, Jacks T. Restoration of p53 function leads to tumour regression in vivo. *Nature*. 2007;445:661–665.
198. Feldser DM, Kostova KK, Winslow MM, Taylor SE, Cashman C, Whittaker CA, Sanchez-Rivera FJ, Resnick R, Bronson R, Hemann MT, Jacks T. Stage-specific sensitivity to p53 restoration during lung cancer progression. *Nature*. 2010;468:572–575.
199. Almazov VP, Kochetkov DV, Chumakov PM. [the use of p53 as a tool for human cancer therapy]. *Mol Biol (Mosk)*. 2007;41:947–963.
200. Brown JP, Wei W, Sedivy JM. Bypass of senescence after disruption of p21CIP1/WAF1 gene in normal diploid human fibroblasts. *Science*. 1997;277:831–834.
201. Gire V, Roux P, Wynford-Thomas D, Brondello JM, Dulic V. DNA damage checkpoint kinase Chk2 triggers replicative senescence. *EMBO J*. 2004;23:2554–2563.
202. Bartkova J, Horejsi Z, Koed K, Kramer A, Tort F, Zieger K, Guldberg P, Sehested M, Nesland JM, Lukas C, Orntoft T, Lukas J, Bartek J. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature*. 2005;434:864–870.
203. Gorgoulis VG, Vassiliou LV, Karakaidos P, et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature*. 2005;434:907–913.
204. Martinez P, Thanasoula M, Munoz P, Liao C, Tejera A, McNees C, Flores JM, Fernandez-Capetillo O, Tarsounas M, Blasco MA. Increased telomere fragility and fusions resulting from TRF1 deficiency lead to degenerative pathologies and increased cancer in mice. *Genes Dev*. 2009;23:2060–2075.
205. Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature*. 1999;397:164–168.
206. Gil J, Bernard D, Martinez D, Beach D. Polycomb CBX7 has a unifying role in cellular lifespan. *Nat Cell Biol*. 2004;6:67–72.
207. Dietrich N, Bracken AP, Trinh E, Schjerling CK, Koseki H, Rappsilber J, Helin K, Hansen KH. Bypass of senescence by the polycomb group protein CBX8 through direct binding to the INK4A-ARF locus. *EMBO J*. 2007;26:1637–1648.
208. Bracken AP, Kleine-Kohlbrecher D, Dietrich N, Pasini D, Gargiulo G, Beekman C, Theilgaard-Monch K, Minucci S, Porse BT, Marine JC, Hansen KH, Helin K. The Polycomb group proteins bind throughout the INK4A-ARF locus and are disassociated in senescent cells. *Genes Dev*. 2007;21:525–530.
209. Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, Tavare S, Arakawa S, Shimizu S, Watt FM. Autophagy mediates the mitotic senescence transition. *Genes Dev*. 2009;23:798–803.
210. Ben-Porath I, Weinberg RA. When cells get stressed: An integrative view of cellular senescence. *J Clin Invest*. 2004;113:8–13.
211. Blackburn EH. Telomere states and cell fates. *Nature*. 2000;408:53–56.
212. Braig M, Lee S, Loddenkemper C, Rudolph C, Peters AH, Schlegelberger B, Stein H, Dorken B, Jenuwein T, Schmitt CA. Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature*. 2005;436:660–665.
213. Afshari CA, Vojta PJ, Annab LA, Futreal PA, Willard TB, Barrett JC. Investigation of the role of G1/S cell cycle mediators in cellular senescence. *Exp Cell Res*. 1993;209:231–237.
214. Tahara H, Sato E, Noda A, Ide T. Increase in expression level of p21sdi1/cip1/waf1 with increasing division age in both normal and SV40-transformed human fibroblasts. *Oncogene*. 1995;10:835–840.
215. Lazzerini Denchi E, Attwooll C, Pasini D, Helin K. Deregulated E2F activity induces hyperplasia and senescence-like features in the mouse pituitary gland. *Mol Cell Biol*. 2005;25:2660–2672.
216. Malumbres M, Perez De Castro I, Hernandez MI, Jimenez M, Corral T, Pellicer A. Cellular response to oncogenic ras involves induction of the Cdk4 and Cdk6 inhibitor p15(INK4b). *Mol Cell Biol*. 2000;20:2915–2925.
217. Funayama R, Saito M, Tanobe H, Ishikawa F. Loss of linker histone H1 in cellular senescence. *J Cell Biol*. 2006;175:869–880.
218. Rogakou EP, Sekeri-Pataryas KE. Histone variants of H2a and H3 families are regulated during in vitro aging in the same manner as during differentiation. *Exp Gerontol*. 1999;34:741–754.

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