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Review

# Telomeres: history, health, and hallmarks of aging

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## SUMMARY

The escalating social and economic burden of an aging world population has placed aging research at center stage. The hallmarks of aging comprise diverse molecular mechanisms and cellular systems that are interrelated and act in concert to drive the aging process. Here, through the lens of telomere biology, we examine how telomere dysfunction may amplify or drive molecular biological processes underlying each hallmark of aging and contribute to development of age-related diseases such as neurodegeneration and cancer. The intimate link of telomeres to aging hallmarks informs preventive and therapeutic interventions designed to attenuate aging itself and reduce the incidence of age-associated diseases.

## INTRODUCTION

Over the last century, advances in public health and medicine have fueled a dramatic rise in life expectancy worldwide. On the current trajectory, 2.1 billion individuals will be older than age 60 by 2050 (United Nations, 2017). This demographic milestone will be accompanied by major increases in age-associated diseases, such as Alzheimer's disease, cardiovascular disease, and cancer (Melzer et al., 2020), which essentially double in incidence every 5 years after age 60. In the absence of new medical and wellness paradigms, the world will experience an unsustainable burden of chronic disease that already extracts a significant social and economic toll. The link between aging and such diseases has motivated fundamental investigations into the mechanisms of aging and strategies to attenuate its effect.

Aging is a progressive degenerative state accompanied by tissue stem cell depletion, tissue inflammation, matrix alterations, cellular senescence, and metabolic dysfunction (López-Otín et al., 2013). These cellular and tissue changes reflect underlying molecular aberrations in mitochondria, proteostasis, intercellular communication, nutrient sensing, epigenetics, and DNA repair that lead to genomic instability and damage, including telomere dysfunction (López-Otín et al., 2013). An ever greater understanding of the diverse molecular mechanisms of aging points to the telomere as an instigator or amplifier of the molecular circuitry driving the aging process and its associated diseases.

Telomeres are composed of repetitive nucleotide sequences that form a "cap structure" that functions to maintain the integrity of chromosomes. Human telomere maintenance-associated gene defects are linked to germline and somatic degenerative diseases such as dyskeratosis congenita, idiopathic pulmonary fibrosis, ulcerative colitis, and others (Calado and Young, 2009). Mice engineered with null or inducible telomerase alleles (Blasco et al., 1997; Jaskelioff et al., 2011) helped researchers to forge a link between telomere dysfunction and aging itself (Blasco et al., 1997; Lee et al., 1998; Rudolph et al., 1999), progeric syndromes (Chang et al., 2004), and chronic inflammatory and degenerative conditions (Chakravarti et al., 2020; Rudolph et al., 2000). Reactivation of endogenous telomerase reverses premature aging in mice with telomere dysfunction (Jaskelioff et al., 2011). Both killifish (Harel et al., 2015) and zebrafish (Carneiro et al., 2016) serve as models for telomere biology studies; their telomere length is similar to that of humans, whereas their telomere dysfunction phenotypes mirror those in murine models.

This review provides an overview of the history and current state of telomere research, highlights mechanistic connections between telomere dysfunction and aging hallmarks, and examines the seemingly pervasive roles of telomeres and telomerase in driving hallmarks of aging, progeria syndromes, and common age-associated diseases such as neurodegeneration and cancer. We describe how the intimate link of telomeres and aging mechanisms informs the development of anti-aging and disease preventive strategies.

### History of telomeres and telomerase

The concept of telomeres was born in the 1930's when McClintock and Muller (Creighton and McClintock (1931) and Muller (1938)) inferred the existence of a unique structure at the ends of chromosomes in *Zea mays* and *Drosophila melanogaster* and hypothesized that it was critical for prevention of chromosome end fusion. Muller coined the term "telomere" from the Greek *telos*, meaning "end," and *meros*, meaning "part"; hence, "end part" (Figure 1). In 1961, it was demonstrated that human fetal cells possessed finite replicative potential of 50 to 60 doublings, subsequently dubbed the "Hayflick limit" or replicative senescence (Hayflick, 1965; Hayflick and Moorhead, 1961). In the early 1970s, Olovnikov (1973) and Watson (1972) introduced the "end replication problem" by observing the asymmetry in linear DNA replication and predicting that each cell division would incur a loss of chromosomal DNA from the termini of the lagging strand because of removal of the terminal RNA primer, leading to progressive chromosomal shortening.

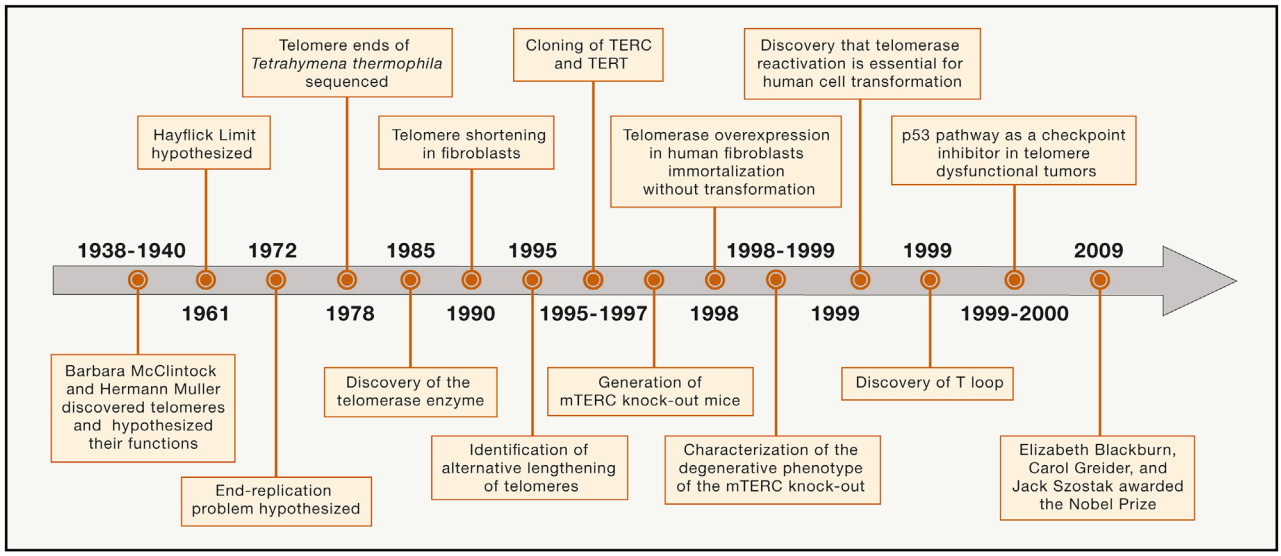


**Figure 1. Timeline of discoveries in the human telomere field**

Blackburn and Gall (1978) sequenced the rDNA of the ciliated protozoan *Tetrahymena thermophila*, revealing termini composed of tandem repeats of hexanucleotide sequences of 5′-CCCCAA-3′ and 3′-TTGGGG-5′ on the complementary strand. Greider and Blackburn (1985) uncovered a novel enzymatic activity capable of adding DNA repeat sequences to chromosome ends and extending telomere length, which is now known as telomerase. The RNA component was cloned from *T. thermophila* (Greider and Blackburn, 1989). In 1990, this journey of discovery culminated in evidence that telomere attrition occurs in parallel with the replicative lifespan of human primary cells in culture, establishing that short telomeres trigger the Hayflick limit (Harley et al., 1990). The role of telomere attrition and damage in inducing permanent cell cycle arrest was further demonstrated using human fibroblasts overexpressing a mutant form of TRF2 (TRF2ΔBΔM) through activation of DNA damage checkpoints (d'Adda di Fagagna et al., 2003).

The next major breakthrough occurred in 1996 with the demonstration that telomerase is a ribonucleoprotein that acts on a 3′ overhang (Lingner et al., 1997). This was followed by cloning of human telomerase reverse transcriptase (hTERT) (Harrington et al., 1997) and the human telomerase RNA component (hTERC) (Feng et al., 1995). The first TERC knockout mouse was generated in 1997 (Blasco et al., 1997). In the ensuing years, mice null for TERC and TERT, alone or in combination with progeria or cancer-relevant alleles, established that telomere dysfunction can drive premature aging, cancer, and various degenerative diseases. These mouse models proved that intact telomeres maintain genome stability, tissue stem cell reserves, organ system homeostasis, and a normal lifespan (Chang et al., 2004; Jaskelioff et al., 2011; Rudolph et al., 1999; Sahin et al., 2011; Wong et al., 2003). In 1998, a capstone study showed that enforced hTERT expression endows unlimited replicative potential to primary human cells, including fibroblasts, retinal pigment epithelial cells, and vascular endothelial cells (Bodnar et al., 1998). Notably, these cell cultures maintain a normal karyotype and show no malignant properties.

Historically, although telomerase was known to be consistently upregulated in cancer, its role in cellular transformation was documented when enforced TERT expression, together with classical oncogenes, promoted full malignant transformation of primary human cells (Hahn et al., 1999). Contemporaneously, genetic models of cancer established that the status of the p53-dependent DNA damage response dictated whether short telomeres promote or suppress cancer *in vivo* (Chin et al., 1999; Greenberg et al., 1999). Further analysis of late-generation *mTERC*$^{-/-}$;*p53*$^{+/-}$ mice (Artandi et al., 2000) revealed a humanized tumor spectrum of epithelial cancers possessing chromosomal rearrangements and nonreciprocal translocations typical of human cancer genomes. Thus, mice engineered to experience telomere-based crisis in the context of deactivated DNA damage signaling (p53 deficiency) illuminated a major mechanism driving the preponderance of epithelial cancers in aged humans and explained why such cancers develop radically altered cytogenetic profiles.

The TERC and TERT knockout mouse models authenticated the role of telomeres in aging and identified a core signaling pathway driving the aging process. First, these models established that telomere dysfunction accelerates signs and symptoms of aging characterized by shortened life expectancy, an aged appearance, declining tissue stem cell reserves, organ atrophy, and diminished capacity to cope with stress, injury, and regenerative demands (Lee et al., 1998; Rudolph et al., 1999). Second, these models highlighted the essentiality of telomere dysfunction in progeroid syndromes and Parkinson's disease (Chang et al., 2004; Lee et al., 1998; Rudolph et al., 1999; Wong et al., 2003). Third, unbiased transcriptomics analyses across diverse tissues of the late-generation *TERC*$^{-/-}$ mouse revealed the p53-PGC pathway of aging, integrating three previously separate theories of aging: genotoxic stress (telomere

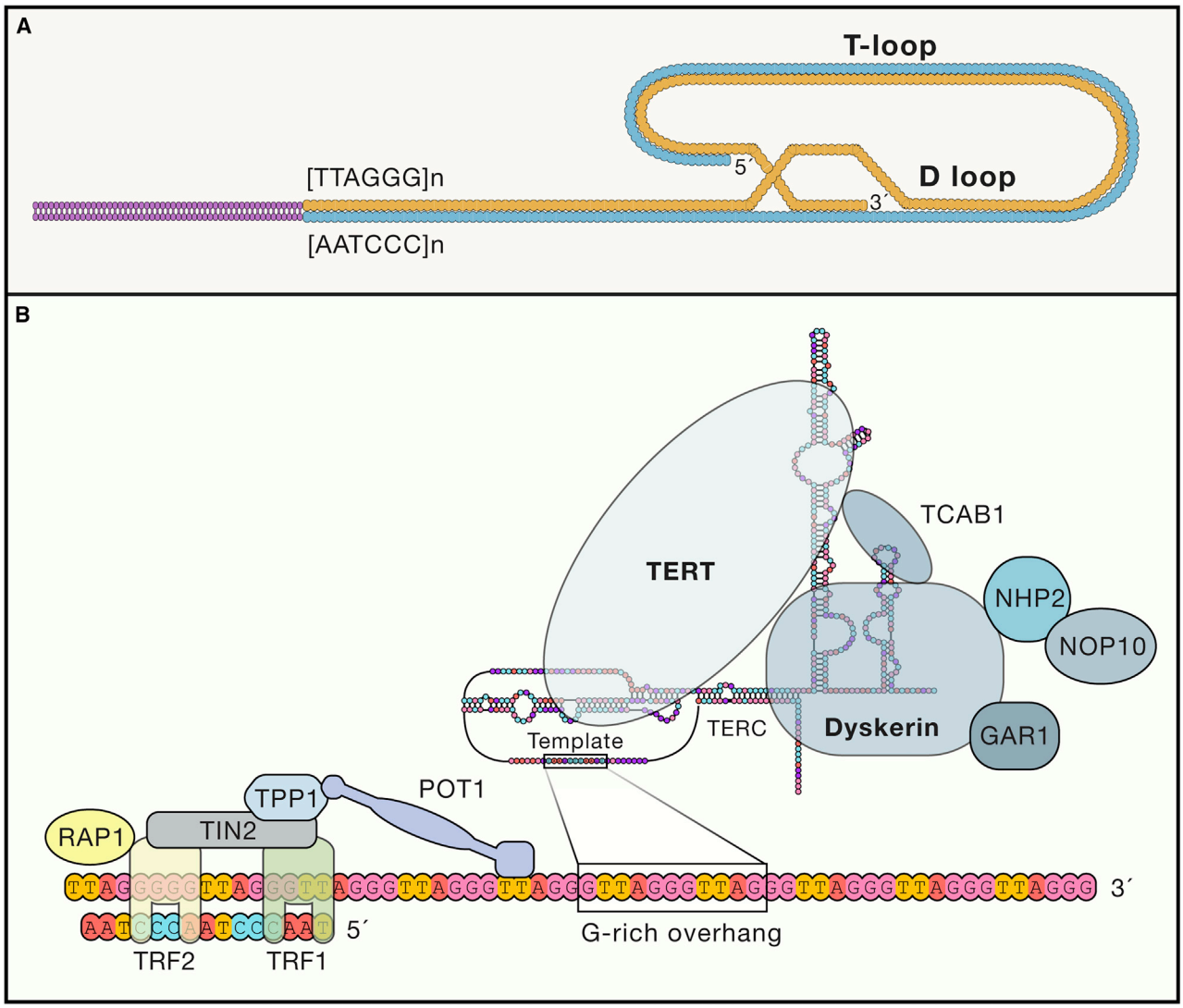


**Figure 2. The vertebrate telomere/telomerase complex**

(A) Structure of the D-loop and T-loop at the telomeric end.

(B) Structure of telomerase. NOP10, nucleolar protein family A, member 3; NHP2, nucleolar protein family A, member 2; TIN2, TERF1-interacting nuclear factor 2; TPP1, telomere protection protein 1; TRF1, telomeric repeat binding factor 1; TRF2, telomeric repeat binding factor 2; POT1, protection of telomeres 1; RAP1, TERF2-interacting protein; TCAB1, telomerase Cajal body protein 1; GAR1, nucleolar protein family A, member 1.

dysfunction), oxidative damage, and mitochondrial decline (Sahin et al., 2011). Finally, an inducible TERT mouse model demonstrated that reactivation of endogenous telomerase reverses advanced premature aging in mice (Jaskelioff et al., 2011). Furthermore, adenoviral delivery of telomerase in aged mice improves cardiac function following acute myocardial infarction, enhances muscle coordination and kidney and liver function, reduces insulin resistance and subcutaneous fat depletion, increases bone mineral density, and extends life expectancy without causing an increase in cancer incidence (Hong and Yun, 2019). This collective body of work across decades and diverse model systems has defined the molecular biology of telomeres and their role in health and disease.

## Shelterin and the telomerase complex

Telomeres maintain chromosomal integrity, which is essential for sustaining the health span and propagation of the species. The evolutionarily conserved function of telomere end protection extends from lower multicellular organisms such as *T. thermophila* to higher-order organisms, including *Homo sapiens* (Roake and Artandi, 2020). Structurally, telomeres consist of tandem repeat sequences of TTAGGG measuring several to tens of kilobases and terminating at the 3′ end in a single-stranded 75- to 300-nt overhang enriched in guanine nucleotides (Figure 2B). Seminal work by Griffith etal. (1999) established that the overhang folds back on itself to form a lariat-like structure called the T loop (Figure 2A).

The telomere is cloaked in specialized proteins dubbed the shelterin complex, a multimer composed of six protein subunits: TRF1, TRF2, TPP1, POT1, TIN2, and RAP1 (de Lange, 2018). This higher-order structure of telomeres functions to inhibit DNA damage signaling from the telomere ends, prevent DNA repair programs from fusing ends via recombination or classical/alternative non-homologous end joining, and regulate the access and activity of telomerase at the termini. Correspondingly, mutations in the aforementioned components can disrupt the shelterin-telomere complex, causing end fusions and premature senescence (van Steensel et al., 1998). Similarly, overexpression of *TRF1* or deregulation of *POT1* impairs telomerase binding to telomere ends, causing telomere shortening (Loayza and De Lange, 2003; Smogorzewska et al., 2000). Conversely, loss of TRF1 also leads to formation of common fragile sites in telomeric DNA because of its inability to recruit the Bloom (BLM) helicase and, therefore, BLM's execution of robust double-strand break repair during replication (Yang et al., 2020).

In normal tissue, telomerase expression is abundant in germ cells (Lee et al., 1998) and present in undifferentiated stem and progenitor cells of the skin (Lee et al., 1998; Rudolph et al., 1999), intestine (Schepers et al., 2011), hematopoietic system (Colla et al., 2015), hair bulge (Sarin et al., 2005), and testes (Rudolph et al., 1999). Differentiated cells such as keratinocytes, fibroblasts, skeletal muscle myocytes, neurons, cardiac myocytes, and spermatids show modest or undetectable TERT levels (Artandi and DePinho, 2010; Pech et al., 2015).

TERC is related to small Cajal body RNAs (scaRNAs) and small nucleolar RNAs (snoRNAs). Although such RNAs are encoded within introns of other genes, *TERC* is a prototypical gene with its own promoter (Feng et al., 1995). TERC RNA is transcribed in a precursor format with a 5′ methylguanosine cap and a poly(A) tail. These precursors are deadenylated by the disease-associated poly(A)-specific ribonuclease (PARN), which promotes TERC maturation and accumulation (Roake and Artandi, 2020). TERC contains an H/ACA domain consisting of a 3-nt sequence, called the Cajal body localization (CAB) box, that is essential for binding to telomerase Cajal body protein 1 (TCAB1). TCAB1 is required for catalytic activity and trafficking of telomerase to Cajal bodies, which aids in trafficking telomerase to telomere ends. Telomerase associates with TERC through its pseudoknot/template domain (PK/T) and CR4/5 domains. Multiple proteins are essential for proper functioning of the holoenzyme, including dyskerin, NHP2, NOP10, and GAR1, which form core components along with other proteins.

In summary, the structure of telomeres, coupled with the highly regulated activity and recruitment of telomerase, ensures proper telomere maintenance in normal cells. At the same time, each of these features is vulnerable to mutations and dysregulation, leading to familial and sporadic diseases. Nevertheless, several questions remain regarding how the telomerase complex senses and is recruited to the shortest telomeres and the specific order in which the different components are assembled. Uncertainty also surrounds how these processes may be differentially regulated between normal and evolving neoplastic cells. Most importantly, a deeper understanding of the regulation of telomerase expression and function is needed to define the contributions of telomerase to normal aging as well as inherited and somatic degenerative disease pathogenesis in humans.

## Crosstalk between dysfunctional telomeres and the hallmarks of cellular aging

Limited telomere reserves serve as a roadblock to cellular immortalization, but loss of telomere function coincides with an age-related decline in fitness and cancer-inducing genome instability. Indeed, telomere dysfunction has been described as one of the nine cellular and molecular hallmarks of aging (López-Otín et al., 2013). In this section, we detail the role of telomeres in processes of aging with an emphasis of how telomeres interrelate with the other hallmarks of aging, serving to drive or amplify these mechanisms (Figure 3). Additionally, we summarize the role of genetic model systems in revealing the interconnectedness of telomeres with other mechanisms and pathways driving aging as well as premature aging syndromes. The following subsections outline how telomere dysfunction links to the mechanisms underlying each hallmark of aging.

### *Cellular senescence*

Senescent cells accumulate in aging tissue and contribute to aging and age-related diseases via various mechanisms. The following are the two most well-studied: the first involves impeding the replicative potential of tissue stem cells (another hallmark of aging, stem cell exhaustion) immune cells (so-called immunosenescence), and stromal cells; the second stems from disrupting organ function through release of pro-inflammatory factors, including but not limited to interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-$\alpha$) (Coppé et al., 2010) by senescent cells. With respect to the latter, the Hayflick limit triggers senescence and proliferative arrest via activation of the p53-p19$^{ARF}$ and p16$^{Ink4a}$-Rb signaling pathways (Shay et al., 1991). Whether and how telomere dysfunction activates the senescence program in low-proliferative stroma tissue during normal aging requires further investigation, although it is tempting to speculate that reactive oxygen species (ROS)-induced damage of telomeres leads to telomere dysfunction-induced focus (TIF) generation and induction of senescence.

### *Stem cell exhaustion*

Tissue stem cell exhaustion is a cardinal feature of aging. In late-generation TERT- or TERC-null mice, progressive telomere erosion leads to p53-dependent apoptotic elimination of tissue stem cells, which contributes to organ atrophy, particularly in highly proliferative tissues with high rates of self-renewal, including the skin, intestine, testis, regenerating injured liver, and blood (Blasco et al., 1997; Chin et al., 1999; Colla et al., 2015; Jaskelioff et al., 2011; Lee et al., 1998; Rudolph et al., 1999, 2000; Sahin et al., 2011). Beyond telomere maintenance, TERT may also affect stem cell biology via non-classical functions of TERT involving activation of the Wingless-related integration site (WNT) pathway, a major regulator of stem cell homeostasis. Specifically, in mouse cells, TERT can physically interact with Brahma-related gene-1 (BRG1) and serve as a co-factor in the $\beta$-catenin complex, resulting in upregulation of WNT network genes (Park et al., 2009).

### *Genomic instability*

Genomic instability, another hallmark of aging, can lead to stem cell exhaustion and provoke inflammation. Telomere

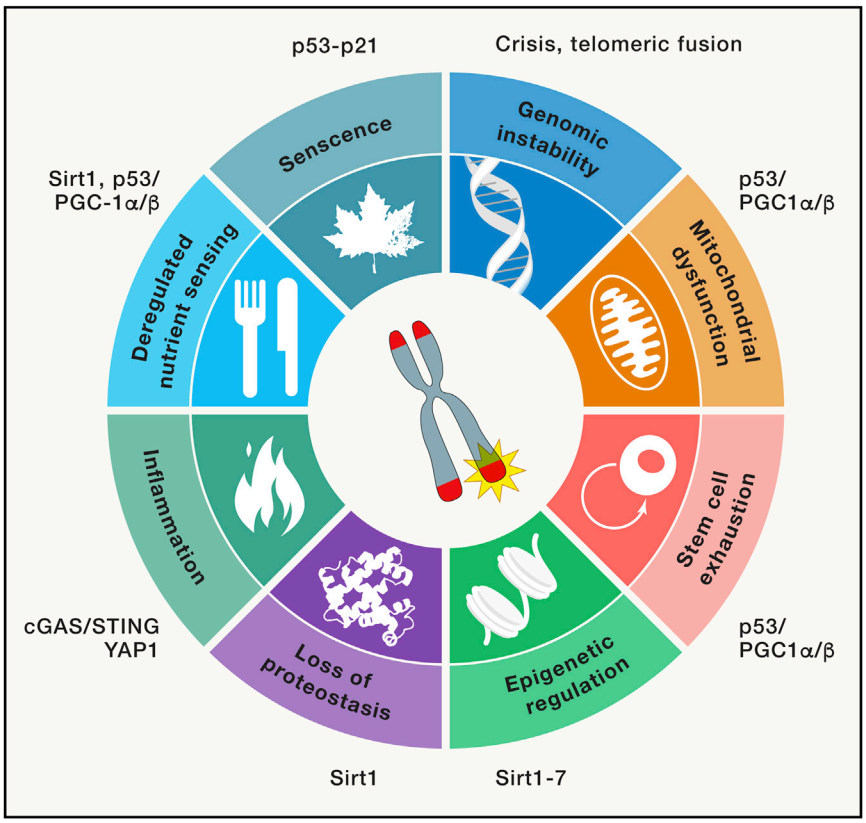


**Figure 3. Relevance of telomere dysfunction to cellular aging hallmarks**
Telomere dysfunction can drive the hallmarks of cellular aging.

dysfunction can fuel chromosomal instability, as evidenced by cytogenetic analysis of late-generation TERC-null cells and tissues; these analyses showed amplifications, deletions, translocations, and anaphase bridge formation in tumors, including colorectal cancer (detailed below; Artandi et al., 2000; Blasco et al., 1997; O'Hagan et al., 2002; Rudolph et al., 1999, 2001). Mechanistically, eroded or uncapped telomeres undergo end-to-end fusion, forming dicentric chromosomes and resulting in breakage-fusion-bridge (BFB) cycles, aneuploidy and tetraploidization, translocations, and amplifications, which create genomic instability through kataegis (localized hypermutations) and chromothripsis (clustered chromosomal rearrangements) during mitosis (Maciejowski and de Lange, 2017). Moreover, loss of p53 enables cells to survive these DNA double-strand break events to produce aberrant chromosomal imbalances and nonreciprocal translocations that drive cancer initiation (see Telomeres and Telomerase in age-related diseases and cancer). These chromosomal abnormalities have been documented in nonmalignant aged stem cell compartments, where the mutational burden strongly correlates with increasing age in human tissues, including colonic crypts and the hematopoietic system (Calado et al., 2012; Hsieh et al., 2013).

### *Mitochondrial dysfunction*

Telomeres and mitochondria are intimately linked (Sahin et al., 2011). In aging, mitochondrial function declines, leading to diminished energy (ATP) production as well as increased intracellular ROS. Diminished energy production causes overall frailty, whereas increased ROS cause cellular damage, including formation of 8-oxoguanine base lesions in DNA (telomeres are guanine rich). Direct evidence of a role for mitochondrial decline in driving processes of aging derives from mice harboring mutations in the mitochondrial DNA polymerase subunit gamma (*POLG*). These mutant mice show reduced mitochondrial abundance and abnormal mitochondrial morphology (e.g., fragmented cristae and disrupted external membrane; Trifunovic et al., 2004) and exhibit premature aging (e.g., alopecia, kyphosis, reduced body weight, reduced subcutaneous fat, reduced bone mineral density consistent with osteoporosis, anemia, and cardiomyopathy). Notably, the premature aging phenotypes associated with mitochondrial dysfunction mirror those of telomerase-deficient mice, p53-hyperactivated mice (Tyner et al., 2002), and PGC1α/β-null mice (Lai et al., 2008). Given the centrality of mitochondria in aging, the overlapping phenotypes of TERC-, peroxisome proliferator activated receptor gamma co-activator (PGC)1α/β-, and POLG-null mice enabled us to link telomeres, mitochondria, and oxidative defense mechanisms in driving aging. Indeed, TERC-null mice have been documented (Sahin et al., 2011) to have impaired mitochondrial function and diminished oxidative defense. Furthermore, transcriptomics analysis of diverse tissues in the late-generation $TERC^{-/-}$ mouse revealed prominent representation of p53 and PGC1α/β target genes, enabling us to identify a common pathway integrating three competing theories of aging: accumulating genotoxic stress, declining mitochondrial function, and increasing oxidative damage. Specifically, telomere dysfunction activates p53, which, in turn, represses PGC1α and PGC1β expression (Sahin et al., 2011; Figure 4). Diminished PGC1α/β expression, in turn, results in decreased mitochondrial biogenesis and function and reduces expression of genes governing oxidative defense. This signaling circuit of telomere→p53→PGC1α/β→mitochondria leads to escalating ROS levels and further ROS-mediated 8-hydroxydeoxyguanosine modification of guanosine bases at telomeres (Figure 4). This axis creates a feedforward loop linking telomere dysfunction, mitochondria, and oxidative stress pathways, resulting in accelerated aging.

### *Epigenetic dysregulation*

Age-dependent changes in the epigenetic landscape include increased local methylation and decreased global methylation, increased H4K16 acetylation, H3K4 trimethylation, and H4K20 trimethylation and decreased H3K9 monomethylation and

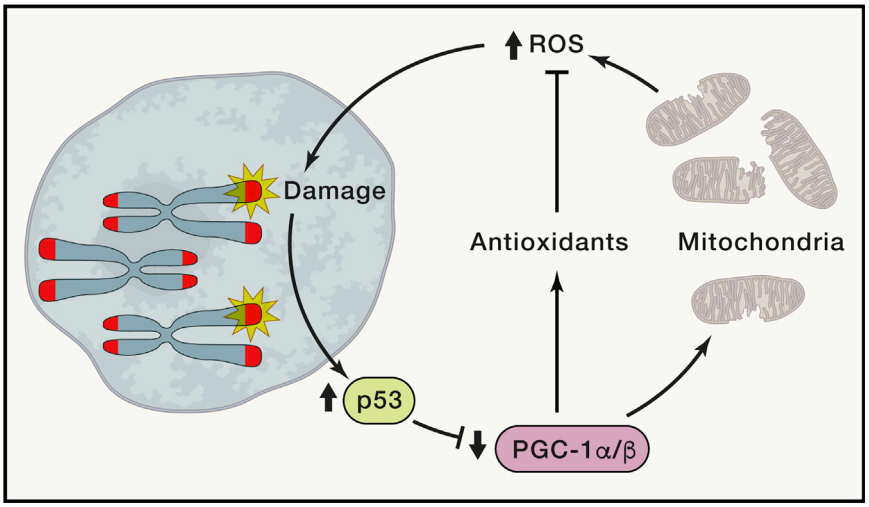


**Figure 4. Telomere dysfunction drives mitochondrial defects**
Telomere dysfunction compromises mitochondrial function and oxidative defense, increasing ROS and creating a feedforward loop involving p53/PGC-1α/β signaling.

H3K27 trimethylation (Barnes et al., 2019). Direct telomere and epigenetic interaction is reflected in the regulation of sirtuins (SIRT), a family of seven (SIRT1–SIRT7) nicotinamide adenine dinucleotide (NAD+) dependent deacetylases regulating lifespan and health span (Amano and Sahin, 2019). In mammals, SIRT1 deacetylates several aging-relevant transcription factors, including p53, forkhead-box transcription factor (FOXO), PGC1α, and nuclear factor κB (NF-κB), which regulate crucial molecular pathways related to stress resistance, metabolism, and oxidative defense (Houtkooper et al., 2012). The interplay between telomere dysfunction and SIRT (SIRT1 and SIRT6) is evidenced by telomere dysfunction-induced p53-mediated repression of all seven SIRTs (Amano et al., 2019). Moreover, telomere dysfunction-mediated p53 activation represses expression of PGC1α/β, which, in turn, regulates expression of mitochondrial SIRT3, SIRT4, and SIRT5. In addition, p53 activation results in transcriptional upregulation of microRNAs (miR-34a-5p, miR-26a-5p, and miR-145-5p) that suppress translation of non-mitochondrial SIRT1, SIRT2, SIRT6, and SIRT7 (Amano et al., 2019). Thus, telomere-regulated epigenetic networks are relevant to aging regulators.

### *Loss of proteostasis*

Telomere dysfunction and the resultant p53-mediated repression of SIRT1 expression are also relevant to the aging hallmark of altered proteostasis. Loss of proteostasis stems from a decline in the activity of the chaperone network that is responsible for proper protein-folding capacity. Mutant mice deficient in the carboxyl terminus of heat shock protein 70 (HSP70)-interacting protein (CHIP), a co-chaperone of the heat shock family, exhibit accelerated aging phenotypes. In mammalian cells, SIRT1-mediated deacetylation of heat shock factor-1 (HSF-1) potentiates transcriptional activation of heat shock genes such as HSP70. Thus, it is plausible that telomere dysfunction-induced repression of SIRT1 and the resultant decreased HSP70 levels impair protein homeostasis and heat shock responses during stress (Westerheide et al., 2009). Protein folding is essential for maintaining neuronal homeostasis; post-mitotic long-lived neurons are extremely vulnerable to misfolded proteins because they are unable to reduce the misfolded protein load through cell division (Muchowski and Wacker, 2005). Deregulated protein folding occurs in several neurological diseases of aging, such as Alzheimer's disease and Parkinson's disease, which exhibit misfolded β-amyloid peptide and α-synuclein, respectively. The accumulation leads to gradual neuronal death and cognitive impairment. Recent studies have shown that overexpression of HSP70 is neuroprotective in *Drosophila* and in mouse and rat models of Alzheimer's disease and Parkinson's disease (Auluck et al., 2002; Kakimura et al., 2002; Klucken et al., 2004).

### *Altered nutrient sensing*

Deregulated nutrient sensing in aging relates to a highly conserved network consisting of IGF-1 and the mechanistic target of rapamycin (mTOR)-S6 signaling pathways and members of the FOXO and AMP-activated protein kinase (AMPK) families of proteins. These pathways reciprocally regulate each other to maintain metabolic homeostasis. AMPK is a central sensor of nutrients that modulates the mechanistic target of rapamycin mTOR signaling and transcriptionally activates FOXO transcription factors and SIRT1 (Saxton and Sabatini, 2017). SIRT1, in turn, can activate FOXO and PGC1α, which are essential for mitochondrial biogenesis (Brunet et al., 2004; Rodgers et al., 2005). Thus, the capacity of telomere dysfunction to affect metabolism stems from its activation of p53 and repression of PGC1α and SIRT1. Indeed, telomere-dysfunctional mice fail to maintain plasma glucose levels under fasting conditions because of defects in gluconeogenesis regulated by p53-mediated repression of PGC1α/β and its downstream effectors glucose-6 phosphate (GLC-6-P) and phosphoenolpyruvate carboxykinase (PEPCK) (Sahin et al., 2011). Enforced expression of mTERT or PGC1α or genetic ablation of p53 elevates expression of PGC1α/β, GLC-6-P, and PEPCK and restores gluconeogenesis (Sahin et al., 2011). Telomere dysfunction-induced mitochondrial impairment increases the dependency of tissues on glucose metabolism (Missios et al., 2014). Increases in glycolysis versus mitochondrial oxidative metabolism may also lead to changes in NAD/NADH pools, further impairing SIRT activity. Additionally, DNA damage signaling and PARP activation, as a consequence of telomere dysfunction, reduce NAD pools and impair SIRT function. Poly (ADP-ribose)polymearse (PARP) and SIRTs compete for the common pool of NAD+ so that inhibition of PARP or genetic ablation leads to increased SIRT function in brown adipose tissue and muscle, as observed in *PARP1* knockout mice (Bai et al., 2011).

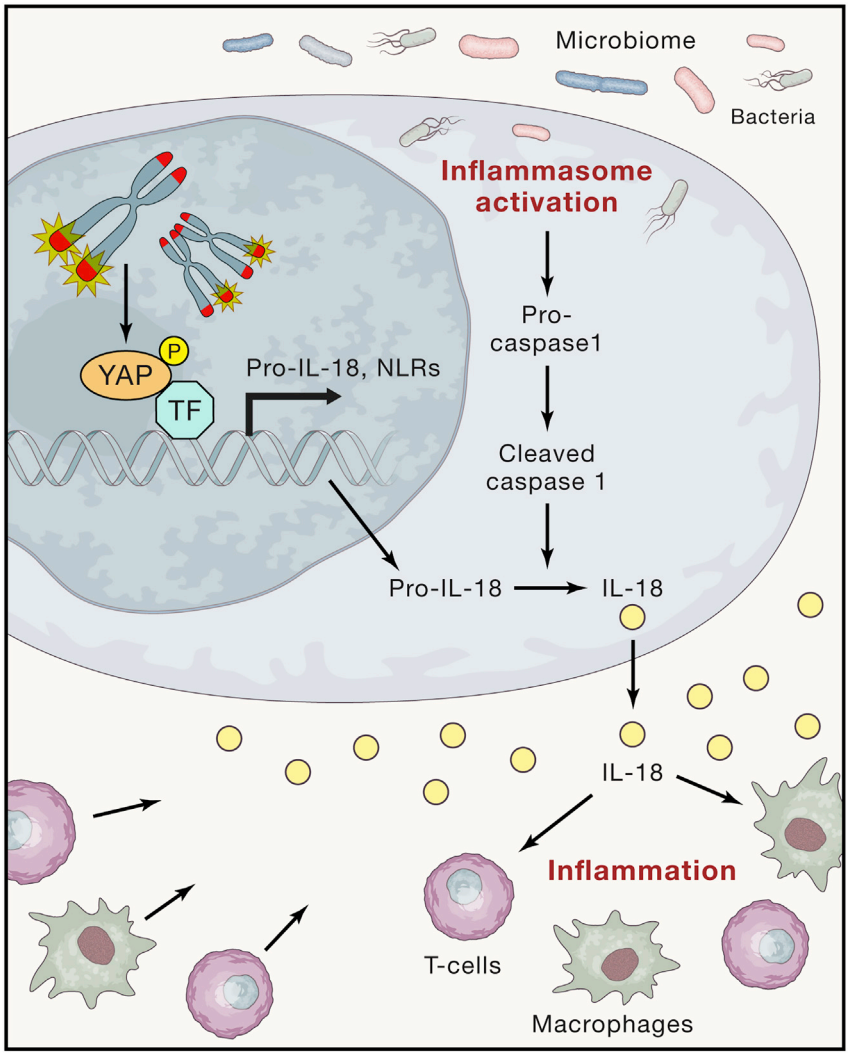


**Figure 5. Telomere dysfunction drives tissue inflammation**

Telomere dysfunction drives tissue inflammation through activation of the ATM/cABL/YAP1 axis and driving secretion of mature IL18 to recruit and potentiate T cells and macrophages. ATM, ataxia telangiectasia mutated; cABL, Abelson murine leukemia viral oncogene homolog 1; YAP1, Yes-associated protein 1; IL-18, interleukin 18.

### *Inflammation*

Aging processes encompass inflammatory signaling from genomically damaged or senescent cells. Telomere dysfunction can initiate and maintain inflammation on several levels. First, telomere dysfunction provokes cellular senescence, which stimulates production and secretion of IL-6 and TNF-$\alpha$, among other inflammatory factors (Coppé et al., 2010). Second, telomere dysfunction results in production of extrachromosomal fragments that can precipitate autophagic cell death via activation of the cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) pathway (Nassour et al., 2019). In the context of cancer specifically, a recent study demonstrated that targeting telomeres with a new drug, 6-thio-2′-deoxyguanosine (6-thio-dG), induces generation of TIFs and release of extrachromosomal DNA into the tumor microenvironment of cancer cells. Uptake of this DNA by dendritic cells triggers the intracellular cGAS/STING pathway and initiates an inflammatory cascade via interferon regulatory factor-3 (IRF3)TANK binding kinase-1 (TBK1)-mediated upregulation of type I interferons, leading to enhanced cross-priming activity and recruitment of interferon $\gamma$ (IFN$\gamma$)-producing CD8+ T cells (Mender et al., 2020). Recent studies have forged a direct link between telomere dysfunction and tissue inflammation (Figure 5; Chakravarti et al., 2020). Specifically, telomere dysfunction activates ataxia telangiectasia mutated (ATM)/Abelson murine leukemia viral oncogene homolog 1 (cABL)-induced phosphorylation of YAP1, resulting in its nuclear translocation. Nuclear YAP1 upregulates inflammation and inflammasome genes such as *pro-IL18*, *NLRC5*, *NLRP1b*, and *NLRP6*. Together with gut microbiome activation of the inflammasome axis, the resultant activated caspase-1 cleaves pro-IL-18 into its mature IL-18 form, whose secretion recruits and activates T cells and initiates inflammation. Treatment of telomere-dysfunctional mice with a YAP1 inhibitor, caspase-1 inhibitor, or antibiotics *in vivo* or reactivation of telomerase in the intestinal compartment leads to a reduction in cleavage of procaspase-1 to caspase-1 and in production of mature IL-18, consequently reducing tissue inflammation. Chakravarti et al., 2020) established a direct link between telomere dysfunction and inflammation that can partially explain the age-related increase in inflammatory response seen in the aged population. This work also highlights the cooperation between cell-intrinsic telomere dysfunction-driven molecular pathways and the microbiome in driving the inflammatory response.

In summary, numerous studies link telomere dynamics to pathways and biological processes underlying all hallmarks of aging. Moreover, many of these stress responses create feedback loops that further damage telomeres, amplifying and accelerating degenerative aging phenotypes. This interconnectedness of telomeres with virtually all hallmarks of aging serves to initiate and escalate the aging process.

## Telomeres and telomerase in age-related diseases and cancer

How telomeres contribute to aging and age-related diseases came to light through the study of mice and individuals harboring germline alterations in telomere maintenance genes. Murine and human telomeres show differences in length and regulation. The laboratory mouse, *Mus musculus*, possesses telomeres up to 10

times longer than those of humans (30–150 kb in mice versus 10–15 kb in humans) (Kipling and Cooke, 1990). Moreover, murine somatic cells exhibit high levels of telomerase activity relative to the low activity found in human somatic cells (Kipling, 1997; Wright et al., 1996). These species differences enabled elucidation of the function of telomeres and telomerase in genome stability, aging, and cancer through engineering and characterization of mice with shorter, human-like telomeres. Specifically, in mice null for TERT or TERC, successive intergenerational crosses progressively shorten telomeres, culminating in telomere dysfunction (end-to-end fusions) by generation 3 (G3) (Artandi et al., 2000; Blasco et al., 1997). This telomere dysfunction coincides with widespread tissue stem cell depletion, progressive tissue atrophy, germ cell depletion, reduced fecundity, impaired adaptive immunity, decreased memory, delayed wound healing, diminished stress responses, increased hair graying and alopecia, diminished cardiac function, a weakened skeletal frame, increased cancer incidence, and overall frailty (Allsopp et al., 2003; Artandi et al., 2000; Chin et al., 1999; Colla et al., 2015; Jaskelioff et al., 2011; Leri et al., 2003; Pignolo et al., 2008; Rudolph et al., 1999; Sahin et al., 2011).

This section summarizes mounting evidence linking telomere functionality to aging processes such as frailty as well as age-related diseases, including cardiovascular diseases (atherosclerosis, vascular dementia, and coronary artery disease), metabolic disease (type II diabetes), neurological disease (Parkinson's disease), and cancer.

### *Telomere dysfunction and telomerase in age-related diseases*

Numerous studies have implicated telomere dysfunction in age-related diseases. First, in highly proliferative tissue such as the skin, gastrointestinal tract, and hematopoietic system, low levels of telomerase in progenitor cell compartments and continual tissue renewal cause progressive telomere attrition over decades. This attrition ultimately triggers DNA damage responses such as cell cycle arrest, apoptosis, impaired differentiation, and/or senescence. Second, lowly proliferative tissue such as heart, brain, and liver could experience ROS-induced damage of telomere sequences, causing attrition and uncapping over time. Specifically, murine and human studies show that oxidative stress itself accelerates telomere attrition in vascular endothelium (González-Guardia et al., 2014), skeletal muscle (Ludlow et al., 2014), cardiomyocytes (Puente et al., 2014), and innate and adaptive immune cells (Vaziri et al., 1993; Kaneko et al., 1996). Additionally, although telomere shortening per se generates TIFs, ROS-induced guanine modification also produces TIFs, reflecting uncapping from shelterin disengagement (Anderson et al., 2019). This is evident from pioneering studies demonstrating that perturbations in shelterin components, such as dominant-negative mutant forms of TRF2, can induce TIFs without changes in telomere length (van Steensel et al., 1998).

The entwined processes of senescence and inflammation may be particularly relevant to the telomere-aging connection. These pleiotropic actions can drive atherosclerosis, type II diabetes, osteoarthritis, and Parkinson's and Alzheimer's diseases. Specifically, a recent study highlighted the role of senescent cells in driving Alzheimer's disease (Bussian et al., 2018). In this study, mice engineered with mutant *Tau* (*MAPTP301SPS19*) that develop neurofibrillary tangles and Alzheimer's-like symptoms show preservation of cognitive function upon genetic or pharmacological removal of $p16^{INK4a}$-expressing senescent astrocytes and microglia (Bussian et al., 2018). The significance of this study lies in the finding that accumulation of senescent cells precedes formation of neurofibrillary tangles, suggesting that senescent cells may influence initiation of tangle formation. Similarly, removal of senescent cells from the BubR1 progeroid mouse model leads to an extended health span (Baker et al., 2011). These studies catalyzed development of "senolytic" agents capable of clearing senescent cells in humans (Ellison-Hughes, 2020). The senescence-inflammation axis may also be active in patients with end-stage heart failure, cardiac hypertrophy, and coronary artery disease (Birks et al., 2008).

### *Telomere dysfunction in inflammatory diseases*

That telomere dysfunction can drive inflammatory diseases in human aging is evident from the study of telomeropathy. Telomeropathy results from germline defects of telomere maintenance genes, including *TERT*, *TERC*, *DKC*, *PARN*, *RTEL1*, *TINF2*, and *POT1*, as reviewed elsewhere (Opresko and Shay, 2017). On the cellular level, telomeropathy is characterized by (1) hematopoietic stem cell depletion leading to bone marrow failure, (2) immunosenescence of lymphocytes, and (3) intestinal stem cell loss resulting in intestinal villous atrophy associated with crypt cell apoptosis, villous blunting, basal plasmacytosis, and intraepithelial lymphocytosis (Jonassaint et al., 2013). On the tissue level, telomeropathy shows increased incidence of idiopathic pulmonary fibrosis, liver cirrhosis, and kidney diseases (Armanios and Blackburn, 2012), all of which are relate to increased inflammation. Given that high ROS levels are associated with telomere shortening and are postulated to be drivers of tissue inflammation, even in the absence of genetic mutations in telomere-maintenance genes, telomere shortening and damage could be instrumental in driving various inflammatory diseases in older individuals without classic germline mutations affecting telomeres (Wiemann et al., 2002). These conditions could stem from dysfunctional telomeres in a few cells of an affected tissue, leading to a local increase in inflammatory cytokines and driving further tissue damage and telomere shortening. Such inflammatory diseases include inflammatory bowel disease (Risques et al., 2008, 2011), pancreatitis (van Heek et al., 2002), non-alcoholic fatty liver disease (Laish et al., 2016), chronic obstructive pulmonary disease (Birch et al., 2016), and liver cirrhosis as a consequence of chronic liver disease (Wiemann et al., 2002), among others. In such conditions, telomere dysfunction may serve to amplify and cooperate with the primary drivers of disease.

Mitochondrial dysfunction and the associated increased ROS are linked to aging and, specifically, to senescence and inflammation. Maintenance of mitochondrial activity has been a prime therapeutic objective because murine studies have shown beneficial effects of PGC1$\alpha$ overexpression on various aging-relevant biological processes, including metabolism and ROS control (Dillon et al., 2012). Uncontrolled ROS are also known to affect gluconeogenesis, fatty acid metabolism, and $\beta$-oxidation, which can drive age-related metabolic diseases, including diabetes; cancer (see Telomere dysfunction, telomerase, and cancer); inflammatory conditions; sarcopenia; neurodegenerative diseases

like Alzheimer's disease, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis; as well as overall frailty.

### Telomere dysfunction, telomerase, and cancer

Cancer is a disease of aging, with 1 in 2 men and 1 in 3 women in the United States receiving a cancer diagnosis by age 80 (National Cancer Institute, 2020). Moreover, epithelial cancers are the predominant cancer types of the aged; these cancers are typically aneuploid and possess many chromosome structure alterations. Curiously, as mice age, the cancer spectrum consists primarily of hematopoietic (lymphoma) and mesenchymal (sarcoma) cancers and few epithelial cancers (Brayton et al., 2012). Also, these murine malignancies exhibit minimal chromosome structural aberrations. These cross-species differences enabled elucidation of a key mechanism driving the preponderance of epithelial cancers in aged humans as well as the basis of genomic instability in these cancers. Specifically, by engineering telomerase-deficient mice possessing shorter human-like telomeres, we demonstrated (Artandi et al., 2000) that telomere dysfunction, in the setting of p53 deficiency, enables cells in crisis to survive chromosomal breakage events fueling amplification, deletion, and translocation of cancer-relevant genes (Figure 6). Strikingly, these TERC/p53-null mice not only show increased cancer initiation but also a humanized tumor spectrum possessing complex karyotypes. These mouse genetic experiments, coupled with subsequent genomic evidence of telomere dysfunction in early-stage human cancers (Ding et al., 2012; Fernandes et al., 2020; Rudolph et al., 2001), established that telomere dysfunction is a major mechanism driving epithelial carcinogenesis in the aged. Stated differently, the absence of this mutational mechanism in the mouse (long telomeres and promiscuous telomerase expression) protects mice from developing epithelial cancers.

These murine models revealed the complex role of telomeres in the evolution of cancer; specifically, how telomere dysfunction enhances tumor initiation while restricting full malignant progression (Chin et al., 1999; Rudolph et al., 2001). For example, in the intestinal adenoma of adenomatous polposis coli ($APC^{min}$); TERC-null mouse model, telomere dysfunction initially increases adenoma frequency but ultimately constrains progression to macroadenomas and increases survival (Rudolph et al., 2001). This constrained malignant progression relates prominently to activation of p53 in aspiring cancer cells experiencing telomere dysfunction. Inactivation of the p53 tumor suppressor is one of the most frequent events in human epithelial cancers (Brosh and Rotter, 2009), and the status of p53 can dictate whether telomere dysfunction enhances or suppresses cancer development. Specifically, late-generation $TERC^{-/-}$ mice null for p53 show an increase in cancer incidence and decreased survival (Artandi et al., 2000; Chin et al., 1999), whereas late-generation $TERC^{-/-}$ mice null for Ink4a/Arf show a significant reduction in tumor incidence and increased survival (Greenberg et al., 1999). Notably, even though Ink4a/Arf-null mice lack p19ARF, which signals p53 during oncogenic activation, a functional p53-dependent DNA damage response is retained in these mice (e.g., apoptosis) (Kamijo et al., 1997). Thus, the role of telomeres in tumor initiation depends on the integrity of p53-mediated DNA damage signaling and associated cellular checkpoint responses.

Recent single-cell DNA sequencing of human cancers has uncovered that many cancers evolve as a consequence of a single punctuated genomic event (Gao et al., 2016; Navin et al., 2011) where bursts of copy number alterations and mutations dominate all clones. Although the mechanisms driving this punctuated evolution or "episodic instability" are under active investigation, mouse and human evidence clearly implicates telomere-based crisis and subsequent telomerase reactivation as a mechanism shaping cancer genomes and driving epithelial carcinogenesis in the aged. Although this episodic instability model is consistent with human genomics data (Sottoriva et al., 2015) on evolving cancers, we acknowledge that the "Vogelstein mechanism" (Fearon and Vogelstein, 1990)— stepwise accumulation of driver mutations—may also be at work here and that the two models are not mutually exclusive.

Additional evidence supporting a telomere-cancer link includes age-dependent telomere shortening in colonic mucosa of aged individuals and shorter telomeres in cancers than in adjacent nonmalignant tissues (Hastie et al., 1990). Additionally, human tumor tissues show elevated TERT and TERC expression compared with matched normal tissues (Meyerson et al., 1997), and malignant transformation of cultured human primary cells requires enforced TERT expression, indicating that telomere maintenance is essential for full malignant transformation (Hahn et al., 1999). Finally, cancer-prone mice engineered with an inducible TERT allele first experience telomere-based crisis followed by subsequent telomerase reactivation. This crisis-reactivation sequence generates more aggressive tumors, establishing that crisis enables acquisition of genomic events that endow a more aggressive malignant phenotype (Ding et al., 2012; Hu et al., 2012). Similarly, transient induction of telomere uncapping in telomerase wild-type mice (by mutant TRF2 protein and disruption of the shelterin complex) increases initiation and progression of hepatocellular carcinoma in carcinogen-treated mice (Begus-Nahrmann et al., 2012). In contrast, telomere shortening in TERC knockout mice increases tumor initiation but fails to induce tumor progression in a model of carcinogen-induced hepatocellular carcinoma (Farazi et al., 2003). Thus, multiple lines of evidence substantiate a role of telomere-based crisis and telomerase reactivation in cancer initiation and progression, respectively.

### Telomere- and telomerase-dependent mechanisms of carcinogenesis

As noted, loss of telomere function leads to end-to-end fusion. During mitosis, these dicentric chromosomes generate anaphase bridges with subsequent chromosome breakage, leading to the BFB cycle first proposed by Creighton and McClintock (1931). The surviving BFB daughter cells accumulate cancer-relevant chromosomal alterations during crisis and ultimately activate telomerase to dampen DNA damage signaling and quell rampant chromosomal instability, enabling full malignant progression. A TERT-inducible model of prostate cancer has experimentally validated this cancer genome evolution concept. In this cancer model (Ding et al., 2012), critically short telomeres result in increased cancer cell apoptosis and decreased proliferation, producing smaller and less aggressive tumors compared with telomere-proficient controls. In these chromosomally unstable high-grade prostate intraepithelial neoplasias, experimental reactivation of telomerase results in malignant progression, including acquisition of new phenotypes such

as bone metastasis. In contrast, telomere-intact controls (which did not experience crisis followed by telomerase reactivation) exhibited only local invasion (Ding et al., 2012). Moreover, genomic analysis of tumors experiencing crisis showed additional pro-metastasis mutations (e.g., SMAD4 deletion and additional perturbations to the transforming growth factor β [TGF-β] pathway). Thus, telomere-based crisis drives chromosomal instability to initiate cancer, and subsequent telomerase reactivation enables full malignant progression.

Large-scale sequencing studies show that TERT promoter mutations are the most frequent noncoding mutations in human cancer (Fredriksson et al., 2014; ICGC TCGA Pan-Cancer Analysis of Whole Genomes Consortium, 2020; Rheinbay et al., 2020); such mutations are highly enriched in cancers originating in tissues with relatively low rates of self-renewal (Killela et al., 2013). For example, melanoma shows highly recurrent TERT promoter mutations with an average 4-fold increase in TERT expression (Horn et al., 2013), and primary glioblastoma shows TERT promoter mutations in over 80% of cases (Killela et al., 2013). The most common recurrent single-nucleotide mutations in the TERT promoter occur at G228A and G250A, which generate *de novo* ETS consensus binding motifs to recruit GA-binding proteins(GABP) and potentially other E26 transformation specific (ETS) family of transcription factors to elevate TERT expression (Bell et al., 2015). Additional mechanisms driving increased TERT expression in cancer also result from crisis-induced chromosomal alterations (Artandi et al., 2000), which produce focal amplifications of the TERT locus in hepatocellular carcinoma (Totoki et al., 2014). Finally, gene therapy studies have shown that CRISPR-mediated correction of the TERT promoter to wild-type sequences significantly prolongs the survival of glioma-bearing mice (Li et al., 2020).

In addition to the genomic mechanisms driving TERT upregulation, oncogenic signaling pathways can enhance TERT gene transcription. Cellular MYC (c-MYC) can bind Myc binding elements in the TERT promoter to induce TERT transcription and increase telomerase activity in primary human fibroblasts, although enforced TERT expression alone is an insufficient substitute for c-MYC in promoting transformation (Greenberg et al., 1999; Wang et al., 1998). Similarly, activated WNT signaling cooperates with Krüppel-like factor 4 (KLF4) to activate TERT transcription (Hoffmeyer et al., 2012). Although key developmental and cancer-related pathways can activate TERT, there is also evidence that TERT, in turn, can activate these pathways in *cis*. Activation of telomerase in TERT overexpression mouse models showed that proliferation of quiescent hair follicle stem cells and kidney podocytes can occur independent of telomere length, TERC function, or TERT reverse transcriptase function (Flores et al., 2005; Sarin et al., 2005). Mechanistic studies revealed that TERT directly binds TCF elements and enhances MYC and WNT transcriptional programs (Choi et al., 2008; Park et al., 2009), pathways known to govern stem cell homeostasis and drive cancer (Dang, 2012; Reya and Clevers, 2005). In addition, TERT interacts with the NF-κB complex and binds IL-6 and TNF-α promoter elements, increasing cell proliferation and resistance to cell death (Ghosh et al., 2012). The increased cell proliferation and resistance to cell death upon ectopic TERT and TERC expression are attenuated through repression of p65. Functionally, G1 TERC-null mice were more resistant to endotoxic shock than their littermate controls, with more than 50% of mice surviving lipopolysaccharide-induced shock compared with 25% of controls, which suggests that telomerase regulates the NF-κB inflammatory response independent of telomere length. These studies point to the interactions of oncogenic signaling molecules and TERT in regulation of cancer-relevant circuits. Further investigation is needed to assess the role of these circuits in human cancer.

Mutations in and/or altered expression of shelterin proteins have been detected in cancer, although their precise roles in cancer initiation and progression are not yet determined. In early-stage chronic lymphocytic leukemia, telomere dysfunction is linked to disease initiation and end-to-end fusions (Chang, 2013). Accordingly, in this disease, there is reduced expression of shelterin proteins, including TRF1, RAP1, and POT1 as well as TIN2 and TPP1. POT1 is the most mutated shelterin-related protein in human cancers, with somatic mutations present in 5% of chronic lymphocytic leukemia cases. Somatic mutations in POT1 or RAP1 can occur in familial glioma, familial melanoma, Li-Fraumeni-like syndrome, parathyroid adenoma, cardiac angiosarcomas, mantle cell lymphoma, and hepatocellular carcinoma (Fernandes et al., 2020). TRF1 and TRF2 are upregulated in various tumor types, including cancers of the lung, breast, colon, stomach, and kidney, suggestive of a supportive role of shelterin proteins in human cancer.

### *Alternative mechanisms of telomere maintenance in cancer*

Although telomerase is the most common telomere maintenance mechanism in cancer, cancer cells also utilize a telomerase-independent recombination-mediated mechanism called alternative lengthening of telomeres (ALT) (Henson et al., 2002). ALT occurs in 5%–15% of human cancers, particularly in osteosarcoma and glioblastoma, and is typically associated with a poor prognosis (Heaphy et al., 2011). ALT lengthens telomeres using DNA homology-directed repair mechanisms in which telomeric DNA templates are copied from sister chromatids or non-homologous chromosomes. Essential factors for ALT include MRE11, RAD50, NBS1, FEN1, MUS81, and FANCD2 (Cesare and Reddel, 2010). Interestingly, ALT-positive cells are commonly defective in their ability to sense cytosolic DNA, and studies have demonstrated that extrachromosomal telomere repeats (ECTRs) elicit an IFN response through the cGAS-STING cytosolic DNA-sensing pathway (Chen et al., 2017). Thus, cancer cells employing ALT have additional anti-proliferative barriers to overcome, such as senescence induction and innate immune surveillance, because of constant production of ECTRs. Interestingly, although ALT is an important factor that drives tumorigenesis in the absence of telomerase reactivation, it is less efficient in driving aggressive malignancy and metastasis. Evidence derives from a study where mouse *mTerc*$^{-/-}$ *Ink4a/Arf*$^{-/-}$ fibroblasts that were passaged extensively showed development of ALT. Although ALT-positive cells maintained telomeres, they were unable to form lung metastases; however, upon mTERC transduction, telomerase-positive cancer cells acquired metastatic potential, establishing that ALT-positive and telomerase-positive cancers are not biologically equivalent (Chang et al., 2003).

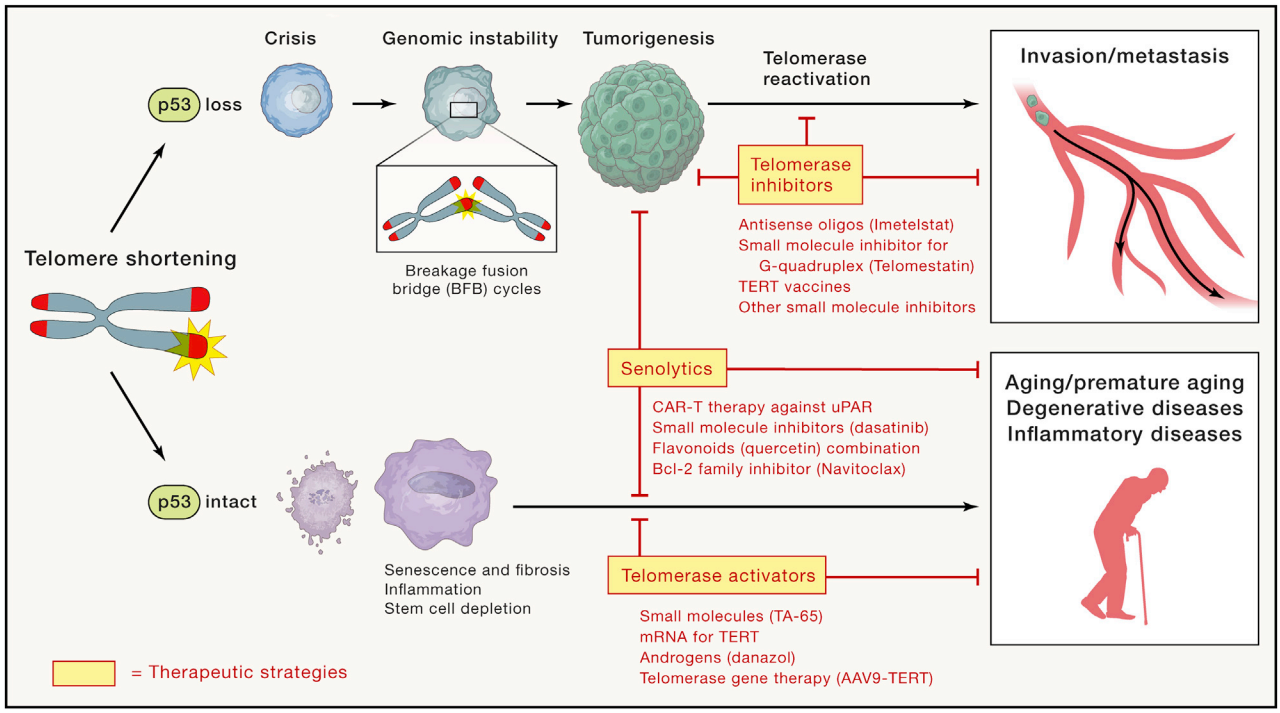


**Figure 6. Telomere/telomerase in aging, cancer, and potential therapy**

Telomere shortening leads to senescence, fibrosis, inflammation, and stem cell depletion in the presence of a functional p53 checkpoint. These processes lead to aging and other degenerative and inflammatory diseases. Telomerase activators and senolytics can inhibit these processes and inhibit aging and age-related diseases. Shortened telomeres also lead to telomeric fusion and cycles of break-fusion-bridges. In the absence of a p53 checkpoint, these events lead to tumorigenesis. Further activation of telomerase leads to progression to invasion and metastasis. Telomerase inhibitors and senolytics inhibit processes that can thwart tumor progression, invasion, and metastasis.

The ALT pathway can also serve as a resistance mechanism for telomerase inhibitor therapies. In a lymphoma-prone ATM mutant mouse model engineered with a tamoxifen-controlled TERT allele (TERT-ER) (Hu et al., 2012), extinction of telomerase activity resulted in re-entry of lymphoma cells into telomere-based crisis. Although telomerase extinction cured two-thirds of mice, the remaining mice developed recurrent tumors possessing hallmark features of ALT (Hu et al., 2012). Interestingly, these recurrent tumors acquired amplifications and deletions in genes involved in homologous recombination that are known to play essential roles in ALT as well as amplification of the PGC1β locus, which may signify ongoing mitochondrial stress in ALT-positive cells (Hu et al., 2012). These mechanistic insights inform potential regimens combining telomerase inhibitors with agents targeting homologous recombination or mitochondrial mechanisms. With respect to the latter, ALT-positive cancer cells, which show diminished mitochondrial function and associated high intracellular ROS, are particularly susceptible to agents that target antioxidant proteins.

## Outlook for telomerase therapy and future perspectives

The link between telomere dysfunction and the hallmarks of aging, the incidence of age-associated diseases, and development of inherited and acquired degenerative conditions has catalyzed interest in telomerase restoration therapy as a potential antiaging strategy (Figure 6). The optimal application of such therapy would likely be in the form of transient telomerase induction to enable restoration of telomere reserves and healing while avoiding the potential for fueling cancer that might come from constitutive telomerase activation. Indeed, in preclinical mouse models with long telomeres, constitutive TERT expression increased the lifespan by 40%, although these mice also harbored extra copies of p53, p16, and ARF (Tomás-Loba et al., 2008), which enhanced their cancer resistance. It remains to be determined whether the increased lifespan and disease-modifying activities of enforced TERT expression relate to TERT's actions on telomeres or to its activation of WNT, which may enhance stem cell reserves.

Advancing knowledge of the molecular networks regulated by telomerase and rejuvenation of prematurely aged mice with telomerase activation (Jaskelioff et al., 2011) has fueled interest in development of agents that activate TERT expression for antiaging therapy. Several small molecules have been identified that appear to activate TERT (Figure 6), including TA-65 (Harley et al., 2011) and histone deacetylase inhibitors (Won et al., 2004), although a clear understanding of their mechanisms of action is lacking. TA-65 (cyclastragenol) is a natural compound isolated from various plant species in the genus of *Astragalus*. Ongoing human trials of TA-65 have reported improved macular function (Dow and Harley, 2016), reduced high-density lipoprotein levels, and reduced levels of the inflammation markers C-reactive protein and TNF-α (Fernandez et al., 2018). In addition, hormonal

agents such as danazol (an antiestrogenic and antiprogestogenic) and 5α-dihydrotestosterone (an androgen), which can increase telomerase levels, are being tested in individuals with telomeropathies (Calado et al., 2009; Townsley et al., 2016). Finally, recent development of agents targeting TERC stability may offer new therapeutic options for telomeropathies. Specifically, PAPD5 degrades TERC by 3′ oligoadenylation, priming these transcripts for destruction by the RNA exosome. As mentioned earlier, PARN, involved in de-adenylation and maturation of TERC, is mutated in several diseases, including dyskeratosis congenita. A PAPD5 small-molecule inhibitor increased telomere length in induced pluripotent stem cells from individuals with dyskeratosis congenita and was well tolerated in mice over an extended period of time (Nagpal et al., 2020).

A particularly intriguing application of pulsatile telomerase activation therapy would be treatment of progeroid diseases such as Werner and Bloom syndromes. This concept emerges from the astonishing lack of degenerative phenotypes in mice null for genes connected to inherited degenerative conditions in humans, including () ataxia-telangiectasia, which results from mutational inactivation of ATM; (2) Bloom syndrome, caused by a mutation in BLM of the RecQ helicase family; and (3) Duchenne muscular dystrophy (DMD), which arises from mutation of dystrophin. Strikingly, when each of these alleles was crossed to the TERC knockout mouse, the salient phenotypes of these syndromes surfaced as telomeres reached shorter, more human-like lengths (typically at G2–G3) (Chang et al., 2004; Du et al., 2004; Sacco et al., 2010; Wong et al., 2003). For example, WRN/TERC-null mice recapitulated the hallmark features of Werner syndrome, including kyphosis, pathological long bone fractures, alopecia and hair graying, defects in stem cell and immune compartments, senile cataracts, hematopoietic deficiencies such as pancytopenia and unbalanced response to immunogens, and metabolic deficiencies, including insulin resistance (Chang et al., 2004).

As noted, telomere-intact mouse models with mutations in the *WRN* or *BLM* genes do not exhibit any degenerative phenotypes, providing a rationale for telomerase activation therapy to delay or reduce the intensity of symptoms and extend life expectancy. Along similar lines, in individuals with germline mutations affecting telomere maintenance (e.g., *DKC*), telomerase activation therapy could alleviate progressive symptoms such as anemia, pulmonary fibrosis, and gastrointestinal dysfunction. Another group of diseases with limited treatment options in which telomerase activation may play a beneficial role is chronic inflammatory diseases such as liver cirrhosis, pancreatitis, and ulcerative colitis (Figure 6). Telomere dysfunction at disease onset can drive tissue inflammation, which, in turn, can accelerate telomere shortening, creating a feedforward loop that ultimately leads to disease recurrence and even cancer brought about by genomic instability, p53 loss, and telomerase reactivation. Here again, telomerase activation at very early stages of disease prior to entry into telomere-based crisis could prevent disease flares and carcinogenesis. Telomerase activation may also be useful in treating neurodegenerative diseases, given the strong improvement in brain health in mice following genetic induction of telomerase (Ding et al., 2012; Jaskelioff et al., 2011). In addition to telomerase activation, it may also be possible to ameliorate telomere dysfunction-induced organ degeneration and mortality by impairing the function of checkpoints that mediate the tissue-disruptive effects of dysfunctional telomeres. Along these lines, it has been shown that the selective inhibition of p21-dependent cell cycle arrest (Choudhury et al., 2007) or Puma-mediated apoptosis (Sperka et al., 2011) could prolong maintenance of tissue integrity and lifespan in telomere-dysfunctional mice without increasing cancer development. Also, deletion of Exo1 increases tissue maintenance and lifespan in telomere-dysfunctional mice by inhibiting formation of chromosomal fusions and BFB cycles that drive induction of DNA damage and activation of p53 checkpoints in telomere-dysfunctional mice (Schaetzlein et al., 2007).

Contrary to the potential applications of telomerase activation in antiaging therapy, the increased telomerase activity observed in most cancers has led to development of antitelomerase therapeutic agents. Several strategies to target TERT in cancer have been engineered, including antisense oligos, vaccines, and small-molecule inhibitors (Ruden and Puri, 2013; Figure 6), but no antitelomerase agents have reached randomized phase III trials. This limited efficacy may be attributable to the time required for telomeres to shorten to a length that can induce tumor shrinkage. In addition, alternative strategies to inhibit telomerase could generate a more meaningful effect in the clinic. To start, cancers with intact p53 would be more suitable for telomerase inhibition because of their functional checkpoint machinery, which would trigger senescence. This strategy still requires caution because preclinical animal studies have demonstrated that TERT inhibition can lead to activation of the ALT pathway in lymphoma (Hu et al., 2012). Thus, combined telomerase and ALT pathway-suppressing drugs (Flynn et al., 2015) could minimize emergence of resistance. In the subset of cancers that are ALT positive, targeting relevant immune circuits may also enhance response rates and outcomes. That is, because of the persistent production of cytosolic DNA in ALT-positive cancer cells (Chen et al., 2017), it is tempting to speculate that these tumors may be more responsive to immunotherapy because cytosolic DNA upregulates IFN signaling via activation of cGAS-STING. In preclinical studies, the nucleoside analog 6-thio-dG, which incorporates into newly synthesized telomeres to cause telomeric DNA damage, can render cancers that are resistant to anti-PD-L1 treatment susceptible to immune checkpoint therapy (Mender et al., 2020). To summarize, antitelomerase therapy remains a viable anti-cancer strategy based on its potential role in the inhibition of advanced malignancies, although the genotypic and biological context will be important to determine, along with specific combination therapies for synergistic activity.

In closing, the pursuit of the fundamental knowledge of chromosome structure and cell biology has illuminated mechanisms central to many major human diseases and aging itself. The telomere field has been an exemplary model of basic science and multidisciplinary convergence, revealing the role of telomeres and telomerase in the hallmarks of aging and the pathogenesis of cancer. Many knowledge gaps remain, such as elucidating the mechanisms governing telomerase expression and activity, the non-canonical functions of TERT, and the interplay between telomere dysfunction and pathological processes such as inflammatory, fibrotic, and degenerative diseases. Regardless of

whether telomere dysfunction initiates the disease or is only a participant, telomeres clearly play an integral pathogenetic role in human disease. Such an elemental role encourages the development and rigorous testing of telomerase activators for treatment of aging and age-associated diseases as well as assessment of efficient telomerase inhibitors for treatment of advanced cancers. The hallmarks delineated here outline a framework to stimulate further study of the role of telomeres and telomerases, which may help confront the lethal disease ultimately suffered by all: aging.

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## DECLARATION OF INTERESTS

R.A.D. is a co-founder, director, and advisor of Tvardi Therapeutics and co-founder and advisor of Asylia Therapeutics, Nirogy Therapeutics, and Stellanova Therapeutics.

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